LEFT VENTRICULAR HYPERTROPHY AND MYOCARDIAL PROTECTION WITH PERHEXILINE DURING CARDIAC SURGERY

by

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ABSTRACT

Myocardial protective strategies during cardiac surgery continue to improve yet they remain imperfect. Patients with left ventricular hypertrophy (LVH) are considered to be at greater risk of myocardial injury post cardiac surgery. Perhexiline is an anti-anginal agent known to modulate myocardial metabolism towards a more efficient glucose metabolic pathway. This metabolic modulation may improve myocardial protection.

In this thesis I present a multi-centre double-blind randomised placebo controlled trial evaluating the role of perhexiline as an adjunct to standard myocardial protection in patients with LVH secondary to aortic stenosis undergoing an aortic valve replacement. Perhexiline does not augment myocardial protection. Magnetic Resonance Spectroscopy based energetic studies, echocardiographic and functional assessments in a homogenous patient cohort show no added benefit with perhexiline therapy in LVH. Therefore perhexiline should be limited to those patients refractory to maximum medical therapy.

Metabolomic assessment of LVH has shown no change in the metabolomic profile within the myocardium. However any changes that do exist may be subtle. In LVH there is an increased activity of some innate cardioprotective mechanistic pathways in patients that do not sustain a low cardiac output episode post cardiac surgery. Further examination of these cardioprotective regulators is warranted.

To my parents,

for the sacrifices they have made to create the opportunities I have had

and

to my wife, Trianka

for all her support and encouragement

ACKNOWLEDGMENTS

I would like to thank a number of people who have helped me complete the work that is incorporated in this thesis. Firstly I would like to thank the British Heart Foundation, who funded my fellowship in cardiac surgery and thereby funded the main clinical trial (HYPER), and all the work associated with my research and work towards this thesis. I would also like to thank the University Hospital Birmingham Charities Foundation who helped fund a sub-study of the main HYPER trial.

This thesis would not have been possible without the support and guidance of my supervisors; Prof Domenico Pagano, Consultant Cardiothoracic surgeon, Queen Elizabeth Hospital and Principal Investigator of the HYPER trial; Mr Neil J. Howell, Consultant cardiothoracic surgeon, Queen Elizabeth Hospital; Prof Nick Freemantle, chair of Biostatistics, University College London and Prof Mark Viant, Chair of Metabolomics, University of Birmingham. They have all advised and guided me uniquely in the different aspects and components of my research. I would also like to thank Mr Aaron Ranasinghe, Consultant Cardiothoracic surgeon, Queen Elizabeth Hospital for his continued support during my research and for his additional guidance on this thesis.

I am grateful to a number of individuals who helped me run the clinical trial smoothly according to the trial protocol. This would firstly include all the consultant surgeons namely Messer's Tim Graham, Stephen Rooney, Ian Wilson and George Mascaro. Running a clinical trial as per the trial protocol also required the help and cooperation of a number of individuals in multiple disciplines and this includes all the secretarial staff, staff at the pre-screening clinics, all the staff in theatres especially the anaesthetists, operating department assistants and perfusionists, and all the nursing

staff on the intensive care unit and the ward. I would like to thank Dr Melanie J. Calvert for her help with developing the randomisation programme and periodically checking it's accuracy. The perhexiline concentrations were measured in the Toxicology Unit at Llandough Hospital, Cardiff and I thank Dr Alun D. Hutchings and Emma Bennett for their help with this.

I would like to thank my predecessors in cardiac research who have helped develop the department of cardiac surgery at the Queen Elizabeth Hospital into one that is geared for clinical research. I would particularly like to thank Mr Nigel Drury for helping me with the clinical trial at its initial stages.

I am also grateful for the support from other cardiac centres; University Hospital Coventry and Warwickshire, Coventry and Royal Sussex County Hospital, Brighton and namely Mr Mike Lewis, Consultant cardiothoracic surgeon, Brighton and Mr Sunil Bhudia, Consultant cardiothoracic surgeon, Coventry who were extremely supportive towards establishing a multi-centre trial and for their encouragement towards my research. At these centres, I would like to thank research nurses Ailie Mackenzie and Nina Cooter, Royal Sussex County Hospital, Brighton and research nurse Geraldine Ward at Coventry for their administrative help with the trial at each centre.

I am indebted to all those individuals who were patient and tolerant enough to teach me the new scanning and laboratory techniques that I have used to complete various portions of my research, outlined in this thesis. Namely this includes Dr Roger Beadle, and Dr Michael Kuel, Registrars in cardiology, who taught me the techniques of Magnetic Resonance Spectroscopy and for performing the Transthoracic Echocardiography studies in patients enrolled into a sub-study of HYPER. With regard

to the laboratory work I would like to thank Prof Mark Viant's group in Biosciences, namely Dr Jennifer Hill initially, who taught me the principles of metabolomic analysis and then Dr Ralf Weber who facilitated the metabolomics study and performed the complex metabolomic analysis of small ventricular biopsies. I would also like to thank Dr Dan Tennant and his group in Clinical Cancer Sciences for facilitating the work on key master regulators and helping me with the techniques of Western blotting and analysis.

Finally I would like to thank the patients who enrolled into the clinical trial, without whose informed consent and participation this research would not have been possible.

EXTENT OF PERSONAL CONTRIBUTIONS

The majority of the work presented in this thesis was performed during my period as a British Heart Foundation clinical research fellow in cardiac surgery. I was appointed to this position at the University of Birmingham through competitive entry and took up this position in December 2009 for a period of 3 years. During this time a large portion of my work was related to a clinical trial evaluating the role of perhexiline in myocardial protection in patients with left ventricular hypertrophy (HYPER trial). Other areas of research outlined in the reminder of this thesis stemmed from this trial.

Chapter 1

The introduction and background information to the research presented in this thesis is entirely my own work. The hypotheses outlined at the end of this chapter have been generated through discussions with my supervisors during the conceptual stages of the various components of this research.

Chapter 2

This trial was designed by Prof Domencio Pagano and Mr Neil Howell and was developed on the same principles as the preceding trial (CASPER), evaluating the role of perhexiline during coronary artery surgery and followed the same principles to the preceding trials that were conducted in the department, evaluating myocardial protection. The original trial protocol and grant application were written by Mr. Neil Howell and Mr. Aaron Ranasinghe. The ethical approval and approval from the MHRA were obtained by my predecessor Mr Nigel Drury. Funding and approvals were obtained prior to my appointment. The power calculations and randomisation schedule

for the trial were undertaken my Prof Nick Freemantle and Dr Melanine Clavert prior to my appointment.

I submitted a substantial amendment to the ethics committee and MHRA, to expand the trial to other centres, which also included obtaining local trust approvals. Following my appointment I was responsible for all aspects of trial management. This included the management of local and multi-centre trial logistics and the financial management of the BHF and charities grants. I undertook training in Good Clinical Practice related to all aspects of clinical research including ethics, consent, trial methodology and investigator responsibilities. I also completed courses in database management in Microsoft Access and statistical analysis in SPSS, at the University of Birmingham.

In the first 2 months Mr. Nigel Drury assisted with trial management. I recruited patients into the trial, which included screening patients, consenting, randomising and prescribing the trial therapy. I was responsible for the management of trial therapy thereafter. I coordinated with the administrative staff regarding scheduling of cases and provided a first port of call for all trial patients for reporting of potential side effects and therapy management. I managed the trial and patients recruited into the trial, as per the trial protocol and adhered to the trial protocol in the pre-operative, peri-operative and postoperative stages; in addition I was responsible for the reporting of serious adverse events.

I collected trial patient's tissue samples, processed them and stored them for later analysis in accordance with the Human Tissue Act 2007. In theatre, I managed the patients' haemodynamic status and this continued onto the Intensive Care Unit were I managed the patient for a further 4 – 6 hours post operatively.

I collected all the data and entered it into an MS Access database that I wrote, in accordance with the Data Protection Act 1998. This data was cleaned and used for presentation at the blinded end-points committee meeting that I chaired, the data safety monitoring board meetings and for the final trial statistical analysis. I also submitted data monthly to the UK Clinical Research Network database.

The expansion of the trial to other centres involved establishing relations and collaborations with the departments involved with the trial at each new site, and education of the local administrative and nursing staff, which I provided. I visited these sites regularly to establish these networks at the initial stages, and then subsequently for every case that was performed at each site, to manage the patient as per the trial protocols as outlined above.

Chapter 3

The presentation of the main clinical trial results in this chapter is entirely my own work. The statistical analysis plan was drawn up following discussions between Prof Nick Freemantle and myself. Prof Nick Freemantle was responsible for the statistical analysis of the main trial outcomes and the futility analysis. I performed the remainder of the analyses related to the secondary end-points presented in this chapter.

Chapter 4

I conceived and designed the study outlined in this chapter with advice from Mr. Aaron Ranansinghe. I wrote the study protocol and collaborated with Dr Roger Beadle who had previous experience in performing Magnetic Resonance Spectroscopy. Through this collaboration I learnt the techniques of MRS, which included acquiring and analysing

the spectra. I prepared the documentation required for ethical approval and obtained approval from the University of Birmingham. I recruited all the participants enrolled into this study. I performed the MRS scan with the help of Dr Roger Beadle and then performed the spectral analysis. Subsequently I performed all of the data analysis outlined in this chapter.

Chapter 5

This study was conceived as a sub-study to the main HYPER trial. I was involved with the groundwork for obtaining approval from the ethics committee and regulatory bodies as part of a substantial amendment to the main trial. The techniques I learnt from the preceding validation study on MRS were applied in this study. I recruited all of the patients enrolled onto this study similar to the principles of recruitment outlined above, managed their trial therapy, and coordinated with the administrative staff regarding scheduling of cases. I took blood for serum perhexiline monitoring, and the doses were adjusted by a blinded contact.

The baseline and follow-up echocardiography scans as per the protocol, were performed by Drs Roger Beadle and Michal Kuel. I performed all of the other baseline and follow-up investigations. I performed the MRS scans with the help of Drs Roger Beadle and Michal Kuel. I collected all the data and performed all of the analysis presented in this chapter.

Chapter 6

This study was conceived following discussions I had with Mr Neil Howell and this study led to consolidating collaborations made with Prof Mark Viant's group. The tissue

samples used in this study were obtained from patients recruited into the HYPER trial. I processed the tissue samples at the time they were obtained for later analysis. During the conceptual stages of this study, I spent time in the lab understanding the principles of metabolomics and learning some of the laboratory techniques. This was initially under the supervision of Dr Jennifer Hill. During this time, the measurement and analysis of small myocardial tissue samples using mass spectrometry metabolomic techniques were optimised. I was involved in these discussions and contributed from a clinical perspective.

The tissue samples used in this study were prepared for analysis, and the data gathered from these experiments were analysed by Dr Ralf Weber. The highly specialized laboratory techniques and statistical analysis used in this study extend beyond my capabilities. However I was involved with the interpretation of these results from a clinical context.

Chapter 7

This study was conceptualised following discussion with Mr Neil Howell and Dr Dan Tennant and consolidated existing collaborations with Dr Dan Tennant's group. The tissue samples for this study were obtained from patients recruited into the HYPER trial. I prepared the tissue samples once obtained, for later analysis.

During this study, I attended Dr Dan Tennant's laboratory and initially learnt the techniques of extracting proteins through Western blotting. I used these techniques to prepare the tissue and perform the Westerns presented in this chapter under the supervision of Dr Dan Tennant. The data obtained from these Westerns, were analysed with the help of Dr Dan Tennant.

Chapter 8

The discussion related to the preceding chapters and conclusions drawn from my research presented in this thesis, is entirely my own work. The avenues for future research in this field are drawn from discussions I have had with my supervisors and collaborators.

ABSTRACTS, PRESENTATIONS AND PUBLICATIONS

Abstracts and presentations

Metabolomic analysis of the myocardium in left ventricular hypertrophy secondary to aortic stenosis

Society of Cardiothoracic Surgeons of GB & I- Annual meeting – Edinburgh, March 2014 – Oral presentation

Multi-centre double-blind randomised controlled trial of perhexiline as a metabolic modulator to augment myocardial protection in patients with left ventricular hypertrophy undergoing cardiac surgery

Society of Cardiothoracic Surgeons of GB & I - Annual meeting – Edinburgh, March 2014 – Oral presentation

Publications

Multi-centre double-blind randomised controlled trial of perhexiline as a metabolic modulator to augment myocardial protection in patients with left ventricular hypertrophy undergoing cardiac surgery

European Journal of Cardiothoracic Surgery – October 2014 – *in press*

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ABBREVIATIONS

ACC	acetyl-CoA carboxylase
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMPK	adenosine monophosphate-activated protein kinase
ANOVA	analysis of variance
AS	Aortic stenosis
ASR	annual safety report
ATP	adenosine triphosphate
AUC	area under the curve
AVR	aortic valve replacement
BHF	British Heart Foundation
CABG	coronary artery bypass grafting
CAC	citric acid cycle
CK-MB	creatine kinase muscle-brain type isoenzyme
$C_{OH\text{-}Px}$	concentration of hydroxyl-perhexiline
CONSORT	Consolidated Standards Of Reporting Trials
CPB	cardiopulmonary bypass
CPT	carnitine palmitoyltransferase
C_{Px}	concentration of perhexiline
CV	coefficient of variation
CVP	central venous pressure
DSMB	data and safety monitoring board
ECG	electrocardiogram

ETC	electron transport chain
FFA	free fatty acid
FT-ICR	Fourier transform ion cyclotron resonance
G3P	glyceraldehyde 3-phosphate
G6P	glyceraldehyde 6-phosphate
GCP	good clinical practice
GIK	glucose-insulin-potassium
GTP	guanosine triphosphate
ICH	international conference on harmonisation
ICU	intensive care unit
IMP	investigational medicinal product
IQR	interquartile range
IVS	inter-ventricular septum
LCOE	low cardiac output episode
LDH	lactate dehydrogenase
LVEF	left ventricular ejection fractions
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVPW	left ventricular posterior wall
MAP	mean arterial pressure
MHRA	Medicines and Healthcare products Regulatory Agency
mPTP	mitochondrial permeability transition pore
MRS	magnetic resonance spectroscopy
MS	mass spectrometry
NAD^+	nicotinamide adenine dinucleotide

NADH	nicotinamide adenine dinucleotide reduced form
NMR	nuclear magnetic resonance
OR	odds ratio
PAWP	pulmonary artery wedge pressure
PCA	principle component analysis
PCI	percutaneous coronary intervention
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
PGK	pyruvate dehydrogenase kinase
PPARα	peroxisome proliferator-activated receptor alpha
QEH	Queen Elizabeth Hospital, Birmingham
QP	qualified person
RISK	reperfusion injury salvage kinase
ROS	reactive oxygen species
RSCH	Royal Sussex Country Hospital, Brighton
SAE	serious adverse event
SCTS	Society of cardiothoracic surgeons of GB & Ireland
SUSAR	suspected unexpected serious adverse reaction
TOE	Transoesophageal echocardiogram
TTE	transthoracic echocardiogram

1 INTRODUCTION

1.1 Aortic stenosis

1.1.1 Pathology

Aortic stenosis (AS) results from calcification of the aortic valve leaflets, producing rigid cusps. This is a degenerative condition and is more common in the elderly population. Less commonly a bicuspid aortic valve, a congenital abnormality predisposes the aortic valve to early calcific degeneration and hence affects young adults. Rheumatic fever causes progressive fusion of the commissures, and eventual thickening and calcification of the aortic cusps, leading to AS.

The commonest cause of aortic stenosis in adults is degenerative calcification (Selzer 1987; Dare, Veinot et al. 1993; Roberts and Ko 2005). This is an active inflammatory process with thickening of the subendothelium and fibrosis (Olsson, Dalsgaard et al. 1994; Otto, Kuusisto et al. 1994; Mohler, Chawla et al. 1999; Olsson, Thyberg et al. 1999; Mohler, Gannon et al. 2001; O'Brien, Shavelle et al. 2002; Rajamannan, Subramaniam et al. 2003). The lesions contain lipoproteins, which accumulate and calcify. The calcification starts at the base of the cusps and progresses to the valve leaflets. This calcification causes the leaflets to be stiff and less mobile leading to a smaller effective valve orifice area, without commissural fusion.

1.1.2 Natural history

The natural progression of aortic stenosis is individual dependent and there is marked individual variability in haemodynamic progression. It is known that there is a long

latent period, where there are no clinical manifestations of signs or symptoms. During this latent period morbidity and mortality associated with AS is low.

Faggiano et al estimates the progression of AS using results from invasive and non-invasive studies (Faggiano, Aurigemma et al. 1996). Once moderate stenosis is established, jet velocity increases by 0.3m/s and mean pressure gradient increases by 7mmHg per year with a resultant decrease in valve area of 0.1cm^2 per year (Cheitlin, Gertz et al. 1979; Wagner and Selzer 1982; Jonasson, Jonsson et al. 1983; Otto, Pearlman et al. 1989; Roger, Tajik et al. 1990; Davies, Gershlick et al. 1991; Peter, Hoffmann et al. 1993; Rosenhek, Binder et al. 2000).

Degenerative calcific aortic stenosis is considered to progress more rapidly than stenosis affecting a congenitally bicuspid aortic valve or aortic stenosis associated with rheumatic fever (Vaturi, Porter et al. 1999; Rosenhek, Binder et al. 2000). As progression is individual and pathology dependent, regular echocardiographic follow-up is essential in those who are asymptomatic but have mild or moderate AS, to establish the rate of progression of the disease (Bonow, Carabello et al. 2008).

Otto et al in a prospective echocardiographic study describes the rate of progression of AS in asymptomatic patients with a jet velocity of > 2.6m/s and shows that event free survival was 93%, 62% and 26% at 1, 3 and 5 years respectively (Otto, Burwash et al. 1997). However they highlight that a baseline jet velocity of > 4m/s allows an event free survival of only 21% at 2 years compared to 84% for those with a jet velocity of ≤ 4m/s. This relationship between disease progression relative to jet velocity is evident in other studies. Rosenhek et al describe the progression of 128 consecutive patients classified as having severe aortic stenosis (Rosenhek, Binder et al. 2000). In this study event free

survival was 67% at 1 year and dropped to 33% at 4 years. Furthermore they showed through multivariable analysis that the extent of aortic valve calcification was an independent predictor of outcome. Furthermore they state that 79% of patients who had moderate to severe aortic valve calcification and a jet velocity that increased by ≥ 0.3m/s within a year, underwent surgery or died within 2 years of the observed increase.

Following the latent period, symptoms of AS; angina, syncope, dyspnoea and symptoms of congestive heart failure develop. Following the onset of symptoms survival is dramatically reduced with an average survival of 2-3 years if left untreated (Ross and Braunwald 1968; Schwarz, Baumann et al. 1982; Turina, Hess et al. 1987; Horstkotte and Loogen 1988; Kelly, Rothbart et al. 1988; Iivanainen, Lindroos et al. 1996) with an associated high risk of sudden death. The risk of sudden death is rare in echocardiographic studies with prospective follow-up, and thought to occur without preceding symptoms at a rate of < 1% per year (Ross and Braunwald 1968; Horstkotte and Loogen 1988; Pellikka, Sarano et al. 2005).

1.1.3 Pathophysiology

Stenosis of the aortic valve occurs gradually and increases over time. This advancing stenosis leads to an increasing obstruction of the left ventricular outflow tract. Thus, over time there is a gradual increase in the systolic pressure within the left ventricle. Increasing left ventricular pressure overload over time, leads to a pathological response of left ventricular hypertrophy (LVH); increased left ventricular wall thickness, maintaining a normal chamber volume (Sasayama, Ross et al. 1976; Gaasch 1979; Spann, Bove et al. 1980).

Laplace's law outlined below, illustrates the effect on circumferential wall stress by the relationship between pressure, chamber radius and wall thickness

$$Wall\ stress = \frac{pressure \times chamber\ radius}{wall\ thickenss}$$

Left ventricular hypertrophy allows the left ventricular wall stress to remain low, despite the increase in intra-cavity systolic pressure. Therefore the afterload (LV systolic wall stress) remains within the normal range (Krayenbuehl, Hess et al. 1988). This allows the ejection fraction to be maintained, as an increase in afterload would have an inverse effect on ejection fraction. Therefore if the hypertrophic process is inadequate and wall thickness does not increase proportional to intra-cavity pressure, wall stress increases resulting in a low ejection fraction (Gunther and Grossman 1979; Krayenbuehl, Hess et al. 1988).

Ejection fraction is also affected by the contractility of the myocardium. Depressed contractility will produce a lower ejection fraction. In these patients surgical treatment of AS may not have the same beneficial effects as compared to patients with a low ejection fraction secondary to increased wall stress (Carabello, Green et al. 1980).

Left ventricular hypertrophy renders the myocardium to be less compliant (Gaasch, Levine et al. 1976). Furthermore LVH causes an increased wall thickness and low volume/mass ratio. These features result in an increased end-diastolic pressure without chamber dilatation (Gaasch, Levine et al. 1976; Murakami, Hess et al. 1986). An increased end-diastolic pressure is consistent with features of diastolic dysfunction (Gaasch 1994).

Left ventricular hypertrophy is an adaptive feature to AS, and may seem to counteract the increased pressure overload, however the pathophysiological responses are more a result of LVH rather than the aortic stenosis per se. These responses are further explored later in this chapter.

The stenosis would lead to inadequate flow out of the left ventricle and hence reduce the overall cardiac output or may maintain a fixed cardiac output. This in turn causes reduced coronary perfusion. Furthermore there is also reduced perfusion to the cerebrum. A conjunction of these features leads to the symptoms and signs outlined below.

1.1.4 Clinical manifestations

The narrowing of the aortic valve secondary to degenerative calcific aortic stenosis has clinical features consistent with reduced ejection of blood from the heart. There are usually no symptoms until the aortic stenosis is moderate to severe. When symptomatic, angina, dyspnoea and syncope or pre-syncope features of dizziness or light-headedness are the predominate features.

On examination the pulse is of small volume and slow rising. A systolic thrill may be felt in the aortic area. On auscultation an ejection systolic murmur would classically be evident. The murmur is usually longer as the disease is more severe due to the extended ejection time. A single or paradoxically split second heart sound may also be present.

Over time, if heart failure ensues, symptoms and signs of congestive heart failure may be present.

1.1.5 Diagnosis and quantification

Transthoracic or trans-oesophageal echocardiography confirms the diagnosis of aortic valve stenosis (Bonow, Carabello et al. 2008). However, quantification requires a series of measurements calculated through echocardiography that include aortic jet velocity, mean pressure gradient and valve area (Bonow, Carabello et al. 2008). Using these measurements, aortic stenosis is categorised into mild, moderate or severe as outlined in Table 1-1.

	Mild	Moderate	Severe
Jet velocity (m/sec)	< 3.0	3.0 – 4.0	> 4.0
Valve area (cm^2)	> 1.5	1.0 – 1.5	< 1.0
Mean gradient (mmHg)	< 25	25 – 40	> 40

Table 1-1 Grading of aortic valve stenosis

The two dimensional (2D) echocardiogram is useful for the evaluation of the valvular anatomy, pathology and function. Doppler echocardiography is used to calculate the maximum jet velocity, pressure gradients and valve area using the continuity equation (Cheitlin, Armstrong et al. 2003). Echocardiography is also recommended for the initial assessment of LV wall thickness, size and function. Thereafter serial assessment of this is recommended using echocardiography (Bonow, Carabello et al. 2008).

These measurements remain a guide to quantify the disease. Hence an absolute valve area or mean gradient is not used as the primary determinant, in deciding surgery. If a patient has a low cardiac output it is possible that the mean gradient and jet velocity is lower than someone with a normal cardiac output and yet they may have severe aortic stenosis. These patients fall under the category of low flow low gradient aortic stenosis

and need further thorough assessment and work up before a therapeutic decision is made.

1.1.6 Management and treatment

1.1.6.1 Management of the symptomatic patient

Aortic valve replacement (AVR) remains the most appropriate form of treatment for patients with severe symptomatic calcific aortic valve stenosis and more so for those patients who are not too high risk for conventional open valve surgery.

In symptomatic patients AVR improves symptoms and survival (Murphy, Lawson et al. 1981; Schwarz, Baumann et al. 1982; Lund 1990; Connolly, Oh et al. 1997; Kvidal, Bergstrom et al. 2000). Survival is comparative in patients with moderate LV function, secondary to excessive afterload secondary to pressure overload, to those with normal LV function. However in those with severe LV impairment secondary to afterload, there would be survival benefit, but symptomatic benefit may be limited by the residual LV dysfunction (Smith, McAnulty et al. 1978; Connolly, Oh et al. 1997).

Therefore AVR is indicated in all symptomatic patients with severe AS and should be performed as soon as possible after symptom onset (Appendix 9.1).

1.1.6.2 Management of the asymptomatic patient

Patients who are asymptomatic may have a spectrum of aortic disease from mild to severe and may also have varying degree of calcification. It has been recommended that these patients need to be monitored for the development of symptoms and assessment of disease progression (Bonow, Carabello et al. 2008). Trans-thoracic echo is used for the serial assessment of asymptomatic patients in addition to routine clinical follow-up.

There still remains controversy and contention regarding the optimal timing for aortic valve replacement in the asymptomatic patient in relation to the risk/benefit balance.; likely long-term complications of structural deterioration associated with implanting a biological valve in a relatively young patient (Emery, Erickson et al. 2003) or the long-term complications associated with a mechanical valve implantation (Kvidal, Bergstrom et al. 2000).

The current AHA guidelines (Appendix 9.1) recommend AVR for those with severe AS with LV systolic dysfunction. Furthermore it is indicated in asymptomatic patients with severe AS undergoing any other form of cardiac surgery. Furthermore AVR is reasonable under these circumstances, when the AS is moderate. AHA guidelines also suggest that AVR may be considered in asymptomatic patients with an abnormal response to exercise testing or if there is likelihood of rapid progression and in patients with extremely severe AS.

1.1.6.3 Cardiac catheterisation

Cardiac catheterisation to perform pressure studies, is indicated in those patients where echocardiographic evidence is not confirmatory regarding the diagnosis or severity of AS or when there is a discrepancy between non-invasive tests and clinical finding regarding the severity of AS (Bonow, Carabello et al. 2008). Coronary angiography is most useful in assessing the coronary circulation and hence recommended in patients undergoing an AVR for AS, who have a risk of coronary artery disease. Furthermore it aids in surgical planning for an AVR.

1.2 Myocardial metabolism

1.2.1 Fuels for the heart

Myocardial metabolism consists of a number of processes and includes glycolysis, free fatty acid metabolism, the citric acid cycle and oxidative phosphorylation which all contribute in forming high energy phosphates that are utilised in order for myocardial contraction to occur. The key fuels the myocardium utilises are glucose and free fatty acids and their usage is dictated by their overall availability and the state the myocardium is in i.e. aerobic or anaerobic metabolism.

1.2.2 Glycolysis

Glycolysis is the first step in a cascade of processes necessary to metabolise glucose, both in aerobic and anaerobic conditions. The end product of glycolysis is further oxidized to provide energy. The process that takes place in glycolysis is outlined in Figure 1-1, whereby a single glucose molecule is split to form 2 molecules of pyruvate.

Initially glucose taken up into the cardiac myocytes' cytosol is trapped and converted into glucose 6-phosphate by the enzyme hexokinase. Glycolysis begins at the point at which glucose 6-phosphate is split into fructose-1,6-bisphosphate, influenced by the enzyme phosphofructokinase (PFK). Each bisphosphate is then split further into 2 molecules of glyceraldehyde-3-phosphate. Each glyceraldehyde-3-phospate is finally converted into pyruvate. Through glycolysis, there is a net release of 2 pyruvate molecules, 2 ATP molecules and $4H^+$ ions.

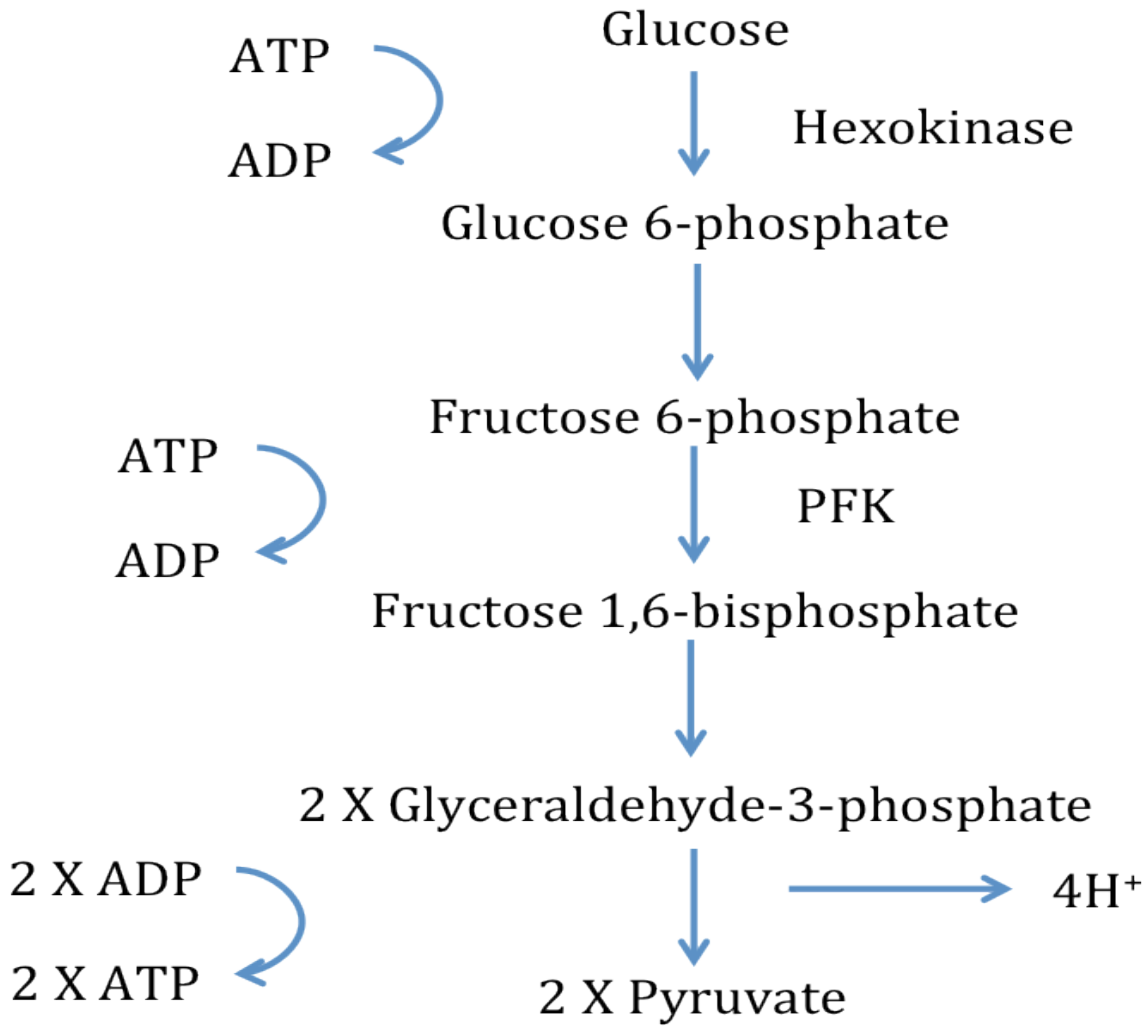


Figure 1-1 Glycolysis pathway

1.2.3 Role of pyruvate

Pyruvate is formed by glycolysis under aerobic conditions. In the aerobic heart, pyruvate is further oxidized through oxidative decarboxylation to Acetyl-CoA by combining with Coenzyme A under the action of the enzyme pyruvate dehydrogenase, found in the inner mitochondrial membrane. This stage forms 2 carbon dioxide molecules and 4 hydrogen atoms, which later enters the oxidative phosphorylation cascade to form ATP. Acetyl-CoA enters the citric acid cycle. Pyruvate dehydrogenase is activated by increased heart work and catecholamines. It is inhibited by $NADH_2$

formation during hypoxia or ischaemia. Furthermore pyruvate dehydrogenase is also inhibited by fatty acid oxidation, in turn slowing down glycolysis (Lopaschuk, Rebeyka et al. 2002).

Under anaerobic conditions pyruvate forms lactate. When aerobic conditions exist, lactate is reversibly converted back into pyruvate by the enzyme lactate dehydrogenase (Figure 1-2).

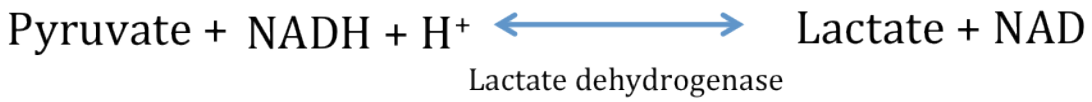


Figure 1-2 Lactate formation

1.2.4 Fatty acid metabolism and β-oxidation

The extent of fatty acid metabolism is proportional to the concentration of free fatty acids (FFA) in the circulation. FFAs are transported in the blood stream to cardiomyocytes, bound to albumin (van der Vusse, van Bilsen et al. 2000). FFA metabolism occurs only in the mitochondrion; therefore the 1st step in the metabolism of FFA is their entry into the mitochondrion.

The carrier substance carnitine is involved in the passage of FFA into the mitochondrion. The first step is activation of intracellular fatty acid by CoA to from fatty acyl-CoA derivatives. As the mitochondrial membrane is not permeable to these acyl-CoA molecules a staged transfer occurs using carnitine to form acylcarnitine, which is transferred into the mitochondrial space (Kantor PF 2001; Opie and Lopaschuk 2004). The sequence of events that occur is outlined below in Figure 1-3, where palmitate is considered to be the FFA.

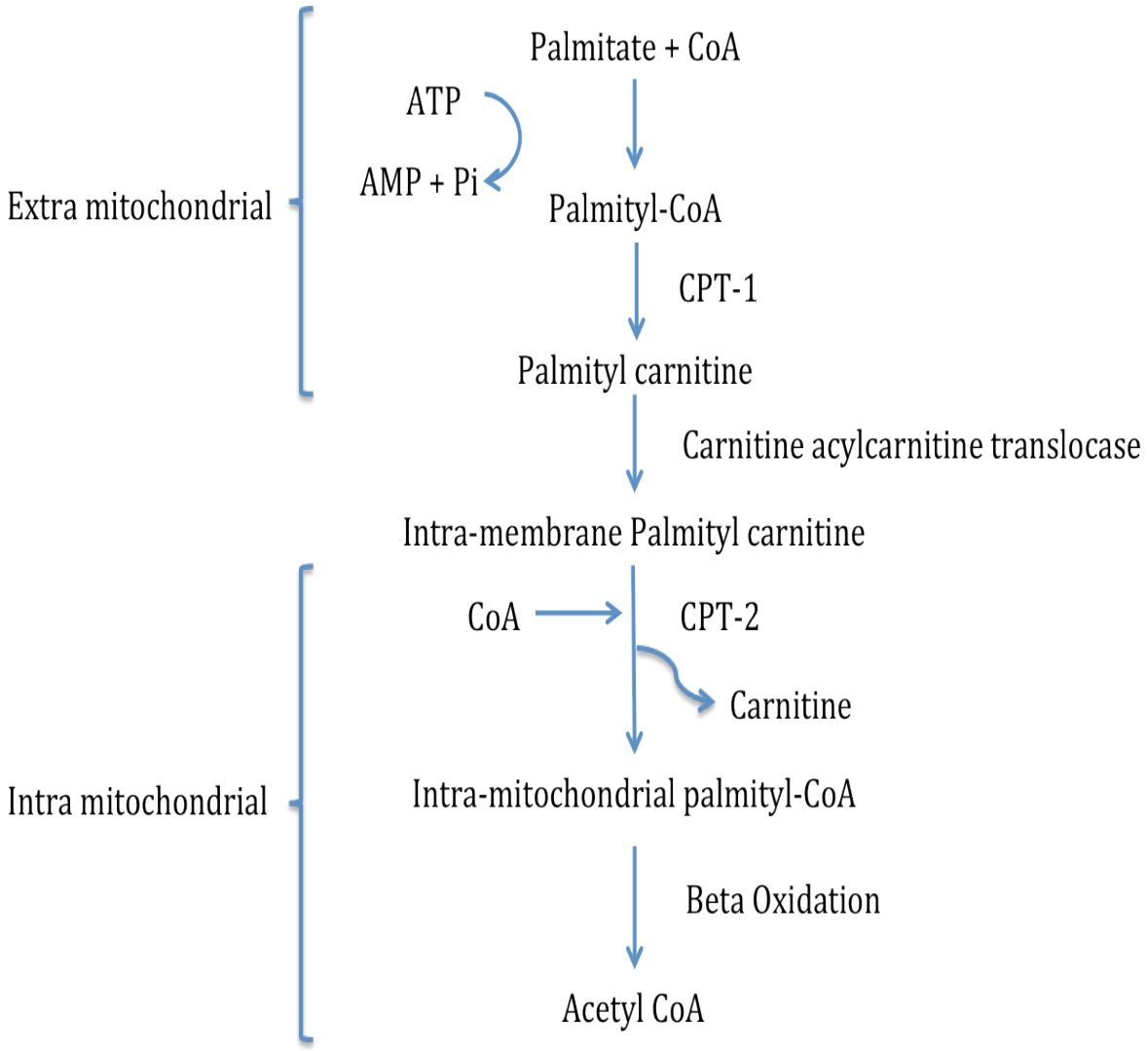


Figure 1-3 Free Fatty Acid metabolism

The enzyme carnitine palmitoyltransferase 1 (CPT-1) catalyses one of the key steps in the conversion of FFA to a form amenable for translocation into the mitochondrion. Then the enzyme carnitine acylcarnitine translocase is involved in the translocation of palmityl carnitine to within the space between the inner and outer mitochondrial membranes. Carnitine palmitoyltransferase 2 (CPT-2) is then involved in the reaction between intra-mitochondrial palmityl carnitine with CoA, liberating intra-mitochondrial palmitoly-CoA and carnitine (Figure 1-3). Carnitine is then exported back into the intra-mitochondrial space.

Therefore once inside the mitochondrion, FFA splits away from carnitine and is degraded and oxidized. FFA is degraded into Acetyl-CoA through the process of β-oxidation.

If excess FFA reaches the mitochondrion, there is a risk of uncoupling between oxidation and phosphorylation causing oxygen wastage. To prevent this occurring, malonyl-CoA is involved in limiting excess FFA entering the mitochondrion. Malonyl-CoA inhibits the action of CPT-1, reducing FFA metabolism (Dyck and Lopaschuk 2002). Malonyl-CoA is synthesised by the enzyme acetyl-CoA carboxylase. Acetyl-CoA carboxylase is inhibited by another enzyme, AMP protein kinase.

β-oxidation converts the intra-mitochondrial long acyl-CoA into acetyl-CoA. There is progressive oxidation of carbon atoms in the long fatty acyl-CoA molecule by oxidizing the beta carbon (second carbon from the right) of the fatty acyl-CoA, releasing acetyl-CoA. At this point, another CoA attaches to the remaining portion of the fatty acid molecule to form a new fatty acyl-CoA. This shorter fatty acyl-CoA gets further oxidized as before, to release another acetyl-CoA. This process continues until the original fatty acyl-CoA is fully oxidized.

During this process of β-oxidation each FFA molecule releases approximately 9 acetyl-CoA molecules and approximately 32 extra hydrogen atoms are removed. Acetyl-CoA released through β-oxidation then enters the citric acid cycle.

During anaerobic conditions, there is impaired β-oxidation. Intermediates of FFA metabolism such as acyl carnitine and acyl-CoA accumulate and damage cardiac cell membranes. Furthermore during ischaemic conditions the action of malonyl-CoA is inhibited, as AMP protein kinase is activated and in turn inhibits acetyl CoA carboxylase.

Therefore in ischaemia, more long chain fatty acids enter the mitochondrion as acyl-CoA. This in turn utilizes any remaining residual oxygen for FFA metabolism.

1.2.5 Citric Acid Cycle

The citric acid cycle (CAC) is the next series of reactions, where Acetyl CoA is broken down resulting in CO_2 and H^+ atoms being released. Figure 1-4 describes this process. Initially oxaloacetate combines with acetyl-CoA to form citrate. The CoA portion is released and is available to form more Acetyl CoA.

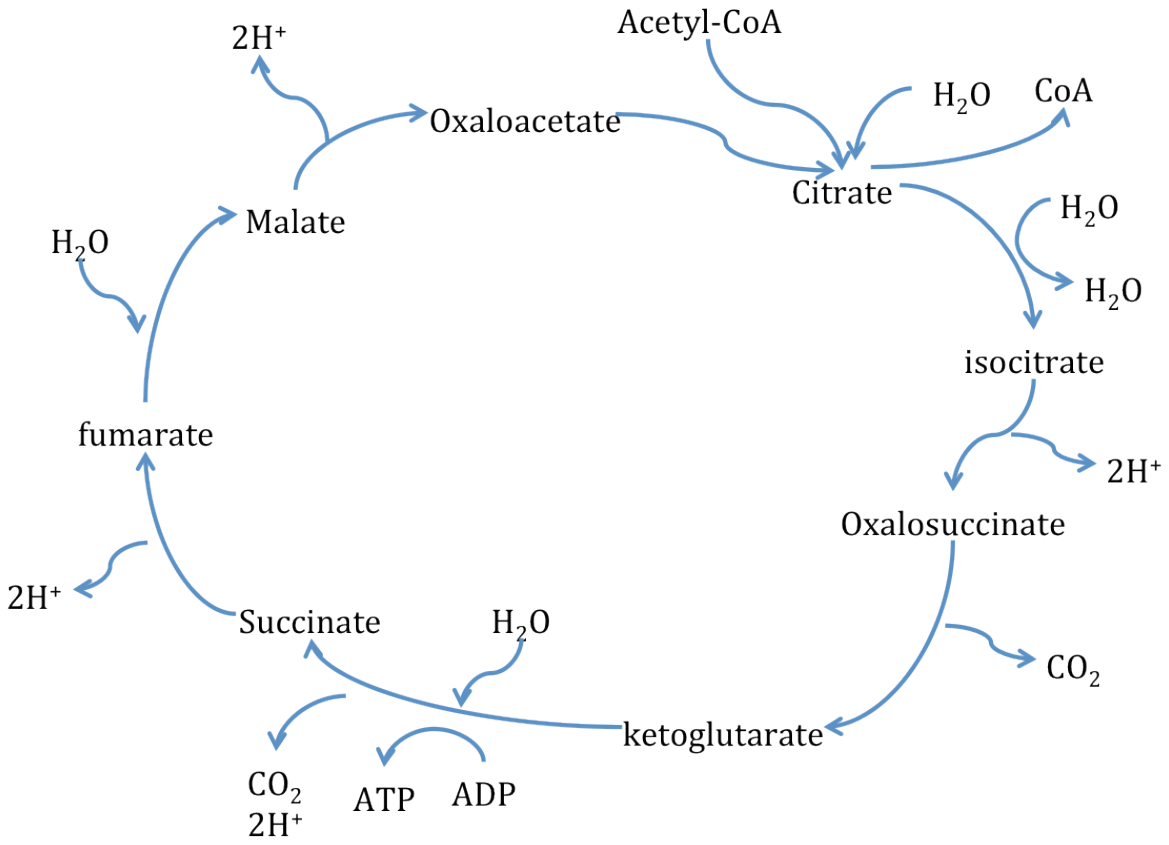


Figure 1-4 Citric Acid Cycle

For each molecule of glucose, 2 molecules of acetyl-CoA enter the CAC; net release of 4 CO_2, 16H^+, 2 CoA molecules and 2 ATPs. For each molecule of FFA, 9 molecules of acetyl-CoA enter the CAC; net release of 18 CO2, 72 H^+, 18 CoA molecules and 9 ATPs. The H^+ atoms released during the CAC are subsequently oxidised through oxidative phosphorylation to produce ATP.

1.2.6 Oxidative phosphorylation

The hydrogen atoms released during glycolysis, formation of Acetyl-CoA and during the CAC undergo a reaction catalysed by the enzyme dehydrogenase to form $NADH^+ + H^+$. Each H^+ is released in packets of 2 and undergoes the reaction as show in Figure 1-5.

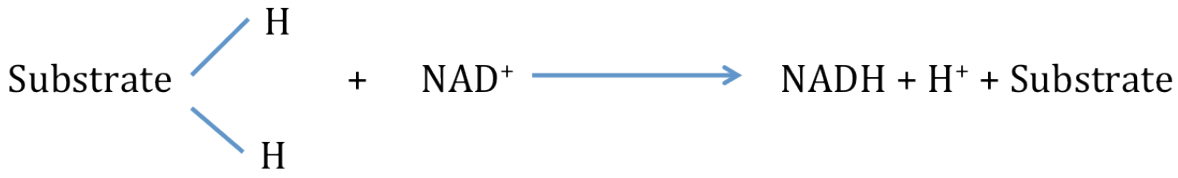


Figure 1-5 $NADH_2$ formation

Hydrogen ions formed during the earlier outlined processes combine with NAD^+ and enter the cascade of oxidative phosphorylation. A small number directly enters the oxidative process without combing with NAD^+.

Formation of ATP occurs in the mitochondrion as a result of this cascade of reactions named the chemiosmotic mechanism and this manner of ATP formation is termed oxidative phosphorylation.

$NADH^{+}+H^{+}$ ($NADH_2$) is oxidized and split in the mitochondrion to form hydrogen ions and electrons. The electrons enter a series of enzymatically catalysed reactions called the electron transport chain within the inner membrane of the mitochondrion. The electrons are shuttled between a series of electron acceptors until it reaches cytochrome oxidase. Cytochrome oxidase gives up 2 electrons, which combine with dissolved oxygen to form hydroxyl ions. These hydroxyl ions and the earlier released hydrogen ions eventually combine to form water. During the transport of the electrons through the electron transport chain energy is released, which is used to pump out the hydrogen ions from the inner mitochondrial membrane into the space between the inner and outer mitochondrial membrane (intra-mitochondrial membrane) (Guyton and Hall 2000).

This creates a high concentration of positively charged hydrogen ions within this chamber and also creates an increased negative electrical potential in the inner mitochondrial matrix. This causes the hydrogen ions to flow from the inter-membrane space into the inner mitochondrial matrix through a large protein molecule called ATP synthetase, positioned through the inner mitochondrial membrane and protruding into the inner mitochondrial matrix (Opie and Lopaschuk 2004). The energy derived from this hydrogen ion flow is used by ATP synthetase to convert ADP into ATP, by combing ADP with an ionic phosphate radical (Pi).

The newly formed ATP undergoes facilitated diffusion through the inner mitochondrial membrane via the ADP-ATP carrier called translocase. Thereafter ATP undergoes simple diffusion through the outer mitochondrial membrane into the cytoplasm. Similarly ADP is transferred in the opposite direction for conversion into ATP.

For each 2 electrons that are transported through the chemiosmotic mechanisms explained above, up to 3 ATP are synthesised utilising up to 1 molecule of oxygen. In this way the oxidation of $NADH_2$ is coupled to phosphorylation of ADP.

1.2.7 ATP utilisation

ATP energizes the synthesis of important cellular components, such as protein synthesis. Furthermore it energizes muscle contraction and active transport across membranes. The majority of ATP synthesized is used for heat production, and only 20-25% of ATP hydrolysed is actually used in mechanical work (Opie and Lopaschuk 2004). The rest is used for contraction and other essential mechanisms i.e. calcium uptake by sarcoplasmic reticulum. Up to 15% of ATP may be used by the sodium-potassium pump. A small percentage is used for the generation of action potentials, phosphorylation of proteins, glycogen and triglyceride turn over, and calcium uptake and release.

ATP wastage occurs in pathological processes where futile cycles speed up or are abnormal. Furthermore excess FFA oxidation increases non-phosphorylating pathways of oxygen uptake, causing oxygen wastage.

1.2.8 Phosphocreatine and ATP

The ATP generated through the processes of oxidative phosphorylation is converted to phosphocreatine (PCr), the form in which ATP is stored. Thereby PCr acts as a reserve of energy (Ye, Gong et al. 2001). ATP is transported to the cytoplasm forming phosphocreatine by the mitochondrial CK isoenzyme, situated on the outside of the inner mitochondrial membrane, close to the ADP-ATP carrier translocase.

When required phosphocreatine is rapidly converted to ATP for utilisation (Figure 1-6). This reaction is catalysed by the enzyme creatine phosphokinase (CK). Under the influence of CK, ATP is provided at the site where ATP is used. This reaction highly favours the formation of ATP, when the slightest amount of ATP is expended. At times when ATP is required the above process allows ATP to be maintained at the expense of phosphocreatine.

Figure 1-6 Formation of ATP from phosphocreatine

1.2.9 Controls in metabolism

The above mechanisms of metabolism have a number of controlling feedback pathways adapted to provide energy when most needed. The cell concentrations of ADP and ATP have a major impact in controlling these pathways. High ATP concentrations inhibit PFK thereby inhibiting glycolysis. Conversely, high ADP concentrations have the opposite effect, promoting glycolysis and thereby promoting the production of ATP. Another mechanism is via citrate produced in the CAC. High citrate concentrations inhibit PFK. Furthermore ATP and ADP are in constant equilibrium. If all the ADP is used in the formation of ATP, then no further ATP can be formed. Therefore equipoise between ATP and ADP is needed to allow more ATP generation.

1.3 Left ventricular hypertrophy

1.3.1 Pathophysiology

Left ventricular hypertrophy initially develops as a physiologically response to pressure overload. Aortic stenosis, which causes gradual reduction in the aortic orifice area, eventually leads to pressure overload. Over time if left untreated, it leads to ventricular contractile dysfunction and dilatation.

Left ventricular hypertrophy is associated with reduced coronary blood flow per gram of muscle (Bache, Vrobel et al. 1981). Furthermore LVH renders the coronary vessels to have less vasodilatory reserve (Bache 1998) (an index of coronary artery vasodilatory response; ratio of peak coronary blood flow to baseline flow), even in the absence of coronary artery disease (Marcus, Doty et al. 1982; Carabello 2002). This lack of normal coronary blood flow and reserve makes the hypertrophied heart more susceptible to the stressors of exercise and tachycardia by producing a maldistribution of blood flow and subendocardial ischaemia. This leads to systolic and diastolic dysfunction of the left ventricle.

Hypertrophic hearts are also more susceptible to ischaemia, with larger infarct and higher mortality rates compared to that seen with no hypertrophy (Koyanagi, Eastham et al. 1982; Gaasch, Zile et al. 1990). There is also evidence of an inappropriate hypertrophic response, particular common in females (Aurigemma, Silver et al. 1994; Carabello 2002). Inappropriate LVH has been associated with high perioperative morbidity and mortality (Orsinelli, Aurigemma et al. 1993).

A key feature of LVH is myocardial fibrosis. This process of fibrosis has been linked to the renin-angiotensin-aldosterone system (Weber and Brilla 1991). This is particularly

evident in LVH associated with hypertension where there is a heterogeneous accumulation of fibrillar collagen. Furthermore there is evidence to suggest that circulating aldosterone and associated hypertension contribute towards cardiac fibroblast involvement necessary for collagen deposition (Weber and Brilla 1991). Moreover fibrosis is also stimulated by chronic pressure overload by activation of procollagen gene expression and collagen protein synthesis (Cuspidi, Ciulla et al. 2006). Angiotensin II increases fibroblast proliferation and in turn alters the fibrillar collagen turnover with deposition of collagen type I and III fibres, ultimately leading to fibrosis (Campbell, Janicki et al. 1995). This deposition of collagen and fibrosis contributes to the ventricular dysfunction and myocardial stiffness.

1.3.2 Clinical manifestations

Angina may result from increased myocardial oxygen demand. Angina may occur with or without coronary artery disease due to the pathophysiological process of reduced coronary perfusion outlined earlier.

Investigation with an electrocardiogram (ECG) may reveal LVH, however there are several confounding factors such as age, gender, race, body habitus etc (Hancock, Deal et al. 2009). A tall R wave (> 25mm) in leads V_5 and V_6 and a deep S wave in leads V_1 or V_2 suggests LVH.

1.3.3 Echocardiographic quantification

The widespread use of echocardiography and more recently its reproducibility due to the technological advances of this technique has allowed this mode of investigation to be a reasonable choice to quantify LVH accurately. Measuring LV dimensions; inter-

ventricular septal diameter (IVSd) and left ventricular posterior wall diameter (LVPWd) at end diastole can assess LVH crudely (Table 1-2).

A more accurate quantification of LVH can be made by estimating left ventricular mass (LVM), assuming that LVM increases with LVH (Table 1-2). This requires the measurement of left ventricular end diastolic diameter (LVIDd) in addition to lVSd and LVPWd. The algorithms used to calculate LVM initially estimates LV cavity volume and subtracts this from the volume enclosed by the LV epicardium (Swamy and Lang 2010). This gives the LV volume, which is multiplied by the muscle density to calculate the LVM. More recently 3D echocardiography has made this assessment less subjective and has eliminated the 2D limitations of LV foreshortening, technical error and other geometric assumptions. However 2D echo remains the more widely used modality to assess LV dimensions. Cardiac Magnetic Resonance (CMR) has become more frequently used due to its advantages in accurately quantifying LVM with good reproducibility (Bluemke, Kronmal et al. 2008). However CMR is associated with greater cost and is less practical compared to 2D echo due to the space and equipment required.

	Mild		**Moderate**		**Severe**	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
IVSd (cm)	1.1-1.3	1.0-1.2	1.4-1.6	1.3-1.5	≥ 1.7	≥ 1.6
LVPWd (cm)	1.1-1.3	1.0-1.2	1.4-1.6	1.3-1.5	≥ 1.7	≥ 1.6
LV mass/BSA, (g/m^2)	103-116	89-100	117-130	101-112	≥ 131	≥ 112

Table 1-2 2D Echocardiographic quantification of LVH

Left ventricular mass index can be calculated by the following equation: -

$$LVMI\ (g/m^2) = (1.04[(IVSd + LVIDd + LVPWd)^3 - LVID^3] - 14g) / \text{Body surface area}$$

This formulae was initially described and validated by Devereux and colleagues (Devereux and Reichek 1977; Devereux, Alonso et al. 1986), who described that LVMI was considered increased if it was > 134g/m^2 in men and > 110g/m^2 in women.

1.3.4 Metabolism in LVH

Metabolism and substrate utilisation in the hypertrophied heart is different to that of the non-LVH heart. It has been implicated that such alterations, including the metabolism of glucose is a contributing factor to the pathophysiology of the cardiac hypertrophy (Cunningham, Apstein et al. 1990; Gaasch, Zile et al. 1990). Allard and colleagues have demonstrated that glycolysis is accelerated in hypertrophied hearts (Allard, Schonekess et al. 1994) and lactate production is greater in hypertrophied hearts during hypoxia and ischaemia (Anderson, Allard et al. 1990), supporting the theory that glycolytic capacity is enhanced in hypertrophy. To validate this further Allard and colleagues performed a series of animal studies to assess the extent of glycogen turnover in hypertrophy and found that glycogen metabolism contributes significantly to myocardial ATP production in hypertrophy and is similar to the normal heart, but overall glucose oxidation may be impaired (Allard, Henning et al. 1997) including an alteration in the exogenous glucose metabolism; increased glycolysis of exogenous glucose and decreased exogenous glucose oxidation. The increased glycolysis but decreased or no change in glucose oxidation causes an uncoupling effect of glucose metabolism and has been supported by a number of other studies (Allard, Schonekess et al. 1994; Wambolt, Henning et al. 1999)

In hypertrophy, the fuel preference tends to switch back towards a foetal metabolic pattern with reliance on glucose metabolism (Razeghi, Young et al. 2001; Kolwicz and

Tian 2011). With an accelerated glycolytic rate there is a greater quantity of NADH produced from glycolysis and is shuttled through the malate-aspartate shuttle (Kolwicz and Tian 2011). Despite these findings the overall rate of lactate oxidation is the same as in non-hypertrophied hearts and conversely, the rate of fatty acid oxidation is decreased (Allard, Schonekess et al. 1994). However, there is now growing consensus that there is an overall downregulation of all metabolic processes associated with LVH.

Additionally, there is evidence to suggest that anaplerosis, a process whereby pyruvate enters accessory pathways to supply carbon based substrates to the CAC is enhanced in hypertrophy (Sorokina, O'Donnell et al. 2007; Pound, Sorokina et al. 2009). This however reduces the efficiency of ATP production from pyruvate.

The increased reliance in glucose following hypertrophy is likely to be a compensatory mechanism to the down-regulation of fatty acid oxidation and oxidative metabolism present in the hypertrophied heart. Animal studies evaluating the role of key master transcriptional regulators such as peroxisome proliferator-activator receptor alpha (PPARα) and peroxisome proliferator-activator receptor gamma co-activator-1 (PGC-1) have shown these to be down regulated in hypertrophy (Barger, Brandt et al. 2000; Lehman and Kelly 2002; Arany, Novikov et al. 2006). Furthermore there is down-regulation of CPT-1 (Depre, Shipley et al. 1998; Sorokina, O'Donnell et al. 2007). This overall down-regulation of key pathways required for FFA oxidation results in reduced FFA metabolism. Furthermore the intracellular energy sensor, AMPK is activated and shown to be a stimulus for glucose uptake and enhances glycolysis by activation of PFK (Marsin, Bertrand et al. 2000).

The adaptive responses that result in an increased glycolytic flux does not necessarily lead to heart failure, providing there is sufficient fuel for oxidative metabolism (Kolwicz and Tian 2011). However further animal and clinical studies are required to test the capabilities of the pathologically hypertrophied heart under stressors of ischaemia and reperfusion.

1.4 Ischaemia and reperfusion

1.4.1 Ischaemia

Ischaemia to the myocardium can be partial or complete. In partial ischaemia there is limited blood flow to the ischaemic region; reduced flow of blood through narrowed coronary circulation or alternative source of coronary perfusion albeit reduced, from collateral circulations. Thus in ischaemic heart disease there is partial ischaemia until total occlusion occurs, to a particular territory of myocardium that does not have collateral perfusion.

During partial ischaemia there is reduced tissue oxygen tension resulting in hypoxia. In complete ischaemia there is no tissue oxygenation and hence anoxia ensues (Opie and Lopaschuk 2004). The extent of myocardial metabolism during ischaemia is dependent on the degree of ischaemia present. During cardiac surgery, once the aortic cross clamp is placed, there is no coronary blood flow hence the myocardium is completely ischaemic.

1.4.1.1 Partial ischaemia

Partial ischaemia is associated with a reduction in mechanical work and depletion of high-energy phosphates such creatine phosphate, with the production of lactate during metabolism. However over time (30-90mins), lactate output decreases (Fedele, Gewirtz

et al. 1988), and there is regeneration of phosphocreatine concentrations (Pantely, Malone et al. 1990), yet contractile work does not return back to normal. Expectantly normal contractile work is regained once normal coronary perfusion is restored. Therefore in periods of partial ischaemia there is a downregulation of the metabolic needs of the myocardium to maintain myocardial viability, thereby resetting the oxygen supply/demand ratio. This period of transient contractile dysfunction is referred to as myocardial hibernation (Rahimtoola 1985).

During this period of partial ischaemia, the main fuel of the heart is free fatty acids. Liedtke et al showed in an animal model with 50% reduction in coronary flow, that FFA oxidation supplies most of the energy of ATP synthesis during ischaemia (Liedtke, Nellis et al. 1978; Liedtke, Nellis et al. 1984).

1.4.1.2 Complete ischaemia

If ischaemia is induced for a long period of time, there is an increase in lactate accumulation, contractile dysfunction and this eventually leads to myocardial necrosis and infarction (Stanley, Lopaschuk et al. 1997).

Complete lack of coronary perfusion and failure to eliminate by-products of metabolism results in cumulative accumulation of lactate causing a rise in intracellular acidosis. Furthermore there is complete dependence on anaerobic metabolism using endogenous substrates, hence a shift towards glycogen utilisation (Stanley, Lopaschuk et al. 1997). Anaerobic glycolysis is controlled by 2 main enzymes, PFK and glyceraldehyde 3-phosphate dehydrogenase. PFK is sensitive to low molecular weight metabolites such as ATP, ADP, and AMP. During ischaemia, the level of ATP falls and those of ADP and AMP

increase, which stimulates PFK resulting in an increase of anaerobic glycolysis (Opie and Lopaschuk 2004).

However, the increasing acidotic environment secondary to a rise in H^+ inhibits PFK and in turn, anaerobic glycolysis. Furthermore the depleting levels of glycogen and glucose delivery add further insult to this limited process and therefore anaerobic glycolysis is halted. The acidotic environment and increasing lactate levels secondary to anaerobic glycolysis also affects glyceraldehyde 3-phosphate dehydrogenase. Therefore metabolism in complete ischemia is short-lived due to this vicious cycle of events.

These effects of ischaemia lead to failure of internal ion regulators; ATP dependent Ca^{2+} pumps within the sarcoplasmic reticulum and plasma membrane fail. Increased acidosis activates the Na^+-H^+ exchange within the plasma membrane. A resultant increase in intracellular Na^+ activates the Na^+-Ca^{2+} pumps within the plasma membrane causing intracellular calcium overload.

1.4.2 Reperfusion

Reperfusion refers to the return of blood to a previously ischaemic tissue. During cardiac surgery ischaemia is electively enforced (for a variable period) and reperfusion occurs on restoration of coronary blood flow. On reperfusion, the unwanted metabolites built up during the period of ischaemia and cardioplegia washout via the coronary sinus. Reperfusion is vital to prevent irreversible ischaemic injury. However it is evident that reperfusion too can exacerbate the pre-existing ischaemic injury, and cause further damage, known as reperfusion injury.

1.4.2.1 Reperfusion injury

Reperfusion injury cannot be taken in isolation, but is coupled and associated to the pre-existing damage caused during ischaemia dependent on the length of ischaemia. Reperfusion injury is primarily associated with the re-introduction of oxygen hence the oxygen paradox (Hearse, Humphrey et al. 1973). This theory was further consolidated when Hearse et al demonstrated a change in the ultrastructure of the myocardial cell and decline of its function with the introduction of oxygen to anoxic myocardium (Hearse, Humphrey et al. 1975). A number of other experimental studies have supported the concept that Reactive Oxygen Species (ROS) and free radicals are the primary component of reperfusion injury (Lucchesi 2001). Re-oxygenation, inevitable with reperfusion causes the release of reactive oxygen species and oxygen free radicals such as O_2^- which reacts with hydrogen peroxide (H_2O_2) to form a more potent free radical, OH. Free radicals can transfer easily between cell membranes, are cytotoxic, and all components of the cell are susceptible to attack including lipids within the cell membrane and membrane proteins. This oxidative stress results in cell damage and causes reduced cell viability. It is thought that the non-uniform introduction of oxygen during reperfusion makes it more likely for ROS and oxygen derived free radical formation (Lucchesi 2001). Endogenous antioxidants and free radical scavengers such as superoxide dismutase, catalase and glutathione peroxidase are able to reduce the extent of cell injury due to ROS, but are eventually overwhelmed by the extent of oxidative stress and are hence unable to provide adequate protection.

In addition to oxidative stress through ROS and oxygen free radicals, reperfusion injury is also due to the neutrophil mediated tissue damage. Neutrophils are activated by chemotactic factors i.e. C5a, IL-8 and platelet activating factors. Once activated the

neutrophils can cause further injury by forming oxygen derived free radicals and release of cytotoxic lysosomal enzymes. A vicious cycle ensues where free radical formation aggregates more neutrophils that cause small capillaries to plug off (Engler, Dahlgren et al. 1986; Engler, Dahlgren et al. 1986) resulting in the no-reflow phenomenon, which in turn contributes to the release of more ROS and greater oxidative stress. Further insult to injury is caused by the activation of platelets from the release of platelet activating factors causing further decrease in perfusion.

Moreover, the activation of the complement system plays a further role in reperfusion injury. Ischaemia induces the activation of the complement pathway and the extent of activation may depend on the length of ischaemia. This complement activation is present into and during reperfusion. Activation of the complement pathway generates anaphylatoxins C3a, C4a and C5a, which cause an inflammatory reaction due to their chemo-attractant properties, causing further damage to the injured tissue through alteration in vascular permeability, smooth muscle contraction and histamine release (Lucchesi 2001). C5a is a potent neutrophil activating peptide. In animal models C5a concentration is higher during reperfusion and is associated with increased neutrophil accumulation within the ischaemic zone (Shandelya, Kuppusamy et al. 1993; Ivey, Williams et al. 1995). It is evident that complement activation also results in activation of neutrophils enhancing the neutrophil dependent tissue injury (Lucchesi 2001). Furthermore free radical generation during reperfusion activates the complement system and its derived mediators of injury.

A probable common pathway to cell damage and injury is associated to the activity of the mitochondrial permeability transition pore (mPTP); formed by a complex of voltage dependent anion channels (VDAC), adenine nucleotide translocase (ANT) and

cyclophillin-D (CyP-d). This pore is thought to exist between the inner and outer mitochondrial membranes. Calcium overload causes opening of the mPTP (Crompton 1999). Intracellular acidosis, increased concentration of inorganic phosphates, loss of ATP, loss of adenine nucleotides all influence mPTP opening. Opening of the mPTP results in loss of the inner membrane potential causing free diffusion of solutes leading to loss of the ionic haemostasis. Furthermore mPTP opening causes the mitochondrion to become uncoupled (Zorov, Filburn et al. 2000; Halestrap, Clarke et al. 2004) resulting in mitochondrial ATP hydrolysis (Crompton 1999). This initiates a vicious cycle of impaired energy metabolism, deregulation of calcium haemostasis leading to further mPTP opening, ultimately leading to necrotic cell death. In addition to necrosis, mPTP opening leads to release of cytochrome-c and other pro-apoptotic molecules. Therefore even after mPTP closure, cell death continues down an apoptotic pathway rather than a necrotic pathway (Halestrap, Clarke et al. 2004).

1.4.2.2 Effects of ischaemia-reperfusion injury

Reperfusion injury causes cell swelling and contraction band necrosis and is associated with high intracellular calcium concentrations (Black, Gralinski et al. 1994). The extent of irreversible tissue injury due to reperfusion is dependent on the duration of the initial ischaemic insult. If the ischaemic insult is short and has not resulted in irreversible injury, myocardial tissue necrosis is not evident on reperfusion. However there may be a period of reduced myocardial contractility known as myocardial stunning. This is reversible and improves with time.

As a result of the ischaemia-reperfusion injury intracellular calcium overload ensues (Shandelya, Kuppusamy et al. 1993; Ivey, Williams et al. 1995). During the period of ischaemia there is depletion of ATP and high-energy phosphates. This stimulates

anaerobic glycolysis causing a gradual increase in intracellular acidosis and lactate production. Intracellular acidosis is counteracted by activation of the Na^+-H^+ pump, which would increase the intracellular Na^+ concentration (Lazdunski, Frelin et al. 1985). Lack of ATP would inhibit the Na^+-K^+ ATPase activity, which would increase the intracellular Na^+ concentration further (van Echteld, Kirkels et al. 1991). This causes the Na^+-Ca^+ exchange to work in reverse, to expel the high intracellular Na^+, increasing the intracellular concentration of calcium (Stone, Darley-Usmar et al. 1989). Furthermore, during ischaemia long chain fatty acids accumulate and incorporate into the membrane; reducing membrane stability and increasing permeability, resulting in calcium overload (Wu and Corr 1992). Moreover the storage and release of Ca^+ from the sarcolemmal membrane and sarcoplasmic reticulum is affected during ischaemia due to deficiencies in ATP. This depressed mitochondrial state leads to calcium overload (Osada, Netticadan et al. 1998).

The processes that lead to calcium overload occur during ischaemia and are made worse during reperfusion (Brooks, Conrad et al. 1995; Meissner and Morgan 1995; Dhalla, Temsah et al. 2001). Calcium overload causes cardiac dysfunction, cellular damage and cardiac contracture (Steenbergen, Murphy et al. 1990; Billman, McIlroy et al. 1991) and is therefore detrimental during reperfusion.

There is evidence to suggest that oxidative stress due to oxygen derived free radicals and ROS attack calcium cycling proteins and other structures involved in the homeostasis of intracellular calcium i.e. sarcoplasmic reticulum, sarcolemmal membrane, Ca^{2+} pump ATPase, L-type Ca^{2+} channels and other calcium regulating pumps (Dhalla, Temsah et al. 2001) leading to calcium overload. Increased intracellular calcium activates proteases and phospholipases that are involved with changes in the

structure of the cardiomyocyte. With time, this leads to increased calcium within the mitochondrion resulting in diminished energy production. These changes translate clinically to myocardial dysfunction due to myocardial cell damage and metabolic derangement.

1.4.2.3 Clinical manifestation of ischaemia-reperfusion injury

Clinically, reperfusion injury causing four types of cardiac dysfunction: myocardial stunning, no-reflow phenomenon, cardiac arrhythmias and lethal reperfusion injury (Yellon and Hausenloy 2007). Stunning refers to the a recoverable state of mechanical dysfunction, despite the absence of irreversible injury and despite restoration of coronary blood flow (Braunwald and Kloner 1982). No-reflow phenomenon is the inability to restore blood flow to the ischaemic territory secondary to impedance of microvascular blood flow (Ito 2006). Arrhythmias can be life threatening but effective treatments exist (Manning and Hearse 1984). All the above processes that include; mitochondrial calcium overload, oxidative stress, restoration of pH, and ATP depletion contribute to lethal reperfusion injury (Yellon and Hausenloy 2007). Targeting the common final pathway to cell injury (mPTP), activating the RISK pathway and exploring ischaemic pre and postconditioning remain potential avenues to limit lethal reperfusion injury, however the latter mechanisms are beyond the scope of this thesis.

1.4.3 Cell signalling during ischaemia-reperfusion

During ischaemia there is recruitment of glucose transporters GLUT-1 and GLUT-4, which increases the uptake of glucose across the cell membrane. There is also an acceleration of glycolytic flux due to activation of PFK-1 secondary to an increase in AMP and decrease in ATP during ischaemia.

During reperfusion, FFA oxidation is preferred over glucose. There is a decreased level of malonyl-CoA hence limited inhibition of CPT-1 activity. Malonyl-CoA is reduced due to the inhibition of ACC. Inhibition of ACC is by a specific AMP-dependent kinase (AMPK) activated by the AMP accumulated during ischaemia (Kudo, Barr et al. 1995; Kudo, Gillespie et al. 1996).

1.4.4 Ischaemia and hypertrophy

The presence of LVH is associated with greater susceptibility to ischaemia (Otterstad 1993; Otterstad, Davies et al. 1993; Maron, Olivotto et al. 2009). This renders this population of patients undergoing cardiac surgery, at a higher risk of ischaemia-reperfusion injury. However there is considerable variation to the susceptibility of the hypertrophic heart to ischaemia. A multitude of factors influence this and include, associated abnormalities in coronary vasculature, previous ischaemic injury before the cardiac surgical ischaemic insult, the consequences of ventricular fibrillation at the onset or during ischaemia or inherent biochemical and metabolic derangements present with hypertrophy (Sink, Pellom et al. 1981; Peyton, Jones et al. 1982). Gaasch et al showed that hypertrophied hearts in dogs, were particularly vulnerable to ischaemia if they were in a failing state and have a reduced capacity for anaerobic glycolysis contributing to diastolic dysfunction (Gaasch, Zile et al. 1990).

Friehs and colleagues in an animal model of pressure overload hypertrophy demonstrated that glucose uptake was impaired pre-ischaemia and remained lower during re-perfusion (Friehs, Cao-Danh et al. 2005); related to restricted GLUT-4 (molecule that augments glucose uptake) translocation due to impaired insulin signalling causing reduced recovery of contractile function. Therefore the altered

reliance on glucose metabolism is linked to the susceptibility of the hypertrophied myocardium to ischaemia.

The effects of ischaemia-reperfusion seen in normal hearts (outlined in sections 1.4.1 and 1.4.2) are exaggerated in hypertrophied hearts and include accelerated loss of high-energy phosphates, greater accumulation of tissue lactate and acidosis, accelerated calcium overload and ischaemic contracture (Allard, Flint et al. 1994; Friehs and del Nido 2003). This susceptibility to ischaemia-reperfusion could also be secondary to the pathological alteration in the structure and composition of the left ventricle; changes to the collagen matrix (thickening of the collagen layer), increase in diffusion distance between myocytes and vessels, alteration in the coronary circulation and decreased myocardial capillary density. Friehs and colleagues have shown an increased vulnerability to ischaemia/reperfusion associated with diminished microvascular supply (Allard, Flint et al. 1994; Friehs and del Nido 2003). Diminished microvascular density can affect overall substrate utilisation; experimental work has shown that vascular endothelial growth factor can stimulate capillary growth and through this post ischaemic contractile function is preserved (Friehs and del Nido 2003)

1.5 Cardiac surgery

Conventional cardiac surgery is performed on a still heart allowing a bloodless field and better surgical manipulation. This requires the heart to be arrested, commonly by using cardioplegia solution. During this period of elective cardiac arrest, the cardiopulmonary bypass machine takes over the work of the heart and supplies blood to the head and body. Once the surgical procedure is complete, the heart is reperfused and allowed to regain its function and cardiopulmonary bypass is discontinued.

1.5.1 Patient demographics undergoing aortic valve replacement

Aortic valve replacement is a common cardiac procedure and over the last 10 years the demographic population of patients undergoing AVR in the UK continue to change. The SCTS 6th National cardiac surgical database report has shown that there has been a 50% increase in both isolated AVR and AVR+CABG procedures between 2001 to 2008 (Bridgewater and Keogh 2009). Furthermore the average age of patients undergoing both procedures has increased over time; in 2008 mean age undergoing isolated AVR and combined AVR+ACBG was 68 and 72 years respectively. In addition the proportion of elderly patients has increased further.

The proportion of females undergoing AVR and AVR+CABG in the UK is 40% and 30% respectively (Bridgewater B, Keogh B et al. 2008). This proportion has not changed over time. The majority of isolated AVR cases are performed on an elective basis (75%), with just 2% being done as an emergency with the remaining being on an urgent basis. This proportion is similar for AVR+CABG surgery. The proportion of patients undergoing AVR for stenosis has not changed over time and stands at 62% for isolated AVR, with 16% being performed for regurgitation and the remainder consists of mixed disease.

The aortic valve can be replaced with either a mechanical or biological prosthesis or homograft. The latter is rare and only used in special circumstances. The majority of implanted valves tend to be biological with 70% for an isolated AVR and 82% for a combined AVR+CABG procedure. Between 2004 and 2008 there has been an increase in the use of biological prostheses. Typically a biological implant was reserved for the elderly patient but more recently there has been an increase in the number of biological implants being placed in the younger population overall, with the greatest increase in

patients aged 61-65 years (Bridgewater and Keogh 2009). This could be due to the improved longevity of the modern biological valve.

1.5.2 Aortic valve replacement

Aortic valve replacement is an established procedure (Emery, Emery et al. 2012) and is routinely performed in adult cardiac surgery. The principles and technique of an aortic valve replacement are outline in Appendix 9.2.

1.5.3 Mortality associated with an aortic valve replacement

Mortality associated with an AVR is now considered to be low. In the United States the average peri-operative mortality in the Society of Thoracic Surgeons (STS) database is 2 – 3% for an isolated AVR and 4 – 5% for a combined AVR plus CABG (Surgeons 2012). This is variable in relation to the volume of work at each centre and in centres with low volume the mortality rates are 33% higher (Birkmeyer, Siewers et al. 2002). Goodney et al evaluated the Medicare data involving 684 US hospitals involving 142,000 patients and shows that in-hospital mortality for AVR in patients > 65 years is 8.8% (13% in low volume centres and 6% in high volume centres) (Goodney, O'Connor et al. 2003).

In the UK, a review of the data submitted to the Society for Cardiothoracic Surgery (SCTS) in Great Britain and Ireland over a 5 year period from 2003 showed that in-hospital mortality for isolated AVR and combined AVR + CABG for England was 2-3% and 4-5% respectively in 2008 (Bridgewater and Keogh 2009).

Increasing age has always been associated with a greater mortality and this is more so in a combined procedure. In patients > 85 years undergoing an isolated AVR and combined AVR + CABG mortality was 5.5% and 10.7% respectively between 2004 –

2008 (Bridgewater and Keogh 2009). Gender in particular is associated as a risk factor for poor prognosis, with females having a higher mortality for both isolated AVR and AVR+CABG compared to males (3.2 % and 6.8% vs. 2.6% and 4.6%).

In the UK as seen from the SCTS database, mortality is associated with the urgency of the procedure with a proportional increase in mortality associated with urgent, emergent and salvage procedures for both isolated and combined procedures. An urgent AVR is associated with a mortality of 4.9% as apposed to 1.9% in an elective setting (Bridgewater and Keogh 2009) and mortality in an emergent setting is 10.4% for an isolated AVR.

Mortality for patients undergoing an isolated AVR for stenotic disease is less at 2.6% compared to 3.7% for regurgitant pathology. However for a combined procedure mortality is independent of the underlying haemodynamic pathology.

In addition to the above mentioned mortality figures, review of the SCTS database has shown that a lower body mass index, ejection fraction of < 50%, left main stem disease, previous cardiac surgery, diabetes, hypertension, extra-cardiac arteriopathy, renal disease, angina (CCS class 4), dyspnoea (NYHA class 4) are all associated with a greater mortality.

1.5.4 Consequences and morbidity post aortic valve replacement

The SCTS in their 6th national adult cardiac surgical database reported on data pertaining to post-operative outcomes and survival post isolated AVR and AVR+CABG (sub-divided on risk factors) (Bridgewater and Keogh 2009).

Patients who are elderly have a lower survival rate at 5 years for both an isolated AVR and AVR+CABG (> 80 years having survival rate of 65% post isolated AVR). Conversely those that are < 61 years have a survival rate of > 90% at 5 years. Rate of survival is less for females compared to males, a difference that is more marked following an AVR+CABG with a survival rate of 75-80% as apposed to 85% in males. Similarly urgency is strongly associated with poor mid-term survival with only a 50% survival rate at 5 years post emergency AVR+CABG and ~65% post isolated AVR. Although mortality rates are more favourable post AVR for stenosis, there is no mid-term survival difference based on haemodynamic pathology. Survival rates follow a similar pattern to that of mortality for all other major risk facts i.e. BMI, ejection fraction, left main stem disease etc.

Average post-operative stay for men following an isolated AVR and AVR+CABG is 10.6 and 12.8 days respectively and is greater for women by up to 2 days following a combined AVR+CABG (Bridgewater B, Keogh B et al. 2008). Similarly a non-elective procedure was associated with longer in-patient stays with a mean stay of 19.5 and 18.3 days for an emergency AVR and AVR+CABG respectively. As with overall survival there is no difference in post-operative length of stay based on haemodynamic pathology. As with other makers of outcome mentioned above for mortality, post-op stay follows a similar trend based on individual risk factors.

The overall rate of re-operation for bleeding following an AVR is 5.5% and is higher at 7% for AVR+CABG between 2004 and 2008. There is a similar association with post operative stroke with a higher post-op stroke rate for AVR+CABG at 2.9% compared to 1.9% post isolated AVR. New post-operative haemofiltration/dialysis is 3% and 5% for isolated and combined AVR+CABG respectively. These rates are all higher than those

reported for CABG alone and are reflective of the risk of the procedure and the patients' haemodynamic pathophysiology.

1.6 Myocardial protection

The placement of an aortic cross clamp during elective cardiac arrest requires the myocardium to be adequately protected. Complete cardiac standstill requires cessation of coronary perfusion and therefore the heart is made globally ischaemic. This ischaemic state has a multitude of negative consequences as outlined earlier, and ischaemic time can vary according to the complexity of the procedure. Therefore the primary aim of myocardial protection is to minimize the effects of ischaemia and re-introduce flow at the earliest opportunity, with an aim to achieve minimal myocardial injury during reperfusion. A number of techniques have been developed to achieve maximal myocardial protection. The most reproducible and at present widely used method uses an infusion of cardioprotective solution (cardioplegia) down the coronary arteries to achieve diastolic cardiac arrest.

1.6.1 Cardioplegia

1.6.1.1 A brief history

The advent of the cardiopulmonary bypass machine in 1954 by Gibbon (Gibbon 1954) allowed surgeons to operate in a bloodless field with the consequence of global heart ischemia. This resulted in intermittent cardiac contraction in the face of ischaemia, causing further myocardial injury. This led to the development of a potassium citrate rich blood based solution (Melrose, Dreyer et al. 1955) which when infused down the coronary arteries caused diastolic arrest of the heart and was used clinically during

cardiac surgery in the late 1950s (Gerbode and Melrose 1958; Sones 1958). This soon fell out of favour as it was realised that potassium citrate was more damaging and caused myocardial necrosis (Helmsworth, Kaplan et al. 1959). It was only much later that it was realised, that the high concentration of citrate was the culprit in myocardial injury (Holscher 1967). Therefore until the mid 1970s other means of operating on the heart were adopted such as continuous coronary perfusion, at times with the aid of ventricular fibrillation and intermittent coronary perfusion.

Non-potassium based cardioprotective solutions were being used particularly in Germany in the 1960s. This was mainly a sodium poor, calcium free solution containing procaine and known as the 'Bretschneider solution' (Sondergaard, Berg et al. 1975). Similarly Kirsch and colleagues developed a cardioprotective solution, which was used in clinical practice and was based on a magnesium, aspartate and procaine mixture (Kirsch, Rodewald et al. 1972).

Gradually, potassium based citrate free cardioplegia re-emerged in the mid 1970s. This was after successful cardioprotection with 25mmol/litre of potassium chloride after 1hr of ischaemia in dog hearts (Gay and Ebert 1973). Roe and colleagues reported on 204 patients using potassium based cardioplegia with a mortality of 5.4% (Roe, Hutchinson et al. 1977). Similarly another clinical series of over 100 patients using the same concentration of potassium showed good myocardial protection (Tyers, Manley et al. 1977). Hence potassium based cardioplegia regained popularity and the St Thomas' hospital cardioplegia solution was conceived in the UK.

1.6.1.2 St Thomas' hospital cardioplegia solution

The St Thomas group reported on a series of experiments using isolated rat hearts, on the effects of adding a series of anti-ischaemic agents i.e. Magnesium, ATP, phosphocreatine, to a potassium rich solution and their role in enhancing myocardial protection (Hearse, Stewart et al. 1976). This led to the development of the St Thomas' hospital cardioplegia solution No. 1 (Braimbridge, Chayen et al. 1977) (Table 1-3) and was introduced into clinical practice in 1975. This was later modified to St Thomas' hospital cardioplegia No. 2 (commercially produced as Plegisol) (Jynge, Hearse et al. 1981) and was widely used throughout the United States and UK (Robinson, Schwarz et al. 1995).

Components (mmol/litre)	St Thomas' solution No 1	St Thomas' solution No 2
Sodium chloride	144	110
Potassium chloride	20	16
Magnesium chloride	16	16
Calcium chloride	2.2	1.2
Procaine	1	-
Sodium bicarbonate	-	10
pH	5.5-7.0	7.8
Osmolarity (mOsm/kg H_2O)	300-320	324

Table 1-3 Composition of St Thomas's solution

The St Thomas' solution was infused at 4°C for 2 minuets at the start of ischaemia at a perfusion pressure of 80mmHg until cardiac arrest ensues and was reduced to 50mmHg after arrest (total volume 1000ml). Subsequent infusions of 300-500ml were infused at 50mmHg.

1.6.1.3 Characteristics of cardioplegia solution

Cardioplegia can either be an intracellular or extracellular composition. An intracellular composition consists of one or more of the following: low sodium (10-15mmol/L), high potassium (100-140mmol/L) and low/no calcium. An extracellular composition can consist of one of the following: high sodium (100-140mmol/L), lower potassium (15-25mmol/L) and normal calcium concentrations (0.5-2.5mml/L). The St Thomas' solution is an extracellular composition whereas the commercially available Custodiol, which is the Bretschneider–HTK solution is an intracellular composition.

There are still on-going studies to elucidate which composition will provide better myocardial protection however at present, the extracellular composition seems to provide an overall benefit (Kempsford and Hearse 1989; Galinanes, Murashita et al. 1992) with the added advantage that the extracellular solution can either be purely crystalloid or diluted in blood (with its added advantages).

1.6.1.4 Mechanism of elective cardiac arrest

Cardioplegia aims to arrest the heart in diastole rapidly and safely at the onset of ischaemia. This would provide a flaccid heart, which is the most optimum condition required to operate effectively. Achieving cardiac arrest would reduce the myocardial oxygen consumption/demand and reduce the depletion of useful cellular energy stores.

Cardioplegia causes a depolarised arrest of the heart due to the high potassium concentration in the solution. Infusion of cardioplegia increases the extracellular concentration of potassium, in turn causing depolarisation of the myocytes' transmembrane potential. A gradual increase of the extracellular potassium concentration makes the resting membrane potential more depolarised. At a resting

membrane potential of approximately -65mV (threshold potential) the voltage dependent sodium channels are inactivated preventing the sodium induced action potential that leads to contraction. Hence the heart is arrested in a diastolic state by depolarisation (Chambers and Hearse 2001).

Despite the rapid elective diastolic arrest achieved with potassium rich cardioplegia, its optimum range of concentration for maximum effect is relatively narrow. An increase in extracellular concentration may depolarise the membrane potential further at the expense of activating the slow calcium channels (around -40mV) causing calcium to enter the myocyte. Hence the narrow window of optimal potassium concentration is 10-30mmol/L (Chambers and Hearse 2001). Even at optimum potassium concentration and membrane potentials other sodium channels may operate which may gradually increase intracellular sodium levels, in turn causing calcium channels to open resulting in contracture and further calcium overload (Chambers and Hearse 2001).

An alternative to depolarized arrest (achieved with cardioplegia) is polarised arrest, by maintaining the transmembrane potential closer to the normal resting membrane potential. Polarized arrest can be achieved in the following manner/agents: -

- Sodium channel blockade
- ATP-sensitive potassium channel activation
- Adenosine
- Acetylcholine

In addition to the above-mentioned methods of polarised arrest, inhibition of calcium influx by promoting hypocalcaemia using calcium antagonists and promoting hypermagnesemia can induce polarised diastolic cardiac arrest. These methods of polarised arrest have therefore been suggested as superior to depolarised arrest but

remain contentious. As cardioplegia is the main agent of myocardial protection in the studies outlined in this thesis, further expansion into polarised diastolic arrest is beyond the scope of this thesis.

1.6.2 Hypothermia

Hypothermia is used as an adjunct to cardioplegia and aids to lower the metabolic rate. Shumway and colleagues used profound topical hypothermia (<4°C) only, as means of myocardial protection with good clinical effect (Hurley, Lower et al. 1964). Hypothermia can reduce the ischaemic injury by slowing the metabolic demands (temperature dependent enzyme activity) and depletion of high-energy phosphates during ischaemia. A 10°C drop in temperature can reduce enzyme activity by 50% (Belzer and Southard 1988). Therefore deep hypothermia alone can achieve myocardial arrest and thus myocardial protection (Greenberg and Edmunds 1961; Griepp, Stinson et al. 1973).

Studies on the effect of hypothermia on myocardial metabolism by Buckberg and colleagues showed that at 22°C an arrested heart had a myocardial oxygen consumption of 0.3 ± 0.1 compared to 1.1 ± 0.4 at 37°C (Buckberg, Brazier et al. 1977). Furthermore in a rat heart model Hearse and colleagues demonstrated the additive myocardial protective benefits of hypothermia in addition to cardioplegia during ischaemia arrest (Hearse, Stewart et al. 1980). This was again confirmed in an *in vivo* dog model (Rosenfeldt, Hearse et al. 1980).

Despite the advantages of hypothermia, this too can have negative consequences causing contracture and diastolic abnormalities. At temperatures below 5°C calcium is released from intracellular stores causing contracture (Kurihara and Sakai 1985; Bers

1987). Furthermore high intracellular calcium causes reduced function (Ting, Spotnitz et al. 1994). In addition to ischaemia, hypothermia too causes rapid sodium pump inactivation. Over time an inability to pump sodium into the extracellular space results in an increasing osmotic pressure gradient, which causes cell swelling (Belzer and Southard 1988; Knerr and Lieberman 1993). Therefore it is important to maintain an optimum temperature to allow maximum protection and minimum hypothermic cell injury.

Hearse et al showed that the greatest benefit gained by hypothermia was from 37°C to 24°C (Hearse, Stewart et al. 1976). Further clinical studies concluded that the most optimal temperature was 11-15°C to achieve maximal myocardial protection. In order to achieve this degree of myocardial hypothermia, cardioplegia would need to be administered at around 4°C (Rabinov, Chen et al. 1989)

1.6.3 Other adjuncts and additives to cardioplegia

Although polarised diastolic cardiac arrest can be achieved with additives and techniques briefly mentioned earlier, these could also be used as an additive to standard potassium rich cardioplegia solution with enhanced benefits. Potassium channel openers that promote ATP-sensitive potassium channel activation causing a hyperpolarised membrane potential has been shown to enhance post ischaemic recovery (Jansson, Bomfim et al. 1987; Hosoda, Sunamori et al. 1994; Qiu, Galinanes et al. 1995) and may reduce the impact of potassium induced calcium influx (Lopez, Jahangir et al. 1996). Similarly adenosine has been used as an additive to standard extracellular cardioplegia (Chambers and Hearse 2001). Inhibition of calcium influx, by using calcium antagonists (i.e. diltiazem, verapamil) and magnesium as additives to

potassium cardioplegia has shown to improve myocardial protection (Takemoto, Kuroda et al. 1994; Kronon, Bolling et al. 1997). The calcium channel antagonist may play a role in reducing the calcium influx that is associated with reperfusion injury in normothermic ischaemia (Chambers and Hearse 2001), yet due to their instability and irreproducibility, calcium channel antagonist are not used in routine surgical practice.

Cardioplegia can be administered as a pure crystalloid solution or diluted in blood with blood acting as a delivery agent. Animal models showing the advantage of blood based potassium rich cardioplegia (Follette, Mulder et al. 1978) and subsequent clinically successful studies (Buckberg 1979) established the role of blood cardioplegia. However the debate of blood cardioplegia being superior to pure crystalloid cardioplegia continues (Chambers and Hearse 2001), as previous studies have been suboptimal and conducting an ideal study between blood and crystalloid cardioplegia would involve multiple assessments of all the possible variables between the two and would be a labour intensive process. Disadvantages of blood cardioplegia include the requirement to make up the solution and less scope for the inclusion of other additives.

One of the main advantages of blood cardioplegia was the simultaneous delivery of oxygen to the myocardium during ischaemia. However at lower temperatures (10-15°C) the oxygen dissociation from blood becomes more difficult, allowing < 50% of the available oxygen for release. In comparison, crystalloid solution has a lower biding capacity of oxygen, hence all of the oxygen is available for release (Digerness, Vanini et al. 1981). Studies have shown a beneficial effect of oxygenating cold crystalloid cardioplegia (Ledingham, Braimbridge et al. 1988; Chambers, Kosker et al. 1990; Coetzee, Roussouw et al. 1990) but this is technically difficult and needs correct manipulation to maintain the carbon dioxide content and pH.

Cardioplegia can be delivered cold, a more conservative approach achieving a hypothermic myocardium. However studies have shown that there is equal benefit in delivering warm blood cardioplegia or even a combination of continuous warm blood cardioplegia to induce arrest and then use cold blood cardioplegia to maintain arrest (advocated for more susceptible hearts) (Rosenkranz, Vinten-Johansen et al. 1982; Rosenkranz, Buckberg et al. 1983). There has been and continues to be further debate regarding continuous or intermittent cardioplegia perfusion with neither having a greater overall benefit (Chambers and Hearse 2001). Theoretically continuous cardioplegia perfusion can provide an aerobic, ischaemic free environment however it is technically challenging and can hamper key stages of a cardiac procedure (distal coronary graft anastomosis). If intermittent cardioplegia is used, then the periods of ischaemia should be kept to a minimum, ideally 10-15 mins in duration (Rousou, Engelman et al. 1995; de Oliveira, Boeve et al. 1997; Kawasuji, Yasuda et al. 1997), to reduce the risk of ischaemic injury.

Intracellular acidosis is proportional to the length of ischaemia. An additional advantage of blood cardioplegia is the inherent buffering capacity that can combat this acidosis; hence may be more beneficial in myocardial protection. Therefore the inclusion of bicarbonate in St Thomas's No.2 to make this more alkaline, allows for the gradual drop in pH (to a physiologically normal range) in a hypothermic state (Chambers and Hearse 2001).

1.6.4 Adjuncts to combat oxidative stress

Oxidative stress due to the production of free oxygen radicals during ischaemia and reperfusion has been implicated in myocardial injury. During ischaemia the protective effects of endogenous anti-oxidants systems (superoxide dismutase and catalase) are suppressed (Galinanes, Ferrari et al. 1992). Despite positive experimental studies showing the myocardial protective benefit in using anti-oxidant agents, these have not translated into clinical practice (Chambers and Hearse 2001). Superoxide dismutase and catalase are expensive to produce and unsuitable (large protein molecules) for administration to patients. Similarly, organic anti-oxidants such vitamin E & C, although experimentally have shown to improve post ischemic recovery (Chambers, Astras et al. 1989) have not been taken up into clinical practice. It is thought that free oxygen radical production and oxidative stress is maximal during reperfusion hence oxidative stress combating agents would be most beneficial in a reperfusion solution rather than the cardioplegia solution.

Therefore there are a myriad of variables to enhance standard potassium rich cardioplegia. These variables include: the vehicle of delivery (blood vs. crystalloid), temperature (cold vs. warm), and frequency (continuous vs. intermittent). Furthermore there are a number of established additives that may reduce ischaemic injury and improve myocardial protection. These methods may enhance the capabilities of the cardiomyocyte to deal with ischaemia. Yet there are mechanisms that could improve the metabolism or optimise the metabolism of the cardiomyocyte, which could improve its ability to deal with ischaemia and the stressors of reperfusion.

1.7 Metabolic modulation

Metabolic modulation refers to the manipulation of the cellular metabolism. It can be achieved by enriching cardioplegic solution with metabolites and substrates i.e. high-energy phosphates, amino acids etc., beneficial to myocardial metabolism. By infusion of cardioplegia through the coronary arteries, it is assured that these substrates are reaching the myocardium during induction and maintenance of cardiac arrest. The principle of metabolic modulation is to reduce high-energy phosphate (ATP) depletion during ischaemia or enhance anaerobic ATP production and/or availability (Chambers and Hearse 2001).

ATP and phosphocreatine as additives to cardioplegia have been demonstrated experimentally and clinically to improve myocardial protection, although the mechanism of protection is as yet unclear (Semenovsky, Shumakov et al. 1987; Chambers, Braimbridge et al. 1991; Chambers, Haire et al. 1996). Exogenous ATP should not be able to gain access into the cell hence their action may be extracellular and act similar to chelating calcium, reducing the calcium concentration (Robinson and Harwood 1991). In addition exogenous high-energy phosphates may reduce oxidative stress (Zucchi, Poddighe et al. 1989).

Other substrates such as glutamate and aspartate, amino acids involved in the Kreb's cycle and malate-aspartate shuttle are involved in the production of ATP. Early experimental and clinical studies have shown that glutamate and aspartate may improve myocardial oxygen consumption and anaerobic metabolism particular in the energy depleted heart (Rosenkranz, Okamoto et al. 1984; Rosenkranz, Okamoto et al.

1986; Pisarenko, Portnoy et al. 1987). Yet further evaluation for routine clinical use is necessary.

The addition of glucose and thereby promoting the anaerobic glycolytic pathway producing ATP, can hypothetically improve myocardial protection. Hence glucose has been added to cardioplegia solution with mixed benefits. In the absence of coronary flow, anaerobic glycolysis can lead to lactate accumulation and acidosis (Chambers and Hearse 2001). Other intermediates of glycolysis such as pyruvate and fructose diphosphate have shown to be beneficial as additives to cardioplegia (Wikman-Coffelt, Wagner et al. 1991; Cargnoni, Condorelli et al. 1992; Schaefer, Prussel et al. 1995). However additives to cardioplegic solution with an aim to achieve metabolic modulation in clinical cardiac surgery have yet to be fully established (Chambers and Hearse 2001). Therefore, metabolic modulation irrespective of the constituents of cardioplegia solution *per se* could be a feasible alternative.

Metabolic modulation independent of cardioplegia aims to promote a more efficient cardiomyocyte metabolism prior to the insult of ischaemia, during ischaemia and into reperfusion, and in turn this would be beneficial during the reperfusion phase. The principle of this modulation is a shift away from potentially harmful free fatty acid metabolism, towards a more efficient carbohydrate metabolism. (Taegtmeyer, Goodwin et al. 1997). Maintaining a more efficient metabolic state whilst utilising carbohydrate substrates for metabolism, may reduce the insult of ischaemia, maintain high-energy phosphate concentrations and therefore allow an enhanced recovery from ischaemia-reperfusion injury.

1.7.1 Glucose-Insulin-Potassium

An intravenous infusion of glucose-insulin-potassium (GIK) was first used in 1962 in a study evaluating the role of GIK in myocardial ischaemia, which showed a reduced infarction size, reduced electrocardiographic signs of ischaemia and prolonged survival (Sodi-Pallares, Testelli et al. 1962). Later, GIK infusion was used in stunned, failing myocardium not responding to other therapies (Braimbridge, Clement et al. 1969; Sheldon, Braimbridge et al. 1969). The mechanism of action of GIK on the myocardium during cardiac surgery is multifactorial.

The administration of GIK prior to the onset of ischaemia can improve myocardial glycogen content allowing for greater ATP and phosphocreatine production, via glycolysis during the period of ischaemia (Iyengar, Charrette et al. 1976; Haider, Schutz et al. 1982). This should allow for greater ischaemic tolerance and recovery post reperfusion. Post cardiac surgery there is higher insulin resistance as part of the hormonal stress response (Rassias, Givan et al. 2002), causing hyperglycaemia. This can be combated by the infusion of insulin as part of the GIK solution (Doenst, Bothe et al. 2003).

A feature of GIK infusion is that it limits the extent of free fatty acid metabolism. Insulin reduces lipolysis thereby reducing free fatty acids in circulation. Furthermore it reduces the levels of acyl-carnitine, reducing membrane injury. In addition to this it limits FFA metabolism by activation of acetyl-CoA-carboxylase (Gamble and Lopaschuk 1997). Activation of acetyl-CoA-carboxylase increases malonyl-CoA, which inhibits the uptake of acetyl-CoA for β-oxidation. This inhibition of FFA metabolism promotes coupled glycolysis and glucose oxidation and reduces the energy inefficient FFA metabolism.

Insulin also increases the expression of glucose transporters, particularly GLUT4. GLUT4 increases the uptake of glucose into cardiomyocytes hence promotes glycolysis (Morgan, Neely et al. 1965). GIK also has a role during reperfusion. Through the process of anaplerosis, GIK is able to replenish the intermediates of the TCA cycle at a fast rate allowing for better energy production and greater turnover of the cycle (Simonsen and Kjekshus 1978; Taegtmeyer, Goodwin et al. 1997). The vasodilatory properties of GIK improves cardiac output, by reducing the afterload (Gradinac, Coleman et al. 1989). Furthermore it has a direct inotropic effect on the heart (Doenst, Richwine et al. 1999).

These multifactorial benefits of GIK have been studied in a clinical setting in both patients with ischaemic heart disease and those undergoing cardiac surgery. An overview of randomised placebo controlled trial in patients that sustained a myocardial infarction, showed a proportional mortality reduction of 28% in those that received GIK; translated to 49 lives saved per 1000 treated (Fath-Ordoubadi and Beatt 1997). However Mehta and colleagues in the CREATE-ECLA trial; randomised 20,201 patients in 470 centres to GIK therapy in patients presenting with STEMI, and showed no overall clinical benefit with no difference in mortality, cardiac arrest and cardiogenic shock (Mehta, Yusuf et al. 2005). Furthermore another meta-analysis looking at patients treated with GIK post MI; in 16 randomised trials spanning 40 years and including 28,000 patients showed no mortality benefit for patient with ST-Elevation myocardial infarction (Mamas, Neyses et al. 2010). However, GIK therapy and its uses have been further evaluated in patients undergoing cardiac surgery.

1.7.1.1 GIK and cardiac surgery

Bothe and colleagues performed a meta-analysis of all randomized trials, using GIK in cardiac surgery. Of 35 GIK trials identified, 11 were included in this analysis; 468

patients underwent coronary artery bypass grafting or valve replacement surgery. Six studies showed significant improvement in post-operative recovery, with a weighted mean relative improvement of cardiac index of 11.4% in those treated with GIK, with reduced inotrope usage. This study concluded that GIK therapy may considerably improve post-operative recovery of contractile function post cardiac surgery (Bothe, Olschewski et al. 2004). A subsequent met-analysis of similar trials included 33 RCTS assessing 2113 patients and showed that GIK therapy was associated with significantly fewer perioperative myocardial infarctions (RR = 0.63, 95% CI 0.42-0.95), less inotropic support requirement (RR = 0.66, 95% CI 0.45-0.96), better postoperative cardiac index (weighted mean difference (WMD) = 0.43, 95% CI 0.31-0.55), and reduced length of stay in the intensive care unit (WMD = -7.96, 95% CI -13.36 to -2.55) (Fan, Zhang et al. 2011).

1.7.1.2 The Birmingham experience

The Birmingham group has conducted a series of studies evaluating the role of GIK therapy in cardiac surgery. The first of a series of trials with GIK was reported in 2006. This double-blind placebo controlled trial randomised 138 patients to the GIK group, and recruited patients who were undergoing first time isolated coronary artery bypass graft surgery. GIK was used as an adjunct to standard cardioplegia solution. This study showed that the incidence of LCOE was lower in the GIK group (GIK group, 22/138 [15.9%]; placebo group, 39/142 [27.5%]; p=0.021). Furthermore there was reduced incidence of inotrope use in the GIK group. There was also a reduced incidence of myocardial injury in the GIK group (GIK group, 16/133 [12.3%]; placebo group, 32/137 [23.4%]; p=0.017). Therefore this study showed GIK improved early post-operative

myocardial performance and reduced the requirement of inotropes and may improve myocardial injury (Quinn, Pagano et al. 2006).

These finding were supported by another double-blind randomized placebo controlled trial looking at the role of GIK individually and in combination with triiodothyronine (T3) and reported on combined data from the previous study (both consisting of identical trial protocols). This too showed improved haemodynamic performance associated with GIK compared to placebo either individually or in combination with T3. Furthermore the use of inotropes and vasoconstrictor usage was similar to the previously reported trial; fewer patients in all treatment groups required inotropic support in the first 6 hours after aortic cross-clamp removal compared with placebo (66/160, 41.3%), GIK (28/157 [17.8%]; $p<0.001$), T3 (14/63 [21.0%] $p=0.03$), and GIK/T3 (9/60 [15.0%]; $p<0.001$), and vasoconstrictor use was significantly higher with the GIK groups ($p<0.001$). Moreover the overall incidence of low cardiac output syndrome was lower in all treatment groups (GIK, 33/157 [21.0%] $p=0.228$; GIK/T3, 10/60 [16.7%] $p=0.186$; T3, 13/63 [20.6%] $p=0.42$; versus placebo 50/160 [31.3%]) but did not reach significance on multiple comparison testing. This study also showed a reduced Troponin I release at 6 hours in all treatment groups (placebo, 5.8 [3.1 to 11.3] ng/mL; GIK, 5.1 [2.7 to 7.7] ng/mL $p=0.19$; T3, 2.74 [1.0 to 5.9] ng/mL $p<0.001$; GIK/T3, 3.3 [1.5 to 7.8] ng/mL $p<0.01$) (Ranasinghe, Quinn et al. 2006). Hence Ranasinghe et al concluded that perioperative GIK can reduce myocardial injury and improve haemodynamics in patients undergoing on-pump CABG; however it is unclear whether the effects seen with GIK are due changes in contractility or afterload.

The above trials and others described in the literature using GIK in cardiac surgery have assessed its effect in patients undergoing CABG only and have not examined the role of

GIK in the presence of quantified left ventricular hypertrophy. This led to the next large trial randomizing patients with quantified LVH secondary to aortic stenosis undergoing aortic valve replacement surgery. This study recruited 110 patients to the GIK arm and of these, 11 patients were diagnosed with a low cardiac output episode showing that GIK was associated with a significant reduction in the incidence of low cardiac output state (odds ratio, 0.22; 95% confidence interval, 0.10 to 0.47; $p<0.0001$). Analysis of the secondary end-points showed that there was significantly reduced incidence of inotrope usage and the cardiac index was significantly higher in the GIK group from the start of treatment until 12 hours from removal of the aortic cross clamp. In keeping with the previous GIK trials, GIK was associated with high incidence of vasoconstrictor requirements (Howell, Ashrafian et al. 2011).

The myocardial protective role of GIK is likely to be insulin dependent and previous studies have explored the more established benefits of metabolic modulation; shift towards the more efficient glucose metabolism. Howell and colleagues explored the role of insulin on antiapoptotic and cardioprotective signalling pathways in addition to protein O-GlcNAcylation and its interaction with insulin.

Phosphorylation and activation of Akt (activated by PI3K and in turn mediated by phosphoinositide-3,4,5 triphosphate, by the action of insulin on PI3K) has been shown to protect against apoptosis after ischaemia/reperfusion in cardiomyocytes (Jonassen, Sack et al. 2001). Furthermore, AMP-activated protein kinase (AMPK); activated by phosphorylation, also has an established role in insulin signalling (Bertrand, Horman et al. 2008) and is thought to have a role in cardioprotection. Protein O-GlcNAcylation refers to modification of serine and threonine residues of nuclear and cytoplasmic proteins by O-GlcNAc (O-linked βN-acetylglucosamine). Experimental studies have

shown high levels of O-GlcNAcylation post ischaemia in isolated perfused hearts, showing that this stress–activated pathway is active in the heart and higher levels of O-GlcNAc improves contractile function and decrease tissue injury after reperfusion (Fulop, Zhang et al. 2007; Liu, Marchase et al. 2007; Liu, Marchase et al. 2007)

In addition to the above positive clinical findings, Howell et al showed that GIK causes insulin signalling pathway activation of Akt and AMPK, which could be associated with improved myocardial protection; significant increase in the ratio of phosphor-Akt to pan-Akt and phopho-AMPK to AMPK in the GIK group. There was also more prominent O-GlcNAcylation in the GIK group (Howell, Ashrafian et al. 2011). These findings add further evidence to the mechanisms of cardioprotection achieved with GIK, in addition to the existing evidence of metabolic enhancement.

Despite the vast amount of data supporting the cardioprotective nature of systemic GIK, GIK is not widely used as an adjunct to standard myocardial protection in cardiac surgery. The administration and peri-operative management of GIK is a labour intensive process that requires medical supervision and input in the peri-operative stage. It requires continuous monitoring and control of blood glucose levels due to the risk of hyperglycaemia (Quinn, Pagano et al. 2006; Ranasinghe, Quinn et al. 2006; Howell, Ashrafian et al. 2011). Hyperglycaemia is well known to have a negative impact post cardiac surgery with increased morbidity and mortality (Doenst, Wijeysundera et al. 2005). Furthermore hyperglycaemia has adverse outcomes post myocardial ischaemia (Ishihara, Inoue et al. 2003; Ishihara, Kojima et al. 2005).

Existing evidence in favour of GIK comes from small studies, some which are inadequately powered or not randomised. Moreover, differing study protocols, regimes

of GIK administration and study populations examined, heighten the heterogeneous manner in which positive cardioprotective conclusions with GIK have been reached. The multiple modalities of action of GIK add to the confusion and debate. Therefore there is a call for multi-centre trials with GIK in patients at risk of low cardiac output syndrome including further examination into the underlying mechanism of actions (Vlasselaers 2011). In the meantime the debate regarding the best myocardial protection strategy continues and other modalities of metabolic modulation, to be used as adjuncts to standard cardioplegia need to be considered.

1.7.2 Promoting pyruvate oxidation

Dichlorocetate (DCA) has been advocated as an agent for short-term metabolic therapy in patients in early heart failure, where the substrate metabolism is near normal. Dichloroacetate inhibits pyruvate dehydrogenase kinase (PDH), in turn maintaining PDH in the dephosphorylated active form, thereby increasing pyruvate oxidation (Whitehouse, Cooper et al. 1974). DCA has shown also to inhibit FFA oxidation by increasing malonyl CoA and inhibiting CPT1 (Stanley, Hernandez et al. 1996). Bersin et al showed that infusion of DCA increased stroke volume and stroke work and an increase in mechanical efficiency from 15.3 to 20.6% (Bersin, Wolfe et al. 1994; Bersin and Stacpoole 1997). Similarly increasing the arterial load of pyruvate by infusing glycerol ester of pyruvate has been shown to reduce the myocardial infarct size during reperfusion in pigs (Stanley, Kivilo et al. 2003). However these experimental studies have not translated to clinical practice and large-scale clinical trials are required to comment on clinical benefit. Furthermore the beneficial effects of DCA appear to be linked to pyruvate oxidation and not glycolysis (Stanley, Lopaschuk et al. 1997).

L-carnitine is involved in the transport of free fatty acids into the mitochondrial space, for fatty acid oxidation. In addition to this role, L-carnitine is involved in regulating pyruvate oxidation (Stanley, Lopaschuk et al. 1997). Administration of L-carnitine and it's analog propionyl L-carnitine has shown to increase glucose oxidation by stimulating PDH activity (Schonekess, Allard et al. 1995). Furthermore these compounds have shown to have cardioprotective benefits in early experimental studies due to their anti-ischaemic properties (Iliceto, Scrutinio et al. 1995; Stanley, Lopaschuk et al. 1997). However, there use in cardiac surgery and their properties in clinical practice is limited.

1.7.3 Trimetazadine

Trimetazadine is a free fatty acid oxidation inhibitor hence has the potential to augment the more efficient glucose metabolism (Fantini, Demaison et al. 1994). Trimetazadine has been clinically used as an anti-anginal drug (Dalla-Volta, Maraglino et al. 1990; Lewandowski 2000; Szwed, Sadowski et al. 2001). Its action is by inhibiting long-chain 3-ketoacyl-CoA thiolase, (Lopaschuk, Barr et al. 2003) a key enzyme in the β-oxidation pathway. Trimetazadine has no impact on the rate of glycolysis during reperfusion (Boucher, Hearse et al. 1994). In patients with ischaemic heart failure, trimetazadine significantly improved left ventricular ejection fraction and enhanced LV wall motion (Belardinelli and Purcaro 2001). These findings have been supported by more recent clinical trials showing an improvement in systolic LV function (Rosano, Vitale et al. 2003).

In animal models, trimetazadine has been beneficial in reducing injury following ischaemia/reperfusion thus could improve recovery of cardiac function. These cardioprotective effects appear to be through activation of Akt signalling pathways

particularly when administered before reperfusion (Khan, Meduru et al. 2010) and the anti-apoptotic features post ischaemia/reperfusion evident in animal studies (Ruixing, Wenwu et al. 2007).

However in June 2012 the European Medicines Agency recommended restricting the use of trimetazadine containing medicines due to concerns regarding the efficacy of trimetazadine. There were also concerns regarding movement disorders such as Parkinsonian symptoms (Agency 2013). Therefore the Agency's Committee for Medicinal Products for Human Use (CHMP) concluded that there was no positive beneficial balance that outweighed the risks associated with trimetazadine, and trimetazadine should only be used as an add-on in patients with angina pectoris, who are inadequately controlled or intolerant to other medications.

1.7.4 Ranolazine

Ranolazine is also an oral anti-ischaemic agent and has shown to be effective in patients with angina, restricting the extent of ischaemic damange (Iliceto, Scrutinio et al. 1995). Its effects are most evident in low-flow ischaemia or normoxia, with no protective benefits during complete cessation of flow (Black, Gralinski et al. 1994). Despite the extensive experimental and animal studies that show significant increases in glucose oxidation and reduction in fatty acid oxidation, it's limitation during no flow makes this unsuitable for practice during cardiac surgery (cessation of coronary perfusion).

1.7.5 Inhibition of free fatty acid oxidation

Inhibition of free fatty acid oxidation would promote the more efficient glucose oxidation pathway and would switch the substrates being utilized towards

carbohydrates. Carnitine palmitoyltransferase 1 (CPT1) is a key enzyme involved in free fatty acid uptake into the mitochondrion. Therefore inhibition of this key step should inhibit FFA oxidation, thereby increasing the oxidation of glucose.

CPT1 inhibition has shown to reduce fatty acid oxidation and increase glucose oxidation (Higgins, Morville et al. 1980; Lopaschuk, Wall et al. 1988; Schwartz, Greyson et al. 1994). These studies used either etomoxir or oxfenicine for CPT1 inhibition and their use in clinical practice is limited. Alternatively perhexiline has been used widely in clinical practice and has shown to be an effective CPT1 inhibitor. Therefore there was potential to translate its cardioprotective benefits as an adjunct to standard cardioplegia for myocardial protection during cardiac surgery.

1.7.6 Clinical use of adjuncts in myocardial protection

GIK therapy in its role as a metabolic modulating adjunct to standard myocardial protection with cardioplegia during cardiac surgery has been widely and extensively evaluated. It has been found to be extremely beneficial. The other metabolic agents described above are less extensively evaluated particularly with cardiac surgery.

As a component of his thesis, Drury performed a systematic review of the literature to examine the extent of use of other metabolic agents as adjuncts to standard myocardial protection during cardiac surgery. Only eight studies were included, 7 using trimetazadine and 1 using trimetazadine and mildronate. None of the other potential agents have been tested clinically in cardiac surgery. He concluded that most of these trials were inadequately designed to effectively judge clinically important outcomes (Drury 2012). The use of perhexiline as an adjunct to myocardial protection has only been studied recently, by our group, in patients undergoing CABG.

1.8 Perhexiline

1.8.1 Overview

Perhexiline was initially developed by Richardson-Merrell Pharmaceuticals in the late 1960s. It was first marketed in France as an anti-anginal agent and it was first marketed in the UK in 1975. Although the exact mechanism of action was not elucidated until the mid 1990s, perhexiline proved to improve symptoms by promoting an efficient cardiac metabolism.

However due to a number of reports related to hepatic and neurotoxicity caused by perhexiline, it fell out of favour and was withdrawn from the UK market in 1985. By 1988 many other countries had followed the trend set by the UK; however in Australia and New Zealand perhexiline continued to be prescribed to patients who were on maximum alternative medical therapy.

Over time, the relation between perhexiline concentration and toxicity was better understood. Furthermore the pharmacokinetics and genetic basis for the metabolism of perhexiline became clearer. This prompted strict monitoring of plasma perhexiline concentrations and the identification of individuals who were poor metabolisers of perhexiline. Therefore at present in the UK, perhexiline is prescribed on a named patient basis with strict plasma concentration monitoring.

1.8.2 Composition and chemistry

Perhexiline is 2-(2,2-dicyclohexylethyl) piperidine (Figure 1-7) and consists of a –CH-CH_2 carbon chain backbone with three six membered rings and has the chemical

formula $C_{19}H_{35}N$. Two of the rings are saturated cyclohexane rings and the other is a pyridine group.

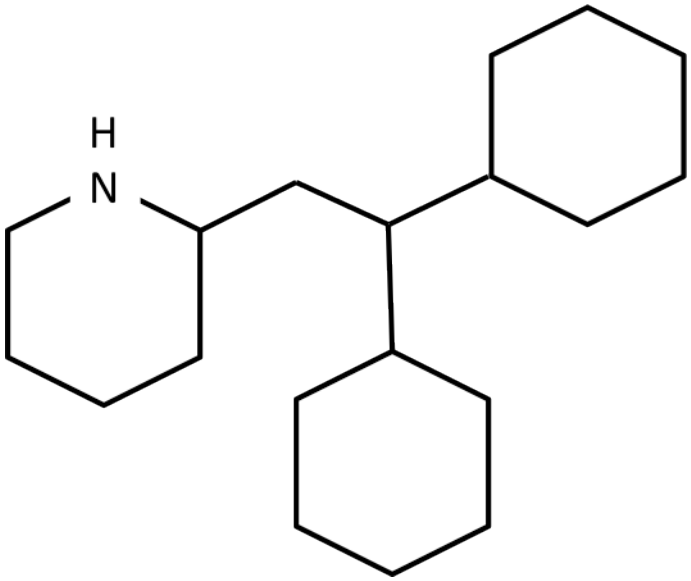


Figure 1-7 Chemical structure of perhexiline

The molecule is amphiphilic in nature as the cyclohexane residues are hydrophobic and the pyridine group is hydrophilic, as it forms hydrogen bonds with the maleate oxygen atoms (Ashrafian, Horowitz et al. 2007).

Perhexiline can be prepared through the catalytic hydration of 2-(2,2-diphenylethyl) pyridine. Pharmaceutical preparations contain perhexiline's maleate salts, although perhexiline hydrochloride is clinically effective. Perhexiline maleate salts contain a racemic mixture of (+) and (-) enantiomers. Perhexiline salts have a white appearance and are odourless. They are aqueously soluble and form a crystalline powder, which melts at 245 °C.

1.8.3 Early work with perhexiline

Over the last 40 years, efforts have been made to understand fully the mechanism of action of perhexiline. The development of perhexiline was based on the promise shown by a related compound, hexadylamine. In 1962 Rowe et al (Rowe, Afonso et al. 1963) showed that hexadylamine increased cardiac oxygen efficiency; increasing coronary vasodilatory properties and cardiac output whilst maintaining constant cardiac work with a reduced cardiac oxygen extraction.

Perhexiline was found to improve cardiac efficiency, but the mode of action through which this was achieved has never been fully understood. A number of *in-vivo* and *in-vitro* experiments in the 1970s have tried to delineate the mechanism of action. Initially perhexiline was found to have vasodilatory properties in both the coronary and systemic circulation, without affecting the femoral blood flow despite a drop in the systemic blood pressure (Hudak, Lewis et al. 1970). A canine right heart perfusion model employed by Cho and colleagues (Cho, Belej et al. 1970) showed that perhexiline increased cardiac metabolic efficiency whist improving the parameters of mechanical cardiac function. Perhexiline given intravenously at 1-2mg/kg decreased the left ventricular work (blood pressure x cardiac output) and increased the index of left ventricular efficiency (change in left ventricular work/change in myocardial oxygen consumption). Similarly direct injection of perhexiline to the circumflex branch of the left coronary artery increased coronary blood flow in a dose dependent manner (Hudak, Lewis et al. 1970). Thus far, animal models have shown that perhexiline improves cardiac efficiency, by improving coronary circulation and hence cardiac function.

A human model to elucidate the effects of perhexiline was undertaken in patients with angina pectoris with angiographically proven coronary artery disease (Pepine, Schang et al. 1974). Patients received 200mg of perhexiline bd for 2 weeks. This study showed that perhexiline decreased heart rate and increased stroke volume and exercise tolerance with reduced angina. Furthermore perhexiline reduced myocardial lactate extraction and enhanced myocardial oxygen extraction. Coronary flow reflected by coronary sinus blood flow was unaltered whilst left ventricular oxygen utilisation was increased. At this stage it was only a hypothetical assumption that perhexiline induced an efficient cardiac metabolism by creating a shift towards carbohydrate utilisation.

1.8.4 Mechanism of action

This hypothesis that perhexiline promoted carbohydrate metabolism over fatty acid metabolism was evaluated in the early 1990s. Free fatty acid metabolism would require more oxygen to generate the same number of ATP; therefore conversion to carbohydrate metabolism seemed a plausible explanation to achieve greater efficiency. Dechamps and colleagues treated *ex-vivo* rat heart preparations and hepatocytes with perhexiline for 72 hours and showed a reduction in β-oxidation of fatty acids (Deschamps, DeBeco et al. 1994). Yet the exact mechanism of promoting carbohydrate metabolism was unclear.

Kennedy and colleagues hypothesised that the mechanism of action was likely to be by the inhibition of carnitine palminotyltransferase-1 (CPT-1) (Kennedy, Unger et al. 1996). The enzyme CPT-1 controls the access of long chain fatty acids to the mitochondrial site for β-oxidation. Similarly other agents that promote carbohydrate metabolism have a similar mode of action. Kennedy et al, demonstrated perhexiline

produced a concentration dependent inhibition of CPT-1 in rat cardiac and hepatic mitochondria *in-vitro*. The experiments used homogenised cardiac and liver tissue from male Sprague-Dawley rats treated with perhexiline. Furthermore there was competitive inhibition of cardiac and hepatic CPT-1 with perhexiline with respect to palmitoly-CoA but non-competitive inhibition with respect to carnitine. Perhexiline was found to be a competitive inhibitor of CPT-1, with the endogenous CPT-1 inhibitor malonyl-CoA. Treatment with the nargase protease, demonstrated that perhexiline acted at an independent mitochondrial site not amenable to proteolysis, unlike malonyl-CoA and other CPT-1 inhibitors (Kennedy, Unger et al. 1996). Moreover, there was also a greater inhibition to cardiac CPT-1 than hepatic CPT-1 with perhexiline.

Therefore perhexiline has been classified as a fatty acid oxidation inhibitor (FAOI), inhibiting fatty acid metabolism in the myocardium at CPT-1 level. Other FAOI include etomixir, oxfenicine and amiodarone. Beyond the scope of the myocardium, FAOI may have a systemic effect and may inhibit fatty acid metabolism in the liver; reducing systemic ketone body levels and mitigating detrimental mitochondrial lipid metabolism (Ashrafian, Horowitz et al. 2007). Due to the competitive inhibition of CPT-1, perhexiline is classified as a partial FAOI inhibitor.

Beyond CPT-1 inhibition, perhexiline has been found to have other mechanistic actions. Palmitate oxidation is inhibited in cardiomyocytes after 48 hours of pre-exposure with perhexiline (Unger, Kennedy et al. 2005). Therefore this study proposed that the increased myocardial efficiency observed in working non-ischaemic rat hearts was independent of perhexiline's effects on CPT-inhibition. Furthermore, perhexiline has a number of electrophysiological effects, yet the relevance and significance is unclear. Barry et al demonstrated that perhexiline altered myocellular electrical activity through

the blockade of voltage gated L-type calcium channels (Barry, Horowitz et al. 1985) and similarly, Grima et al demonstrated the blockade of Sodium channels (Grima, Velly et al. 1988). These electrophysiological effects may demonstrate a negative inotropic effect depending on the degree of inhibition of calcium influx (Barry, Horowitz et al. 1985). Perhexiline also blocks cardiac K+ channels (Rampe, Wang et al. 1995). Rampe et al found that perhexiline blocks both the K+ channels in human embryonic kidney cells and the ultra-rapid delayed rectifier in human atrial myocytes. Furthermore, perhexiline causes a blockade of the Human-Ether-a-go-go Related Gene (HERG), a subunit of the potassium ion channel, and is likely to be the cause of QT prolongation (Walker, Valenzuela et al. 1999).

1.8.5 Metabolism and pharmacokinetics

During the early use of perhexiline as an anti-anginal, the mechanism by which perhexiline was metabolised was not fully understood. Perhexiline metabolism is highly variable between individuals and those that did not metabolism perhexiline adequately developed perhexiline toxicity. This variability in metabolism and associated toxicity led to perhexiline's unfavourable predisposition as an anti-anginal agent. Patients who developed toxicity had neuropathy and liver damage.

Perhexiline is well absorbed orally, with absorption almost complete by 12 hours of the dose. Bioavailability studies in the 1970s showed that perhexiline and metabolites continued to accumulate over time. Furthermore, perhexiline is metabolised primarily through oxidative pathways in the liver and the derivatives excreted by urine are either mono or dihydroxylated variants or their derivatives (Wright, Leeson et al. 1973).

Thus, perhexiline is predominately metabolised to *cis*-monohydroxy derivative (*cis*-OH-perhexiline).

Singlas and colleagues further explored the variability to perhexiline metabolism. They found that patients with neuropathy exhibited higher plasma levels of perhexiline, slower hepatic metabolism and a longer plasma half-life (Singlas, Goujet et al. 1978; Singlas, Goujet et al. 1978). Perhexiline to metabolite ratio was nine times greater in those with neuropathy than those without toxicity. Thus, patients can be broadly divided into 2 groups, those that have no evidence of toxicity and are able to metabolise perhexiline and those that have toxicity and hence poorer metabolisers of perhexiline. The reason for this variation in metabolism was yet to be identified and was thought to be genetic differences between individuals or hepatic damage caused by perhexiline.

Several drug oxidations in humans are controlled by a single gene and exhibit genetic polymorphism (Mahgoub, Idle et al. 1977; Sloan, Mahgoub et al. 1978). The anti-hypertensive drug debrisoquine is extensively oxidised by most individuals and can be used to evaluate the genetic structure of a population with respect to oxidation. Oxidation is regulated by 2 alleles; one for rapid and extensive oxidation and the other for slow impaired oxidation. Those who are homozygous for the slow impaired oxidation are poor metabolisers and have impaired ability to oxidise several other drugs besides debrisoquine. Shah et al demonstrated a clear association between perhexiline induced neuropathy and diminished drug metabolic ability as shown by debrisoquine hydroxylation (Shah, Oates et al. 1982). Those with poor metaboliser phenotype are at a higher risk of developing neuropathy from long-term use of perhexiline and the risk is reduced in extensive metabolisers. Therefore perhexiline

would tend to accumulate in individuals with an impaired drug oxidising ability due to defective metabolic elimination.

Following these initial studies, although the association between a poor metaboliser and high accumulation of perhexiline was known, the molecular substrate for the polymorphism was less clear. In a landmark study in 1988, Gonzalez and colleagues identified the gene responsible for the polymorphic variation predisposing individuals to be poor metaboliser (Gonzalez, Skoda et al. 1988). They showed that poor metabolisers have negligible amounts of the cytochrome P450 enzyme P450db1. Furthermore they identified three variant messenger RNAs that are products of mutant genes producing incorrectly spliced db1 pre-mRNA. This provided a molecular explanation for the commonest defective gene in humans, which is represented as an autosomally inherited trait differentiating poor metabolisers from extensive metabolisers; deficiency in *CYP2D6* activity is inherited as an autosomal recessive trait. This study again was based on patients who had a defect on debrisoquine metabolism. However this same genetic defect predisposed individuals to poorly metabolise more than 30 commonly used medications in different categories. The gene responsible for this polymorphism is a member of the P450 family and named *CYP2D6*.

The metabolism of perhexiline is thus dependent on the *CYP2D6* gene and its activity (Sorensen, Sorensen et al. 2003). Furthermore, the metabolism within each group is highly variable with a 100-fold difference between the poor and extensive metaboliser groups. Individuals can be further classified into three categories: poor metabolisers, extensive metabolisers and ultra-rapid metabolisers (Sallustio, Westley et al. 2002). This categorisation was consistent with *CYP2D6* metabolism and those who were ultra-rapid metabolisers required up to 500 mg/day of perhexiline compared to 10-25mg of

perhexiline for the poor metabolisers. The ratio of cis-OH-perhexiline metabolite/parent plasma perhexiline concentration was used as an indication of perhexiline metabolism. Furthermore a study by Davies et al showed that patients with only 1 functional *CYP2D6* allele had a higher plasma concentration of perhexiline and a lower metabolic ratio compared to patients with 2 functional alleles and concluded that patients with one functional allele have diminished *CYP2D6* metabolic capacity for perhexiline (Davies, Coller et al. 2006).

Therefore a direct correlation between *CYP2D6* activity and perhexiline metabolism (cis-OH-perhexiline metabolite/parent plasma perhexiline concentration) was made and could be used to assess the dosing requirements and identify those at greatest risk of developing side effects.

1.8.6 Side effects and toxicity

The poor metabolisers are most at risk of developing side effects and toxicity and the prevalence of poor metabolisers vary according to race. In European and US Caucasians poor metabolisers constitute 7 – 10% of the population and harbour mutations of *CYP2D6*. In African Americans however, only 1.9% of the population are poor metabolisers (Evans, Relling et al. 1993). In the Chinese, Japanese and Korean population, incidence is even lower at 1% (Gardiner and Begg 2006).

Side effects and toxicity associated with perhexiline is outline in (Table 1-4). Serious toxicity takes up to 3 months to develop. Minor side effects may improve with a reduction in the oral drug therapy regime and does not necessitate in drug discontinuation (Killalea and Krum 2001).

Minor side effect	Major side effects and toxicity
Dizziness	Neurotoxicity/neuropathy
Headache	Hepatotoxicity/liver failure
Nausea	Hypoglycaemia
Vomiting	
Diarrhea	
Tremor	
Lethargy	
Insomnia	
Loss of libido	

Table 1-4 Minor and major side effects of perhexiline

Major side effects and toxicity was observed in patients who were on long-term perhexiline therapy. By August 1983, 80 cases of hepatotoxicity and 131 cases of neuropathy had been reported in the United Kingdom (Shah 2006). Similarly over a 20-year period from 1974, 46 cases of hepatic adverse events due to perhexiline were reported in New Zealand (Pillans 1996). The majority of reported adverse events occurred before the monitoring era. We are also now aware that the side effects observed are likely due to perhexiline accumulation amongst poor metabolisers and hence most evident in patients on long term therapy.

The molecular basis for the side effects observed with perhexiline accumulation is not very well understood. Speculation is that the adverse reactions are a result of excessive CPT-1 and hence fatty acid metabolism inhibition (Ashrafian, Horowitz et al. 2007). Excessive CPT-1 inhibition would lead to upstream cytoplasmic accumulation of fatty acids and their derivatives and decreased downstream derivatives. Excess perhexiline may lead to a pathology termed pseudo-alcoholic hepatitis; changes similar to alcoholic liver disease with cellular changes consistent with macro and microvascular steatosis,

ballooning of hepatocytes and Mallory bodies. Furthermore phospolipidosis and accumulation of triglycerides and fatty acids were identified by studies using ultrastructure and histoenzymological techniques on cultured liver cells (Lageron, Scotto et al. 1981). These changes were also evident in peripheral nerve Schwann cells.

These established cellular changes to perhexiline excess and the reported evidence of toxicity based on long-term perhexiline use required a dose-adjusted regime in perhexiline therapy.

1.8.7 Dosing regime with oral therapy

Poor metabolisers are at greatest risk of developing adverse events. Perhexiline plasma levels need to be monitored and early identification of poor metabolisers based on the perhexiline to metabolite ratio would allow appropriate dose adjustments in this group of individuals. Therefore ideally therapeutic monitoring should be carried out particularly in this group of individuals. Perhexiline is being used extensively in Australia and New Zealand with appropriate plasma monitoring and dose adjustments (Ashrafian, Horowitz et al. 2007). Furthermore metabolic ratio of perhexiline to monohydroxyperhexiline may be used as a diagnostic tool to differentiate ultra-rapid metabolisers, extensive metabolisers and poor metabolisers.

Horowitz et al pioneered a regime to appropriately manage the sub-group of poor metabolisers. They recognised that patients who developed hepatic and neurological side effects had plasma perhexiline concentrations of 720 – 2680 μg/L on doses of perhexiline ranging from 50 – 400 mg/day. Furthermore, this study showed that patients with a plasma concentration of < 600 μg/L (mean dosage approximately 160mg/day) had no side effects (Horowitz, Sia et al. 1986). This maintenance of

perhexiline concentrations could aid long-term therapy with no adverse events. Similarly other studies have corroborated these finding (Pilcher, Cooper et al. 1985; Cole, Beamer et al. 1990; Stewart, Voss et al. 1996).

A gradual loading regime and dose adjustment based on perhexiline to metabolite ratios is ideal to avoid an increase in plasma perhexiline levels. However a fast loading regime exists in patients treated for ACS and may incur side effects (Philpott, Chandy et al. 2004).

We know that perhexiline is metabolised in the liver, and despite the known requirement for *CYP2D6* activity, extra caution should be taken in patients with existing liver disease; perhexiline metabolism may be impaired with underlying liver impairment. Furthermore as perhexiline is metabolized by *CYP2D6* activity, consideration should be made towards other drugs that interfere with this enzyme system i.e. SSRI such as fluoxetine (Alderman, Hundertmark et al. 1997).

It is evident that adverse reactions to perhexiline are consistent with long-term perhexiline therapy in conjunction with poor dose control, in relation to the rate at which perhexiline is metabolised on an individual basis. Therefore short-term therapy without a fast loading regime may prove to be safe and effective particularly in the treatment of patients pre-operatively (Horowitz, Sia et al. 1986).

1.8.8 Pharmacogenetics

It is well established that perhexiline metabolism is controlled by *CYP2D6* activity. Individuals with 2 normal alleles are considered extensive metabolisers. Those with 1

normal allele are intermediate metabolisers but function as extensive metabolisers. Those with 2 poorly functioning alleles are poor metabolisers.

Barclay et al proposed that genotyping was able to identify poor metabolisers and the broad dose of these individuals could be predicted; yet therapeutic monitoring should not be avoided (Barclay, Sawyers et al. 2003). Furthermore, perhexiline has been suggested as an ideal example for pharmacogenetic testing to rescue drugs withdrawn from the market (Shah 2006). However, the use of pharmacogenetics is yet to be well established and would need appropriate ethical approvals and favourable cost-benefit analysis before it is widely accepted for use.

1.8.9 Clinical benefits

Perhexiline is classed as a free fatty acid oxidation inhibitor and promotes carbohydrate metabolism through the mechanisms explained above. Carbohydrate metabolism is an energy efficient system utilising less oxygen and may improve cardiac energetics. Furthermore perhexiline may be able to ameliorate debt and improve coronary perfusion. Therefore perhexiline may be best suited in patients with myocardial oxygen deficiency such as ischaemic heart disease and aortic stenosis. Furthermore energy deficient pathologies such as heart failure and hypertrophic cardiomyopathy may also benefit from perhexiline's efficient metabolic modulation.

1.8.9.1 Ischaemic heart disease

A systematic review was conducted to evaluate the efficacy and tolerability of perhexiline in the treatment of cardiac disease. Although the majority of the trials were of cross over design, this review found that perhexiline is considerably more effective

than placebo when used as monotherapy. It further concludes that perhexiline adds further symptom relief in those patients on maximum conventional anti-anginal therapy. Perhexiline also compared favourably with use of β-blockers (Killalea and Krum 2001). It has been shown that perhexiline is most efficacious when it's used in patients with angina refractory to other medical and interventional therapies (Horowitz, Button et al. 1995). Furthermore it has been used in acute coronary syndromes (ACS) and shown to be beneficial by potentiating platelet responsiveness to nitric oxide and was predictive of resolution of ischaemic symptoms in ACS (Willoughby, Stewart et al. 2002).

1.8.9.2 Heart failure

Heart failure is considered a condition consistent with deranged substrate metabolism (Stanley, Recchia et al. 2005). This deranged metabolism could lead to impaired myocardial energetics (Ingwall and Weiss 2004). Therefore metabolic manipulation with perhexiline may prove to be beneficial in this group of patients.

In a double-blind randomised placebo controlled trial, perhexiline was administered to patients with heart failure on maximum conservative medical therapy (Lee, Campbell et al. 2005). This study showed a significant within group improvement in VO_2max in patients administered perhexiline (16.1 ± 0.6 to 18.8 ± 1.1 mL/kg/min; $P<0.001$), and similar changes between the groups. Perhexiline also improved the quality of life, based on the Minnesota score reduction. Furthermore there was significant improvement in left ventricular ejection fraction from 24% ± 1% to 34% ± 2% when treated with perhexiline. This proved to be a promising metabolic agent for patients on maximum medical therapy for heart failure.

1.8.9.3 Aortic stenosis

With aortic stenosis, a state of relative oxygen debt may exist due to the decreased perfusion to an increasingly hypertrophied ventricle. In a study by Unger et al 15 elderly patients with severe aortic stenosis unsuitable for aortic valve replacement were treated with perhexiline (Unger, Robinson et al. 1997). These patients were followed up for 30 months. In 13 patients, symptomatic status improved (in the 1st 3 months) with 5 becoming asymptomatic. At 12 months actuarial survival was 80%. This study has shown that in patients with aortic stenosis with likely left ventricular hypertrophy, perhexiline has been symptomatically beneficial.

1.8.9.4 Birmingham experience with perhexiline

In a double-blind placebo controlled RCT, Abozguia and colleagues recruited 46 patients with hypertrophic cardiomyopathy and administered oral perhexiline. Cardiac energetics were measured by evaluating the phosphocreatine to ATP (PCr:ATP) ratio using ^{31}P magnetic resonance spectroscopy at a mean follow up of 4.6±1.8 months. This study demonstrated an improved cardiac energetic state with perhexiline therapy, with an increase in PCr:ATP from 1.27±0.02 to 1.73±0.02 versus 1.29±0.01 to 1.23±0.01; $P<0.003$. Furthermore, the primary end point of peak Vo_2 improved from 22.2±0.2 to 24.3±0.2 versus 23.6±0.3 to 22.3±0.2 mL/kg/min; $P<0.003$, with a significant improvement in NYHA class (Abozguia, Elliott et al. 2010). This study in patients with hypertrophic cardiomyopathy demonstrated the clinical benefits of metabolic modulation with perhexiline, including increased cardiac energetics. These beneficial properties of perhexiline may render the ischaemic myocardium less susceptible to injury. The role of perhexiline as a metabolic modulator, to be used as an adjunct to

standard myocardial protection during cardiac surgery (providing a controlled ischaemia/reperfusion phase) thus appeared worthy for further evaluation.

Therefore a randomised double-blind placebo controlled trial was performed across 2 centres to evaluate the role of perhexiline in cardiac surgery. This study recruited 286 patients undergoing coronary artery bypass graft surgery only, over a 3-year period and randomised patients to receive oral perhexiline at least 5 days prior to surgery and assessed the outcome of low cardiac output episodes. This trial found the incidence of a low cardiac output episode in the perhexiline arm was 36.7% (51/139) versus 34.7% (51/147) in the control arm (odds ratio 0.92 [95% CI 0.56‑1.50]; p=0.74). Furthermore there was no significant difference in the incidence of inotrope use or myocardial injury in addition to any other safety outcome measure i.e. reoperation, length of stay etc. Therefore in this large trial, oral perhexiline did not improve myocardial protection in patients undergoing CABG (Drury, Howell et al. 2011; Drury 2012).

The above study was conducted in patients with ischaemic heart disease requiring coronary artery bypass graft surgery. Although no formal left ventricular dimensions were measured in this study, some patients would have pathological left ventricular hypertrophy secondary to hypertension. Yet the majority would have normal left ventricular dimensions with normal left ventricular mass indices. The hypertrophied ventricle, as a result of the pathological processes of hypertrophy secondary to aortic stenosis, as explained earlier is more susceptible to ischaemic injury and the stressors of reperfusion. Furthermore the metabolic profile of the hypertrophied ventricle is vastly different to the non-hypertrophied ventricle (Howell 2010).

In his thesis, Howell studied the metabolic profile of LVH versus non-LVH, by performing an analysis of the key parts of the metabolic transcriptome, which included master transcriptional regulators PGC-1α, PPARα and ERRα. The key fatty acid transport protein, CPT1 and a key glucose uptake transporter, GLUT4 were also analysed. Analyses of these elements were performed on left ventricular core biopsies taken from the anterior wall of the left ventricle on patients with LVH versus non-LVH. A novel metabolomics analysis using Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry was used to assess the metabolome and principal component analysis (PCA) was used to analyze the different metabolic profiles (Howell 2010). This study showed that there was down regulation of the mRNA transcript levels of the key regulators mentioned above including key regulators of glucose and fatty acid oxidation GLUT4 and CPT1 respectively in LVH. Following PCA, it was evident that there was a clear significant separation between the 2 groups (Howell 2010). There was a 2-fold decrease in the level of phosphocreatine and a 2-fold increase in the level of AMP and adenosine in the LVH group.

From this novel study looking at the metabolome of LVH, Howell has demonstrated that in LVH, there is not just down regulation of key master transcription regulators at key stages of both carbohydrate and FFA metabolism, but this down regulation may result in a reduced energetic state. This abnormal metabolomic profile may contribute to the poor prognosis associated with LVH secondary to aortic stenosis. Hence these patients with a hypertrophic myocardium may be more suited to metabolic modulation and therefore gain more from metabolic therapy. Metabolic modulation with perhexiline in patients with LVH secondary to AS has not been previously examined.

1.9 Hypothesis

Patients diagnosed with aortic stenosis who become symptomatic, have a poor prognosis and quality of life, with an increased risk of developing heart failure if left untreated. Currently the most suitable form of treatment for severe symptomatic aortic stenosis is a conventional aortic valve replacement.

Aortic stenosis over time causes the ventricle to undergo an adaptive response of hypertrophy. Hypertrophy is associated with a lower energetic state and impaired cardiac metabolism. Therefore these patients with LVH secondary to longstanding aortic stenosis are at a greater risk of inadequate myocardial protection during conventional cardiac surgery. Morphological and functional changes due to hypertrophy make this risk greater.

Metabolic support through metabolic manipulation can improve the myocardial metabolic state. One method of metabolic manipulation is to try and promote the more efficient carbohydrate metabolic pathway and inhibit FFA metabolism. Perhexiline is known to be a suitable metabolic modulator, through its role in inhibiting CPT-1, one of the key enzymes involved in inhibiting FFA metabolism. This inhibition of FFA metabolism could promote carbohydrate metabolism and increase glycogen stores to be utilised during the ischaemic and early reperfusion period. Furthermore promoting the more efficient glucose metabolism during the re-perfusion phase, could improve myocardial performance.

Therefore through this thesis I aim to elaborate on the following hypotheses: -

1. That pre-operative loading with perhexiline in patients with aortic stenosis and associated LVH undergoing AVR leads to improved myocardial protection.
2. That pre-operative improvement in energetic state leads to an improvement in cardiac performance following AVR, with an improved haemodynamic performance

These hypotheses are evaluated through a double-blind placebo controlled multi-centre randomised controlled trial evaluating the role of perhexiline as a myocardial protective adjunct. The methodology, results of the clinical trial and discussion are outlined in chapters 2, 3 and 8.

I then examine the energetic status of the heart. High-energy phosphate ratios (PCr: ATP) in human myocardium can be measured through ^{31}P Magnetic Resonance Spectroscopy. To validate this and assess the reproducibility of our technique a validation study was performed in healthy volunteers to measure PCr: ATP ratios using ^{31}P MRS and is outlined in chapter 4. Having established this validation study, the following hypothesis is tested and is outlined in chapter 5.

3. Metabolic manipulation with perhexiline in patients with LVH secondary to AS, leads to improvements in high-energy phosphate (PCr: ATP) ratios and an improvement in their functional status.

Left ventricular hypertrophy secondary to AS, is a pathological adaptive process associated with increased morbidity post cardiac surgery. This pathological hypertrophy may result in chronic subendocardial ischaemia and this may change the

metabolome of the myocardium. To understand the metabolome in LVH, I test the following hypothesis: -

4. In LVH due to the pathological processes of hypertrophy, the epicardial and endocardial halves of the myocardium have different metabolomic profiles.

This hypothesis was examined through a novel metabolomics study to quantify the metabolomic profiles within the epicardium and endocardium and is outlined in chapter 6.

Patients with LVH are more susceptible to myocardial injury particularly following the insults of ischaemia and reperfusion during cardiac surgery. This renders some individuals to develop a low cardiac output episode that requires additional inotropic support to maintain adequate haemodynamic parameters. The intrinsic role of some key master regulators within this group is less clear. To examine this further I test the following final hypothesis: -

5. Patients with LVH that do not sustain a low cardiac output episode have an intrinsic upregulation of some key cardioprotective master regulators.

This hypothesis was examined through a basic science laboratory based study, evaluating the role of key transcriptional regulators, measured by the activity of ACC and GSK3β. This study is outlined in chapter 7.

2 CLINICAL TRIAL METHODOLOGY

2.1 Trial design

The trial was designed as a double-blind placebo controlled randomised control trial, between pre-operative oral loading of perhexiline versus placebo. Recruitment began at a single centre, the Queen Elizabeth Hospital, Birmingham. Due to slow recruitment concerns the trial was expanded to become a multi-centre RCT to include 2 further centres in England, initially expanding to the Royal Sussex County Hospital, Brighton (RSCH) and then to University Hospital Coventry and Warwickshire, Coventry (UHCW).

2.2 Ethics and approvals

The trial protocol was approved by the Cambridgeshire 1 Research Ethics Committee (08/H0304/48) and the UK Medicines & Healthcare products Regulatory Authority (16719/0210/001-0004). These authorities also approved subsequent substantial amendments to the protocol, which included expansion to other centres. The trial was registered with clinicaltrials.gov (NCT00989508), the European Clinical Trials Database (2008-002376-95) and the UK Clinical Research Network (5886). The trial was funded by grants from the British Heart Foundation (BHF: PG/08/040 and BHF: PG/10/036) and University Hospitals Birmingham Charities (17-3-695). The trial was sponsored by University Hospital Birmingham NHS Foundation Trust (RRK3535).

2.3 Selection criteria

Patients diagnosed with aortic valve stenosis and awaiting an aortic valve replacement were suitable to be enrolled into the study. As left ventricular hypertrophy secondary to aortic stenosis was the primary pathology being studied, patients with concomitant

coronary artery disease awaiting concomitant coronary artery bypass graft surgery were also eligible for enrolment. The presence of LVH secondary to AS was confirmed using pre-operative echocardiographic data and had to meet the criteria for echocardiographic evidence of LVH. The echocardiograms were either transthoracic or trans-oesophageal and were performed routinely during the work up towards cardiac surgery and in this patient population were also used to assess and quantify the extent of aortic valve stenosis and overall ventricular function. The inclusion and exclusion criteria are outlined in Table 2-1.

Around 30% of patients with AS have concomitant CAD. Excluding this population would have provided a more homogenous patient sample, however these patients are likely to have a further reduced metabolic state and prone to have increased myocardial injury. Therefore inclusion of this group provided an opportunity to assess this sub-group of patients further. Excluding them would increase the recruitment duration to meet the intended target.

Patients having extensive aortic surgery i.e. ascending and/or proximal and/or aortic arch replacement surgery were excluded, although this group may include some patients with LVH secondary to AS. These patients had different disease pathophysiology related to the aortic disease and the procedures undertaken to repair the aortic disease would require a more extensive period on the cardiopulmonary bypass machine, require deeper cooling strategies and other myocardial protection mechanisms to aid recovery following a more prolonged period of ischaemia which includes, at times complete full body circulatory arrest.

Patients who were suitable but required emergency or salvage surgery due to catastrophic events were excluded due to the extensive repairs that are likely, requiring very different surgical and anaesthetic techniques.

Patients with diabetes were excluded. These patients have a different baseline metabolic and energetic state compared to non-diabetics. Therefore regimes for drug intervention and metabolic therapy would be different. Furthermore these patients are likely to have a suppressed microvascular structure and uniquely different myocardial activity.

In patients with LVH, atrial contraction particularly aids ventricular filling during diastole and augments cardiac output. Therefore patients in atrial fibrillation were excluded. In addition, this study relies on the thermodilution principles for measuring cardiac function and this can be inconsistent in patients with AF.

Patients with mixed aortic valve disease; a combination of AS and aortic regurgitation were further evaluated to assess the degree of aortic regurgitation. If the AR was severe these patients were excluded. Severe AR over time changes the haemodynamic status with ventricular dilation and increased stroke volume leading to depressed ventricular function. Therefore patients with AR have a different disease pathophysiology. Similarly patients with other concomitant valvular pathologies would have even more different disease patterns and haemodynamics; hence were excluded.

Perhexiline is metabolised in the liver and due to its pharmacokinetic properties, patients with hepatic dysfunction, renal impairment (serum creatinine > 200μmol/L), pregnancy and those on amiodarone therapy were excluded. Due to the toxicity

associated with perhexiline leading to peripheral neuropathy, patients with pre-existing peripheral neuropathy were excluded.

Inclusion criteria	Exclusion criteria
LVH secondary to AS awaiting AVR	Patient choice
AVR ± CABG with LVH secondary to AS	Pregnancy
Informed consent	Renal impairment (creatinine > 200μmol/L)
	Diabetes mellitus
	Hepatic impairment
	Atrial fibrillation
	Amiodarone therapy with last 3 months
	Peripheral neuropathy
	Intention to perform any other cardiac procedure
	Severe aortic regurgitation

Table 2-1 Inclusion and Exclusion criteria

2.4 Sample size and power calculation

In ideal circumstances such a study could be powered on mortality and/or morbidity. However due to recent developments in operative technique and more so in post operative care, mortality post cardiac surgery has fallen. In 2008, the post-operative mortality in the UK for AVR and AVR ± CABG was 2% and 4-5% respectively (Bridgewater B, Keogh B et al. 2008). Therefore a trial based on mortality alone would require significant resource utilisation and costs, and would be premature. Alternatively, a surrogate marker of outcome such as low cardiac output episode (LCOE) was considered.

In previous trials run in this department assessing myocardial protection using other metabolic therapies, LCOE was used as a primary end-point (Quinn, Pagano et al. 2006; Ranasinghe, Quinn et al. 2006; Howell, Ashrafian et al. 2011). LCOE post cardiac surgery is an indicator of inadequate myocardial protection and could contribute to morbidity and mortality (Hoffman, Wernovsky et al. 2003). LCOE is associated with a depressed haemodynamic state and under these circumstances inotropic support is used to improve the patient's haemodynamic condition and support myocardial function until no further artificial chemical or mechanical support is required. However inotropic support may be associated with permanent myocardial injury resulting in further re-modelling and fibrosis (Fremes, Tamariz et al. 2000).

The incidence of LCOE following coronary artery bypass graft surgery in the UK is approximately 20 – 30% (Quinn, Pagano et al. 2006; Ranasinghe, Quinn et al. 2006; Howell, Ashrafian et al. 2011). The incidence of LCOE post AVR at the Queen Elizabeth hospital recorded in the Patient Administration and Tracking System (PATS) database is approximately 35%.

An estimated sample size of 196 patients was required based on a power of 90% with a α of 0.05 randomising 1:1 between treatment and control arms. This was estimated based on the incidence of inotrope use observed on an earlier trial conducted within the department, assessing myocardial protection with GIK therapy versus placebo, in patients undergoing coronary artery bypass graft surgery; incidence of inotrope use in placebo was 40% and in the GIK group was 19% (Quinn, Pagano et al. 2006). Assuming that perhexiline therapy may show a similar reduction in inotrope usage we intended to recruit 220 patients to provide an adequate power. This would also allow all the

secondary end-points to be analysed in an exploratory fashion with reference to the primary outcome.

2.5 Primary end-point

The primary end point was the incidence of inotrope use ± comparison of cardiac index to show an increase > 0.3L/min/m^2 within the first 6 hours from reperfusion (removal of the aortic cross clamp). The incidence of use was based on predetermined protocols outlined later in this chapter.

A blinded end-points committee was convened at pre-determined recruitment targets, to adjudicate on this end-point (appropriate inotrope use in relation to the presence of low cardiac output episode outlined below). The meetings were set at a target recruitment of 25, 75, 125, 175 and 220 patients.

2.6 Secondary end-points

2.6.1 The incidence of low cardiac output episode (LCOE)

In this study LCOE was defined as hypotension with a cardiac index (CI) < 2.2L/min/m^2 in the presence of adequate filling pressures and adequate heart rate (80-110bpm), and/or inotropic support ± requirement of an intra-aortic balloon pump for > 60 minutes to improve the haemodynamic status, within the first 6 hrs from removal of the aortic cross clamp. Adequate pre-load was defined as a CVP 8 – 12 mmHg and/or Pulmonary Artery Wedge Pressure (PAWP) of 12 – 16 mmHg and hypotension was defined as a Mean Arterial Pressure (MAP) < 60 mmHg.

2.6.2 The electrocardiographic (ECG) evidence of new myocardial injury

An independent blinded cardiologist assessed this outcome. New myocardial injury was defined as the presence of new Q waves (≥ 2mm in depth) in 2 or more contiguous leads, new bundle branch block or loss of R wave progression.

2.6.3 Cardiac troponin release

Cardiac troponin release was measured in the first 24 hrs from aortic cross clamp removal. Comparison of troponin release at 6, 12 and 24hrs of reperfusion to baseline, as an enzymatic marker of myocardial injury was made.

2.6.4 Incidence of inotrope and vasoconstrictor use

The overall incidence of inotrope use within the first 6 hours of reperfusion and 6-12 hours of reperfusion was assessed. The dose per kilogram used for each group was assessed. Similarly the incidence and dose per kilogram of vasoconstrictor use during the same time periods for each group was assessed.

2.6.5 Use of volume expansion and blood products

The volume of colloids transfused to maintain ideal haemodynamics between the groups was assessed. The number of red blood cells transfused and the number of units of blood products used to control bleeding was assessed.

2.6.6 Safety outcome measures

This included variable outcome measures such as length of ward and ITU stay, wound infection, and the incidence of systemic complications i.e. renal failure, stroke, GI complications etc. as outlined in the statistical analysis plan (Appendix 9.3).

2.7 Statistical analysis

A statistical analysis plan was developed prior to starting the trial (Appendix 9.3). The main outcome measures were analysed on an intension-to-treat basis.

Analysis was performed using SAS (version 9.2, SAS Institute Inc., Cary, NC) and SPSS software (version 20.0, SPSS Inc., Chicago, IL). Continuous data was assessed for normal distribution and presented as mean ± standard deviation of the mean or median and inter-quartile range. Student's t-test was used to analyse normally distributed data and alternatively by analysis of variance (ANOVA). Skewed data was analysed by Mann-Whitney U-test. Categorical data was analysed by Fisher's exact test. Paired non-normal data was analysed using Wilcoxon signed rank test. Statistical significance was defined as a $p < 0.05$.

Spearman's rank correlation (a standardized non-parametric measure of the strength of the relationship between two variables) was used to compare IMP concentration with duration of therapy. Kolmogorov-Smirnov test was used to determine normality of data.

The analysis as per the analysis plan was intended to stratify for baseline ventricular function and priority (elective/urgent) status, accounting for surgeon as random effect. However, due to the small number of patients in these groups stratification by ventricular function and priority was not performed but surgeons were included as

random effects. Missing data for outcomes were identified and excluded from the analysis.

All errors including errors to treatment and data entry errors were treated as measurement errors and therefore the locked database was analysed on an intention-to-treat basis. Hence if any error was found during the analysis no further new analyses were conducted on the locked database. Further analyses would be performed only if any supportive analyses could have led to qualitatively different results.

An assessment for futility was conducted. This was performed using the O'Brien Fleming alpha spending function plan, performed to examine the effect of primary outcome including futility; benefits are based upon Lan DeMets plan and harm based upon the power family spending function.

2.8 Investigational medicinal product

The investigational medicinal product (IMP) was perhexiline. Perhexiline and placebo tablets were manufactured by Sigma Pharmaceuticals (Baulkham Hills, Australia) according to the standards of good manufacturing practice and were provided with a certificate of analysis. They were ordered in batches (due to the limited shelf-life of 2 years) through UDG Ltd (South Normanton, UK), a pharmaceutical supply chain specialist who imported perhexiline and placebo on request, into the UK and delivered the IMP to Bilcare GCS (Crickhowell, UK). Bilcare GCS provided the packaging, labelling and Qualified Person (QP) release on the perhexiline/placebo samples.

The tablets were bottled into pre-defined bottle numbers (0-560) according to the randomisation sequence. Each bottle had 34 tablets. Bilcare GCS delivered the packaged

bottles to the UHB pharmacy. All tablets were identical (white/off-white and 8.5 mm in diameter) and were labelled 'PEXSIG'. UHB pharmacy then couriered the batches assigned to the other trial sites to their respective pharmacies.

2.9 Patient recruitment

Recruitment into the trial at UHB began in September 2009. Following concerns with the rate of recruitment the trial expanded and started recruitment at RSCH and UHCW in October 2011 and January 2012 respectively.

2.9.1 Patient screening

Patients were identified from the cardiac surgery waiting list. At the QEH, this included patients from 5 cardiac surgeons. Patients listed as awaiting AVR ± CABG, were screened using the above outlined inclusion and exclusion criteria. An electronic screening log was maintained to track patients on the cardiac surgery waiting list. Screening involved reviewing the indications for referral and assessing the indication for surgery. This included an assessment of the patients' past medical history and echocardiographic findings. At UHCW and RSCH patients were recruited from two additional cardiac surgeons at each centre. This patient population comprised of patients awaiting elective cardiac surgery. Patients suitable for participation were sent an invitation letter (Appendix 9.4) together with a Patient Information Sheet (PIS) (Appendix 9.5) by post, at least 2 weeks before their pre-operative assessment clinic.

At the QEH, patients awaiting urgent cardiac surgery for AVR ± CABG were screened as outlined above, having identified them on the urgent in-patient waiting list. This group included in-patients, who are admitted following an episode of unstable angina, acute

coronary syndrome or exacerbation of their symptoms; unsafe to be discharged and require in-patient definitive treatment. The number of patients awaiting urgent cardiac surgery at any given time is unpredictable. However inclusion of these patients allowed an opportunity to increase recruitment. Suitable in-patients were met on the ward, an invitation letter and PIS were provided and the study was discussed with them. A period of at least 24hrs was then given to the patient to reflect on this information before approaching them for written informed consent. Urgent in-patients were not screened in the other 2 centres due to logistical difficulties and the unpredictable prevalence of these patients.

2.9.2 Enrolment

All elective patients for cardiac surgery routinely attend the cardiac pre-operative assessment clinic approximately 2 weeks before their operation. Patients who were sent PIS regarding the study were approached at this clinic. The study was discussed with them and questions answered. If they were keen to participate written informed consent was obtained at this clinic (Appendix 9.6). Those who provide consent were then randomised to a treatment group.

2.9.3 Randomisation

Patient randomisation was performed on a Microsoft Access database designed by Dr Melanie J. Calvert and Prof Nick Freemantle. This was password protected and encrypted. The randomisation was allocated on a 1:1 ratio to perhexiline or placebo using a blinded minimisation procedure stratified for surgeon and need for concomitant CABG surgery.

The programme allocated a bottle number, which corresponded to the treatment group. If the patient needed a further supply of treatment at a later date, patients' details were entered a second time into the programme and it was able to provide a second bottle number in the same arm of treatment whilst maintaining allocation concealment. Dr Calvert maintained the allocation sequence and remained un-blinded and reviewed the randomisation database at regular intervals to confirm the program was performing accurately.

2.9.4 Dispensing and dosing

Once the randomiser allocated a bottle number, a prescription was written for each patient and this was taken to pharmacy for dispensing. Pharmacy remained blinded throughout the study. The dispensed bottle was handed over or posted to each participant with a patient information sheet explaining the dosing schedule (Appendix 9.7). The dosing schedule was explained in detail to participants during their pre-operative assessment clinic visit.

Each bottle would contain 34 tablets of the Investigational Medicinal Product (IMP). The IMP was administered orally. Participants were dosed on a short loading regime of 200mg bd (twice daily) for 3 days and maintained on a fixed maintenance regime of 100mg bd thereafter until their surgery. IMP therapy was administered for a minimum of 4 days before surgery. Therapy was usually commenced 1-2 weeks before their scheduled operation date.

If there was a delay in their initially scheduled operation date and more therapy was required, a second bottle was provided and participants would continue on their maintenance dose until surgery, unless features of side effects were observed.

Participants were allowed to self-administer the tablets as most patients were only admitted the night before the surgery. Patients were routinely followed up by telephone enquiry to assess compliance and side effects. For in-patients the IMP was prescribed and administered as outlined above.

2.10 Admission

Elective patients were routinely admitted the night before their surgery. Participants were met on admission and compliance with medication was checked. Patients were also asked if they experienced any side effects or if there was any deviation from the dosing regime. The IMP therapy for the night before and the morning of their surgery was prescribed. Thereafter the period leading up to and including anaesthetic induction, the operative procedure and the peri and post-operative management was in line with the HYPER trial protocol.

2.11 Trial protocols

The anaesthetic, surgical and post-operative management protocols were pre-determined and agreed amongst all the consultants in the department. The management of patients requiring inotropic support, the management of LCOE and the trial guidelines recommending inotropic support were standardised for all patients. An extensive description of these procedures and protocols are outlined in Appendix 9.8.

2.12 Trial measurements

All data for the trial was collected prospectively for each patient and recorded on a patient specific data collection sheet. Data collected included: -

- Baseline demographics including medications and risk factors
- Trial therapy duration, compliance and side effects
- Pre-operative echocardiographic measurement data
- Operative data
- Intra-operative and peri-operative haemodynamic studies as outlined below
- Incidence and volume of inotropic and vasoconstrictor support required
- Incidence and volume of fluid administration
- All post operative complications including arrhythmias
- Post operative blood results
- Deviations from trial protocol

All data was recorded prospectively onto a data collection sheet. This data was then entered into a specifically designed Microsoft Access Database and stored. In addition to the above, the following trial measurements were undertaken and recorded for each patient and included baseline blood samples, serial haemodynamic studies, serial blood samples, and TOE assessment as outline in Appendix 9.9.

2.12.1 Quality of life questionnaire

A quality of life questionnaire was introduced into the trial at the time of a substantial amendment to the REC when including another trial centre. This would allow measurement of quality of life markers with the IMP. Hence approximately half of the patients completed a quality of life questionnaire (EQ-5D) (Appendix 9.10) prior to starting trial therapy. This was then repeated at approximately 6-8 weeks post-operatively either in person at their follow up visit or by telephone interview.

The EQ-5D questionnaire was used to calculate a single utility at each time point (pre trial therapy and after surgery). Utilities were calculated using the following methodology.

A score is calculated by subtracting the relevant coefficients from 1 (co-efficients are outlined in Table 2-2). The constant term is used once if there is any dysfunction at all (a response of 2 or 3 on the questionnaire). The N3 term is used once if any dimension has a response of 3.

For example, if you scored 1, 1, 3, 2, 2, then your QoL would be:

$$QoL = 1 - 0.081 - 0 - 0 - 0.094 - 0.123 - 0.071 - 0.269 = 0.362$$

Dimension/Level	**Coefficient**
Constant	0.081
Mobility	
- Level 2	0.069
- Level 3	0.314
Self-care	
- Level 2	0.104
- Level 3	0.214
Usual activity:	
- Level 2	0.036
- Level 3	0.094
Pain/discomfort:	
- Level 2	0.123
- Level 3	0.386
Anxiety/depression:	
- Level 2	0.071
- Level 3	0.236
N3	0.269

Table 2-2 Co-efficients used to calculate EQ-5D utilities

2.12.2 Perhexiline and hydroxyl-perhexiline analysis

The baseline arterial samples collected in the plain vacuette bottles, that were centrifuged and stored at -80°C for the analysis of serum perhexiline concentration were sent in one batch to the Cardiff and Valve University Health Board, Analytical Toxicology Department for the analysis of serum perhexiline concentrations.

Cardiff toxicology laboratories obtained the perhexiline and hydroxy-perhexiline concentrations through gas chromatographic analysis using a Varian 3800 Gas Chromatograph fitted with a CP Sil-8CB column (30Mx0.25mm) and the detection of the compounds was achieved with a Varian 4000 ion-trap mass spectrometer. Analysis was done in batches of approximately 30 samples per batch.

2.12.3 Troponin analysis

Baseline troponin and serial troponin measurement were performed. The Z Serum Sep Clot Activator bottles collected were sent to the Department of Clinical Biochemistry, Queen Elizabeth Hospital, Birmingham. Troponin analysis was performed using the Elecsys Troponin-T assay (Roche Diagnostics, Burgess Hill, UK).

In March 2011, troponin analysis at the Queen Elizabeth Hospital, Birmingham changed to using the High Sensitivity Troponin T analysis using the Cobas immunoassay and Elecsys analyzers (Roche Diagnostics, Burgess Hill, UK).

2.13 Haemodynamic management procedures

2.13.1 Early inotropic support

Inotrope administration was withheld until post CPB haemodynamic measurements were complete. However, sometimes this was not possible due to haemodynamic instability and clinical circumstances based on the subjective features (observational assessment by the operating surgeon) of cardiac performance. If inotropes were required before discontinuation of CPB or soon after separation from CPB, dopamine was used as a first line agent. At times (rarely) based on surgical assessment of contractility, adrenaline was used for inotropic support. The decision to insert an IABP electively before separation from CPB or immediately after was at the discretion of the clinical team. These events were adjudicated at the blinded end points review committee meetings.

2.13.2 Management of a LCOE and institution of inotropic support

A low cardiac output episode (LCOE) was defined as hypotension (MAP <60) with a cardiac index < 2.2 L/min/m^2, in the presence of adequate filling pressures (CVP 8-12 mmHg, PCWP 12–16 mmHg) and heart rate of 70-110bpm; refractory to appropriate intra-vascular volume expansion and following correction or attempted correction of any dysrhythmias.

If these criteria for LCOE were met inotropic support was initiated to improve cardiac output. Therefore once a LCOE is reached inotropic support is required to improve the haemodynamic status of the patient. The appropriate arrival at this end point was adjudicated at a blinded end points review committee meeting, which took place at pre-defined recruitment intervals.

Dopamine (200mg in 50ml 5% dextrose) was used as a 1st line inotropic agent and instituted at a dose of 5-10 μg/kg/min. Further inotropic escalations were at the discretion of the clinical team but usually involved the addition of Adrenaline (2mg in 50ml 5% dextrose) and/or Enoximone.

2.14 Adverse events reporting and trial safety

Adverse events that occurred to participants were reported using pre-determined standard operating procedures for reporting such events (Appendix 9.11). A serious adverse event form was completed for each event and the sponsor was informed through the sponsor's online incident reporting system. The necessary authorities such as the MHRA and the ethics committee were also informed.

Initially the CI/PI was informed of any adverse events (AE), serious adverse events (SAE), adverse drug reactions (ADR) or suspected unexpected serious adverse reaction (SUSAR). Following this the sponsor was informed of the event. The MHRA were informed if required through the MHRA's online eSUSAR reporting system as per their reporting timeframes. The ethics committee and the MHRA were provided with annual safety reports. In the event that code breaking was required, the code breaking SOP would be followed. To avoid reporting of adverse events for known complications associated with cardiac surgery, a separate SOP was written that listed events that did not require reporting (Appendix 9.11).

2.15 Sponsor's internal audit and MHRA inspection

The sponsor for this clinical trial was University Hospitals Birmingham NHS Foundation Trust. The HYPER trial was randomly selected as one of the IMP clinical trials to be

audited internally. This audit evaluated the overall running of the trial and compliance with the current GCP guidelines for the conduct of a clinical trial (ICH 1996; ICH 1997). This audit concluded that the trial was running in accordance with the GCP guidelines, however recommended improving the SOPs and making it more robust for interpretation by anyone other than the research fellow running the trial.

Following this audit the regulatory authority, MHRA carried out an inspection at UHB and selected the HYPER trial as one of the studies they would like to inspect. This again included a thorough assessment and inspection into the running of the trial, including interviews with the Principle investigator and the trial research fellow. Furthermore the MHRA reviewed the practice of SOPs, which included recruitment, randomisation, handing and procurement of the IMP, data collection, and data analysis. The MHRA also assessed the maintenance of the trial master file and reviewed the paper trail associated to each participant. In addition to this, the MHRA conducted a thorough assessment of randomisation. This included a detailed inspection into the practices of IMP handling, dispensing and later destruction of the IMP by the pharmacy at UHB.

The MHRA concluded that the HYPER trial could continue in accordance with the GCP guidelines without restriction or further review, but raised concerns with UHB pharmacy and the handling of IMP. Therefore, a review of the SOPs for handling of IMP by pharmacy was reviewed and updated. Furthermore, the sponsor and myself as the trial research fellow conducted internal audits on pharmacy's documentation, dispensing and handling of IMP following the MHRA inspection.

2.16 Trial expansion

The rate of recruitment was always a concern (outlined in the results chapter) and due to under-recruitment avenues to improve recruitment were considered. One of the main reasons for declining participation in the trial was the requirement to take an IMP. Measures to improve recruitment at UHB, where the trial started were initially made and included the following: -

- Optimise the screening log to ensure that all patients listed for AVR ± CABG were being screened and not missed
- Adequate provision of information by way of an invitation and PIS was sent to all suitable patients well in advance of the PAC appointment
- All suitable patients were met at the PAC and the study discussed in detail including answering any questions regarding the trial
- Patients that needed more time to consider their decision, were given a contact telephone number in the instance they needed to discuss the trial at a later date
- All patients that were uncertain during their PAC consultation were followed up by telephone
- The time required to wait for the dispensing of their IMP was reduced to a minimum and if the patient was unable to wait, this was delivered to them

However, despite maximum measures to improve recruitment at UHB, the recruitment targets set internally were not being met to complete the trial within 3 years. Therefore in an attempt to increase recruitment the trial was expanded to 2 other centres; initially Royal Sussex County Hospital, Brighton (RSCH) and then to University Hospital Coventry and Warwickshire, Coventry (UHCW).

2.16.1 Initial setup

An introductory presentation was made to the cardiothoracic department of each centre. This included the background and the aims of the trial, scope of the trial and intention to collaborate with the department. The trial protocol was reviewed by the surgical, anaesthetic and perfusion departments and disseminated to the nursing department at each centre. The protocol was vetted, queries clarified and rectified and eventually approved once a collective decision was made to collaborate and allow expansion of the HYPER trial to the respective centres. A local PI was appointed and local and national approvals were sought.

2.16.2 Additional approvals

The sponsor was informed of the intention to expand the trial and their approval was sought. Following this, local R&D approval from each trust was obtained. This included a review of the protocol, ethics, standard operating procedures, funding and systems for recruitment and running of the trial. SOPs were tailored to suit each trust, as were the PIS and consent forms. The REC was informed and a substantial amendment to the original ethics was obtained. The MHRA was informed of the trial expansion and approval sought. These approvals were obtained for each trust on separate occasions due to the staggered timing of the expansion.

2.16.3 Local education

Each department involved in the trial were familiarised with the HYPER trial. The trial procedures pertaining to each department were discussed as per the trial protocol and relevant queries clarified.

2.16.4 Local running of the trial

Each centre excluding UHB had a clinical research nurse assigned to the trial. Their role was to screen patients, send out PIS and coordinate with the clinical research fellow regarding trial recruitment. Initial patient screening was performed by the clinical research nurse. This was then checked periodically by myself as the clinical research fellow before invitations and PIS were sent out. Consenting, randomisation and IMP dispensing was conducted on a pre-arranged date. The remainder of the trial was conducted as described above allowing for the exceptions outlined below (section 2.16.5).

2.16.5 Deviations to the protocol

Due to differences in surgical practice and postoperative management between surgeons at RSCH and UHCW, recruitment of patients was restricted to 2 surgeons at each centre. This was accounted for during randomisation and the randomisation programme was set up similar to that used at UHB. Due to logistical reasons of being unable to consent and start IMP therapy on an ad-hoc basis for urgent patients, only elective surgical candidates were approached at the other centres.

Although blood was collected and prepared in the same manner as described above, there was no facility for liquid nitrogen. Therefore biopsies were not taken from these patients due to the inability to snap freeze these samples prior to freezing.

In addition to screening and recruitment the research nurse was involved in the data collection and acquisition of blood samples from beyond the 1st 6 hrs from XC removal.

Serum samples were stored locally in a -80°C freezer and couriered to UHB at the end of the trial.

2.17 Data safety monitoring and futility assessment

An independent data safety monitoring board (DSMB) was appointed and a DSMB meeting was held on the 22nd June 2012 once a predetermined target of 99 patients (45% recruitment) had been recruited and discharged (allowing for all outcome measures to be collected) from the hospital. The DSMB was requested to assess the trial for safety and efficacy and also assess the trial for futility based on the results of the CASPER trial that had concluded earlier. The CASPER trial is discussed in more detail in the following discussion chapters.

The meeting concluded that recruitment has proved to be difficult yet they had no further suggestions to improve recruitment and the recruitment objective would unlikely be achieved in less than 20 months. With regard to the assessment of futility using the O'Brien Fleming alpha Spending approach the board concluded that the trial was unlikely to achieve its scientific objective and was content for the trial to be halted and recommended the trials steering committee to halt recruitment to the trial (Appendix 9.12).

3 RESULTS OF THE CLINICAL TRIAL

3.1 Patient recruitment

Recruitment into the trial started in September 2009 and was initially from a single centre, the University Hospitals Birmingham NHS FT (UHB); patients were recruited from 5 surgeons at this centre. The first patient was operated on, in October 2009. Subsequently the mean rate of recruitment at this centre was 3 cases per month (Figure 3-1). This fell severely below the initial predicted rate of recruitment, to complete the trial well within three years (figure 3-2)

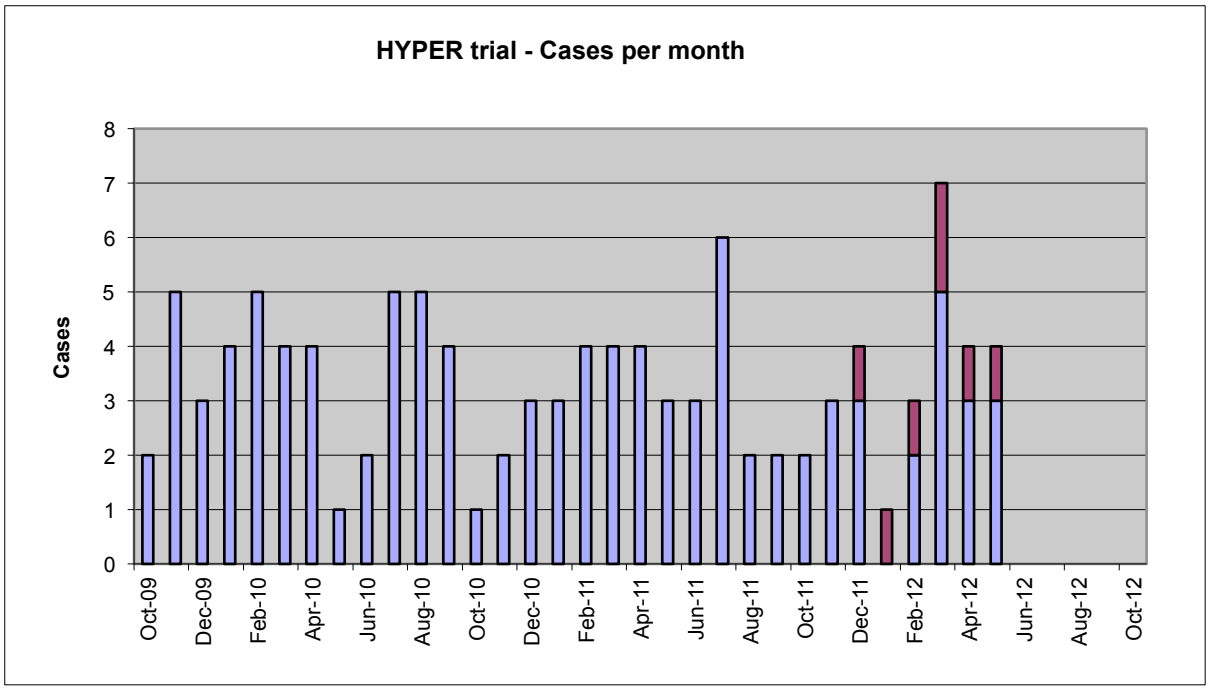


Figure 3-1 Number of cases per month - HYPER trial

This led to attempts to increase recruitment by expanding to other centres. Recruitment at the second centre, Royal Sussex County hospital, Brighton started in October 2011 and the first patient at this 2nd centre was operated on, in December 2011. Despite this additional centre, rate of recruitment was still poor so the trial was expanded further to

a 3rd centre, the University Hospital Coventry and Warwickshire, Coventry. Recruitment in Coventry started in January 2012 and the first patient was operated on, in March 2012.

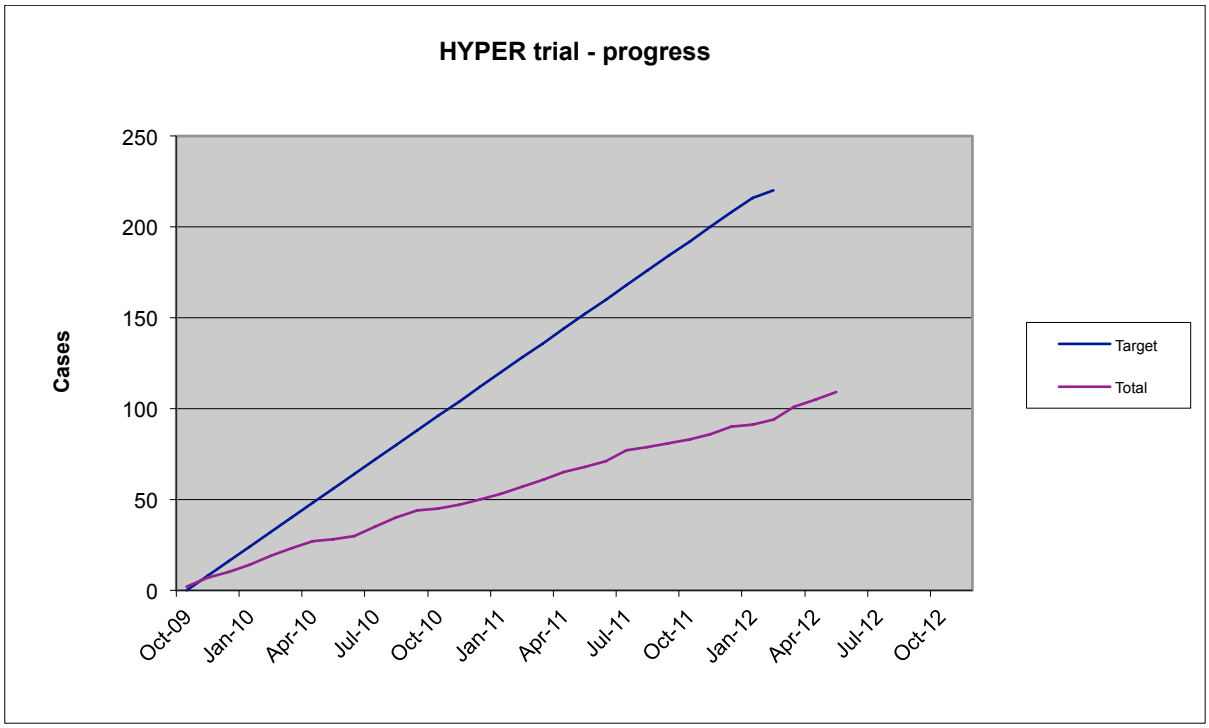


Figure 3-2 Rate of recruitment compared to predicted rate of recruitment

Each additional centre contributed an extra 4 patients each over the duration of trial recruitment at those centres. The highest number of cases in a month was in March 2012 when there were 2 additional cases from centres outside UHB. Prior to the trial being halted in June 2012, 127 patients had been randomised and 112 patients had been operated on. The CONSORT flow diagram (Schulz, Altman et al. 2010) describes the passage of the trial participants.

3.2 Consort flow diagram

The consort flow diagram (Figure 3-3) outlines the number of patients screened for eligibility, the reasons for their exclusion and pathway of patients post randomisation as based on the locked database.

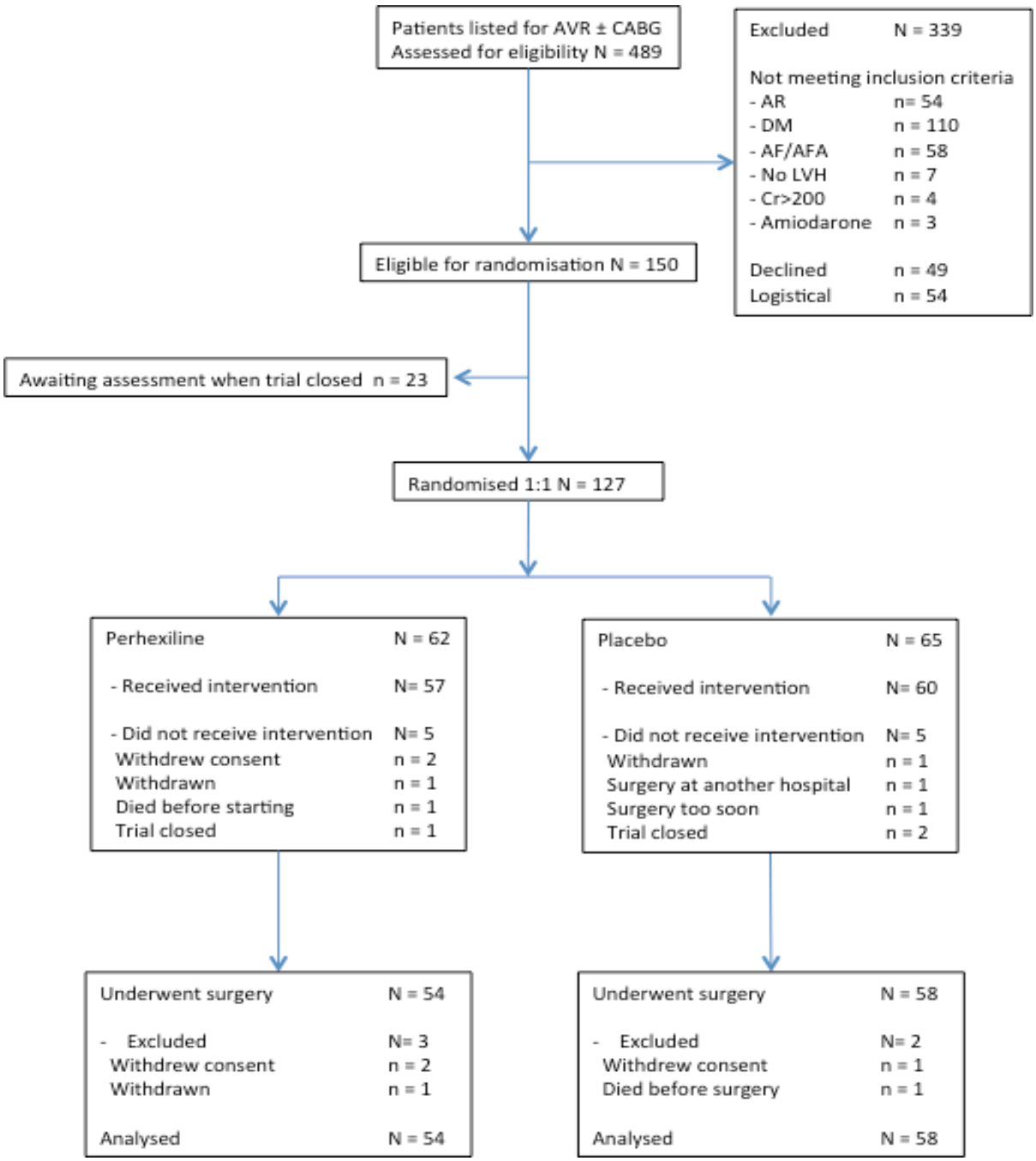


Figure 3-3 CONSORT flow diagram of the HYPER trial

During the period of trial recruitment (Sept 2009 – June 2012), 489 patients were screened for suitability to enter the trial. Of these, 339 were excluded, majority being patients with diabetes (n=110) who otherwise met the exclusion criteria. In addition to the diabetic patients, patients with atrial fibrillation (AF) or those planned to have atrial ablation therapy (AFA) (n=58), and those patients with moderate to severe aortic regurgitation (n=54) met the exclusion criteria. A smaller number of patients n=7, had echocardiographic confirmation that there was no LVH and a similar number had renal dysfunction and were on amiodarone therapy. A total of 49 patients declined to participate in the trial. Another large portion of patients (n=54) fell into a group where it was logistically difficult to recruit them into the trial outlined in (Table 3-1).

Logistical reason for exclusion	**Number**
Operation too soon for intervention	33
Other concomitant procedure	5
Operation in another hospital	3
Unable to contact patient	7
Patient unable to travel for recruitment	3
Other medical reason	3

Table 3-1 Logistical reasons for trial exclusion

Patients required a minimum period of 4 days of trial therapy and this included the loading regime of 3 days. Although efforts were made to identify all suitable patients, an important number (n=33), did not have enough time before surgery to take the trial therapy. Concomitant procedures (initially unplanned) included aortic root surgery or other valve surgery i.e. mitral or tricuspid valve surgery. Other medical reasons included; patients too high risk or had co-existing medical conditions that precluded them from entering the trial i.e. metal health illness.

At the time of trial closure, 23 patients had been identified who were suitable for enrolment and had been sent the patient information leaflets. If the trial continued these patients would have been seen in the pre-assessment clinic to discuss the trial further.

In the locked final database, a total of 62 patients were randomised to the perhexiline arm and 65 to the placebo arm. Of these 57 and 60 patients received and self-administered the trial therapy in the perhexiline and control arm respectively. In the perhexiline arm, 1 patient died whilst awaiting surgery and wasn't started on trial therapy. In the placebo arm, 1 patient had their operation in another hospital and one was operated on too soon, hence trial therapy was not started. One patient in the perhexiline arm and 2 in the placebo arm were randomised but didn't receive trial therapy prior to trial closure. Therefore of 57 patients who received the trial therapy in the perhexiline arm, 54 underwent an operation. Similarly of 60 patients who received trial therapy in the placebo arm, 58 underwent an operation.

3.2.1 Withdrawals

Five patients withdrew consent; four from the perhexiline arm and 1 from the placebo arm. Of these patients, 3 had started trial therapy before they withdrew consent; 2 in the perhexiline arm and 1 in the placebo arm and hence although these patients were followed up as per the trial protocol due to being exposed to the trial therapy, they have been excluded from all analysis.

A total of 3 patients were withdrawn from the trial. Of these, 2 were randomised, didn't receive the intervention but met the exclusion criteria; one had atrial fibrillation and the other post randomisation was scheduled for a Trans-catheter Aortic Valve Implantation

(TAVI), a procedure that doesn't arrest the heart. The third patient was withdrawn due to pre-existing renal impairment (creatinine > 200), but had inadvertently started the trial therapy; although followed up due to exposure to the trial therapy this patient was not included in the analysis.

3.2.2 Patients excluded from the analysis

In the perhexiline arm, 3 patients were excluded from the final analysis due to withdrawal of consent and being withdrawn from the trial as outlined above. In addition to this, 2 patients in the perhexiline arm did not have pulmonary artery flotation catheter (PAFC) inserted at the time of the operation during the majority of the initial 6 hours post cross clamp removal (required to make haemodynamic assessment of cardiac output and index). Therefore a blinded-end points committee decided to exclude these 2 patients from the primary end-point, as there was insufficient evidence to reach a decision on the primary end-point.

In the placebo arm, 2 patients were excluded; one for withdrawal of consent and the other patient had the operation postponed due to ill health and later died of disseminated malignancy before the operation could be re-scheduled.

3.3 Demographics

3.3.1 Pre-operative demographics

Patient demographics are outlined in Table 3-2 and outlines the pre-operative demographics between the groups.

Variable (IQR)/(%)	Placebo (n=58)	Perhexiline (n=54)
Age	72 (66 – 76)	73 (63 – 78)
BSA	1.88 (1.75 – 2.07)	1.7 (1.86 – 1.97)
Male: Female	38:20 (65.5:34.5)	37:17 (68.5:31.5)
Caucasian	56 (96.6)	52 (96.3)
Status		
- Elective	54 (93.1)	53 (98.1)
- Urgent	4 (6.9%)	1 (1.9)
EuroSCORE	6 (4 – 7)	6 (4 – 7)
Logistic EuroSCORE	4.5 (2.4 – 7.25)	3.75 (2.6 – 7.24)
CCS		
- 1	24 (58.6)	38 (70.4)
- 2	20 (34.5)	12 (22.2)
- 3	3 (5.2)	4 (7.4)
NYHA		
- 1	14 (24.1)	12 (22.2)
- 2	26 (44.8)	29 (53.7)
- 3	16 (27.6)	11 (20.4)
- 4	1 (1.7)	2 (3.7)
Previous MI	4 (6.9)	3 (5.6)
Risk factors		
- Hypercholesterolemia	10 (17.2)	18 (33.3)
- Hypertension	23 (39.7)	22 (40.7)
- FHx IHD	6 (10.3)	2 (2.7)
- Previous TIA	4 (6.9)	2 (3.7)
- Previous CVA	1 (1.7)	3 (5.6)

- PVD	1 (1.7)	0 (0)
- CEA	0 (0)	0 (0)
- Smoking		
- Never smoked	13 (22.4)	11 (20.4)
- Ex-smoker	22 (37.9)	18 (33.3)
- Current smoker	3 (5.2)	4 (7.4)
- Asthma	4 (6.9)	4 (7.4)
- COPD	3 (3.4)	6 (11.1)
Medication		
- Aspirin	30 (51.1)	31 (57.4)
- Clopidogrel	5 (8.6)	2 (3.7)
- ACE	13 (22.4)	12 (22.2)
- A2	8 (13.8)	5 (9.3)
- Statin	32 (55.2)	37 (68.5)
- β blocker	15 (25.9)	16 (29.6)
- Ca channel blocker	15 (25.9)	13 (24.1)
- Nitrate	3 (5.2)	3 (5.6)
- Nicorandil	1 (1.7)	2 (3.7)
IABP pre surgery	0 (0)	0 (0)
Pre-op Haemoglobin	13.4 (12.5 – 145)	14.1 (12.8 – 14.8)
Pre-op Creatinine	83 (70.5 – 104.5)	87.5 (75.3 -101.8)

Table 3-2 Pre-operative demographics

3.3.2 Echocardiographic demographics

The pre IMP therapy echocardiographic findings including left ventricular wall dimensions and valve haemodynamics are outlined in Table 3-3. There was no significant difference in the ventricular dimensions or valve haemodynamics between the groups.

Variable (IQR/%)	Placebo	Perhexiline	P value
Ventricular Function			0.42
- Good	54 (93.1)	47(87.0)	
- Moderate	4 (6.9)	6 (11.1)	
- Poor	0 (0)	1 (1.9)	
LVEDV	95.7 (73.4 – 113.5)	98.5 (69.9 – 132.8)	1.0
LVID	4.35 (3.83 – 4.72)	4.6 (4.1 – 4.8)	0.35
IVSd	1.4 (1.2 – 1.6)	1.4 (1.3 – 1.6)	0.99
PWd	1.18 (1.0 – 1.4)	1.3 (1.1 – 1.5)	0.20
Valve V_{max}	4.23 (3.9 – 4.8)	4.4 (3.7 – 5.0)	0.68
LVOT V_{max}	0.9 (0.7 – 1.2)	0.9 (0.9 – 1.2)	0.45
Valve area (m^2)	0.79 (0.6 – 0.9)	0.7 (0.6 – 0.9)	0.61
Peak gradient	76 (63.8 – 94.0)	77 (62.0 – 94.5)	0.77
Mean gradient	43.5 (35.8 – 59.5)	48.5 (34.5 – 56.5)	0.98
Aortic regurgitation			
- Mild	8 (13.8)	8 (19.8)	
- Moderate	3 (5.2)	6 (11.1)	

Table 3-3 Echocardiographic variables

3.3.3 Operative demographics

The operative variables between the groups are outlined in Table 3-4. There was no statistical significance between the groups in each of the variable. In each group, 44% underwent concomitant CABG and the majority of patients had a biological valve implanted. There were 4 IABP inserted and of these, 3 patients were in the perhexiline arm. All balloon pumps were inserted to aid weaning off CPB; either poor haemodynamic performance with low cardiac output or right ventricular dysfunction and/or dilatation on weaning from CPB.

In some patients who underwent CABG, a side-biting aortic occlusion technique was used for the proximal anastomosis of the vein grafts. This was at the discretion of the operating surgeon and was equal in proportion in each group. The additional procedures were unplanned at the time of listing for surgery and hence decided intra-operatively based on intra-operative TOE assessment. One patient in the perhexiline arm underwent unplanned grafting of the left coronary system due to untoward complications intra-operatively.

Variable (IQR/%)	Placebo	Perhexiline	P Value
Procedure			1.0
- AVR	32 (55.2)	30 (55.6)	
- AVR + CABG	26 (44.8)	24 (44.4)	
Additional procedure			
- MV repair	2 (3.4)	1 (1.9)	
- TV repair	1 (3.4)		
- MV replacement		1 (1.9)	
- Unintended CABG		1 (1.9)	
Valve type			1.0
- Biological	50 (86.2)	47 (87)	
- Mechanical	8 (13.8)	7 (13)	
Valve size	23 (21 – 24)	22.5 (21 – 23)	
Technique			0.68
- Side-biting cross clamp	18 (31)	14 (26)	
- Single clamp	40 (69)	40 (74.1)	
Cumulative CPB time	129 (103 – 165)	135 (106 – 192)	0.48
Cumulative cross clamp time	95 (65 – 116)	89 (71 – 120)	0.92
Hot shot administered	28 (48.3)	31 (37.4)	0.35
Tranaexamic acid used	21 (36)	22 (40.7)	0.70
Aprotinin used	9 (15.5)	8 (14.8)	1.0
IABP	1 (1.7)	3 (5.6)	0.35

Table 3-4 Operative variables

3.4 Duration of trial therapy

All patients were required to have a minimum duration of trial therapy of 4 days, which consisted of a loading regime in the initial 3 days. Each bottle of trial therapy consisted of 34 IMP tablets and would last a patient 14 days, which included the loading regime. If the surgery was postponed a second bottle was issued and this would last 17 days on the maintenance regime. A second bottle was issued to 43 patients and this was usually due to the surgery being postponed hence the requirement to continue trial therapy. If the surgery was postponed to a date beyond the duration of therapy possible by one bottle (over 2 weeks) then the trial therapy was stopped and then re-started nearer the new re-scheduled date following the initial loading and maintenance regimes.

The median duration of trial therapy was 8.5 days (IQR 5 – 17.5) for all trial participants in the final analysis. In the perhexiline arm median duration was 8 days (IQR 5 – 11) and in the placebo arm median duration was 8 days (IQR 6 – 14), p= 0.41 and did not meet a normal distribution on a Kolmogorov-Smirnov test of normality ($p<0.005$) The distribution and frequency of trial therapy for each arm is outlined in figures 3-4 and 3-5.

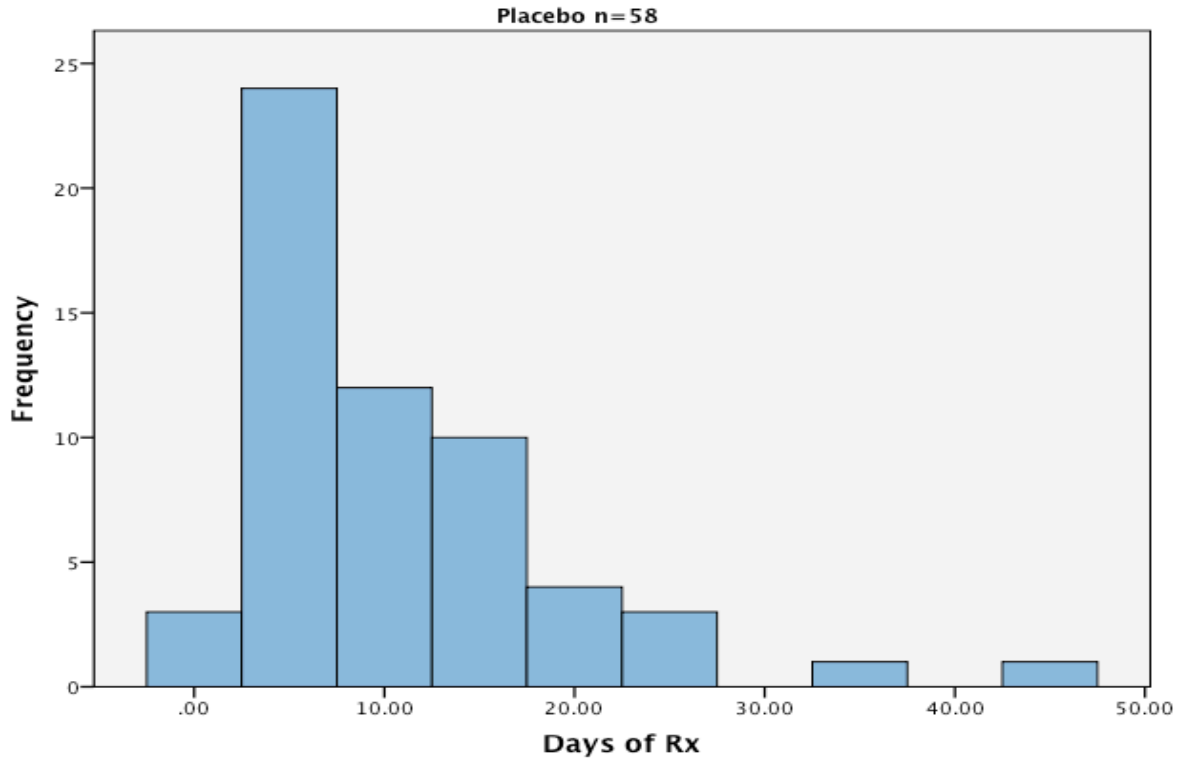


Figure 3-4 Distribution and frequency of placebo therapy

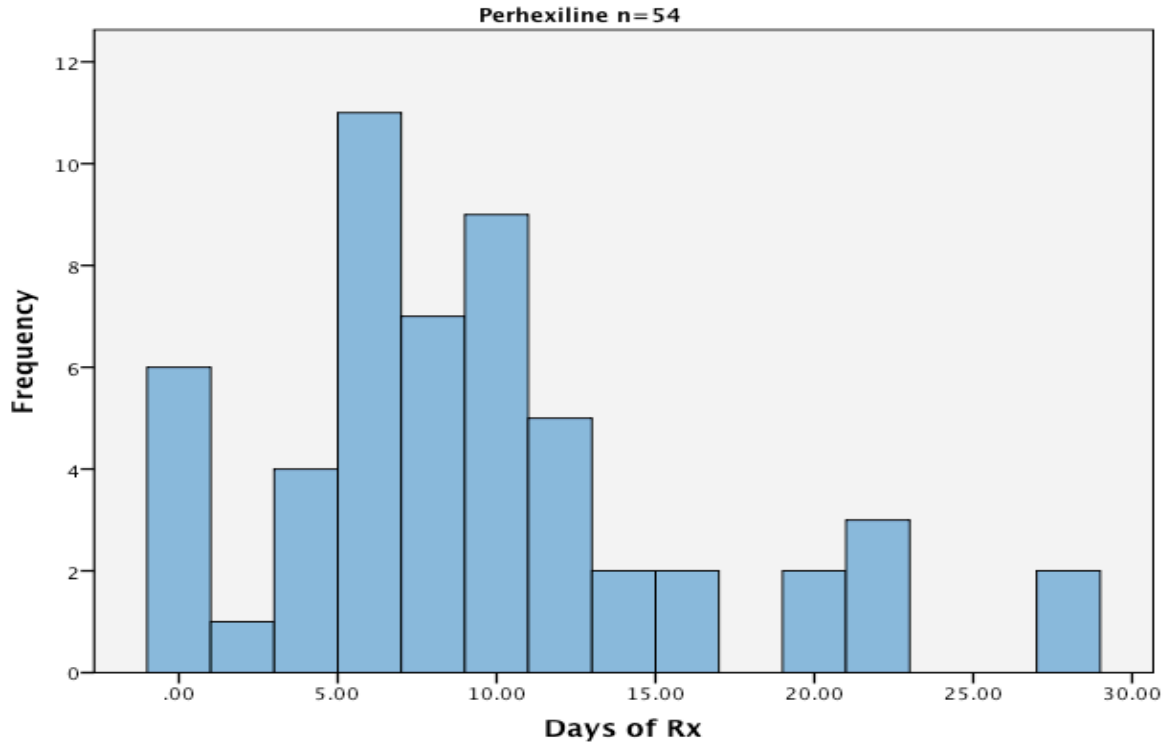


Figure 3-5 Distribution and frequency of perhexiline therapy

Of the 112 patients that received trial therapy, ten patients were not on trial therapy at the time of their operation; four ran out of tablets before their operation and six stopped therapy due to side effects; of the six, five were in the treatment group. Of the four that ran out of tables, three were on placebo and one on perhexiline.

3.5 Serum perhexiline levels

Serum for perhexiline concentration analysis was available in 106 patients, 51/54 (94.4%) in the treatment group and 55/58 (94.8%) in the placebo group. These were all analysed for perhexiline and hydroxy-perhexiline concentrations. All but one patient in the placebo arm had a serum perhexiline concentration of zero mg/L. This patient had perhexiline and hydroxyl-perhexiline concentrations of 0.09mg/L and 0.74mg/L respectively. This patient had been wrongly allocated into the placebo arm as a data entry error prior to locking the database; was analysed within the placebo arm on an intension-to-treat basis without any further analyses thereafter and therefore treated as a unbiased measurement error. Had the results been potentially sensitive to the misallocation of this subject a supportive analysis would have been conducted, but this was judged not to be the case.

In the perhexiline group the median perhexiline concentration was 0.22mg/L (IQR 0.09 – 0.43), ranging from 0 – 1.2mg/L. Twenty patients (39.2%) in the perhexiline arm had a perhexiline level below the therapeutic range (0.15 – 0.6mg/L). Of these, 2 patients had no detectable perhexiline concentration in the serum; one stopped therapy due to side effects of diarrhoea after one day of therapy and the other ran out of tablets 1 month before the operation. Hence 24 patients (47%) were within the therapeutic

range (0.16 – 0.6mg/L) with 7 of these patients being above the therapeutic range (Figure 3-6).

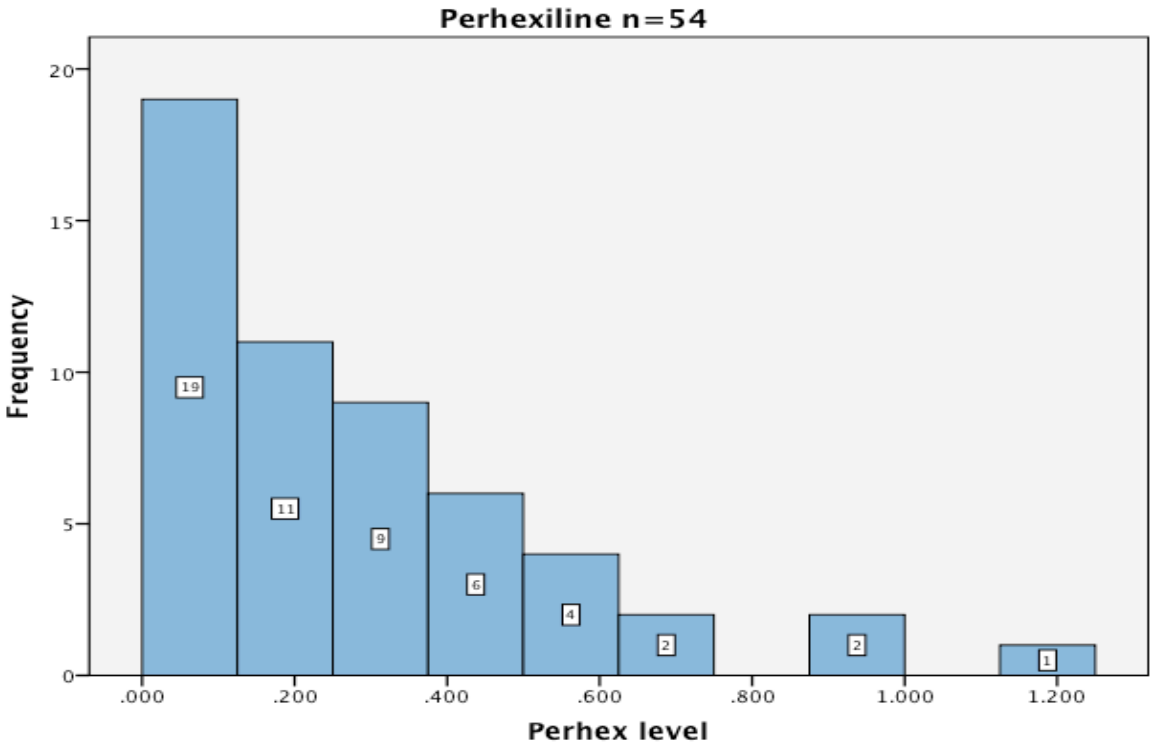


Figure 3-6 Distribution of perhexiline concentrations

The median hydroxy-perhexiline concentration was 1.02 mg/L (IQR 0.72 – 1.22), ranging 0 – 1.67mg/L. Of the 20 patients below the lower therapeutic range, 17 patients had a hydroxy-perhexiline to perhexiline ratio of > 6mg/L and the other had a ratio of 5.92mg/L. These 18 patients fell within the extensive metabolisers group.

There was one ultra-rapid metaboliser in whole cohort and four patients had hydroxy-perhexiline:perhexiline ratio of ≤ 0.3 and fell within the poor metaboliser group. There was 10 and 34 intermediate and extensive metaboliser respectively. Both perhexiline and hydroxyl-perhexiline concentration ranges did not show a normal distribution pattern on a Kolmogorov-Smirnov test of normality, p=0.01 and p=0.02 respectively.

All patients appear to be compliant with trial therapy, yet it is difficult to comment on the level of compliance. Although all patients in the perhexiline arm had detectable levels of perhexiline and hydroxyl-perhexiline, compliance with the strict dosing regime is not measurable. Looking at those who were sub-therapeutic (n=20), median duration of trial therapy in this group was 7 days (IQR 4.25 – 7.25); there was no significant correlation between the length of therapy, and perhexiline concentration using Spearman's rank correlation with a co-efficient of 0.42 (p = 0.63). Similarly analysis of the whole perhexiline arm for correlation between perhexiline concentration and duration of therapy, showed a Spearman's rank correlation co-efficient of 0.15 (p=0.23).

3.6 Side effects to trial therapy

Of 127 patients that receive trial therapy, 11 patients reported side effects outlined in Table 3-5. All but one patient with side effects were in the perhexiline arm and side effects consisted of dizziness with a combination of nausea. Other side effects reported included diarrhoea and itching. The patient in the placebo arm reported a tingling sensation down the arm.

Side effects reported	Perhexiline	Placebo
Dizziness and nausea	3	
Dizziness alone	4	
Diarrhoea	2	
Rash and itching	1	
Tingling		1

Table 3-5 Side-effects after starting trial therapy

One patient that had dizziness and nausea halved the maintenance regime to 1 tablet daily with resolution of side effects. Two patients with dizziness, one with diarrhoea

and one with itching had side effects associated to the loading regime, which settled by day 4. One patient with nausea and dizziness had no benefit with a halved regime and stopped trial therapy. Another 5 patients did not want to trial a reduced regime and stopped trial therapy after noticing the side effects.

At the time of surgery, of the 6 patients who stopped trial therapy and were in the perhexiline arm, one patient didn't have serum for perhexiline concentration analysis and one was sub-therapeutic (serum concentration of perhexiline < 0.16mg/L); all others were within the therapeutic range.

3.7 Primary outcome

The primary end-point was defined as the incidence of inotrope use ± comparison of cardiac index to baseline to show an increase > 0.3L/min/m^2 within the first 6 hours from reperfusion. This was adjudicated by a blinded end-point committee based on the haemodynamic studies conducted in the 1st 6 hours from removal of the aortic cross clamp. These haemodynamic studies included data on the heart rate and rhythm (paced or native), cardiac output, cardiac index, mean systemic and pulmonary artery pressures, filling pressure (central venous and pulmonary artery wedge pressure), and temperature. This information was evaluated in conjunction with metabolic status including lactate and base deficit.

This committee judged if an inotrope was started appropriately based on pre-defined criteria (outlined in the methodology). The presence of a low cardiac output episode inevitably results in an inotrope being initiated to improve haemodynamic performance.

Of 112 patients that underwent surgery, 110 patients had a PAFC in place to obtain the haemodynamic measurement needed to adjudicate on the primary end point. Therefore 2 patients who were both in perhexiline arm were excluded from this analysis.

Of 110 patients, the blinded end-points committee judged that 30 patients in total had inotropes started appropriately and hence had achieved a low cardiac episode, to have the inotropes started. Of the 30, 16 were in the perhexiline arm and 14 in the placebo arm. This primary end-point showed no statistical significance in the incidence of appropriate inotrope usage OR 1.65 (CI 0.67 – 4.06) p=0.28.

3.8 Haemodynamic assessments

Haemodynamic assessments were measured at baseline (prior to institution of CPB), prior to administration of protamine, approximately 10 minutes after protamine administration and then at 2 hourly intervals for 6 hours after aortic cross clamp release (2, 4, 6 hours) and finally at 9 and 12 hours after aortic cross clamp release.

3.8.1 Heart rate and filling pressure

The heart rate pre CPB was based on the native heart rate of the patient. To attain an ideal constant heart rate prior to CPB discontinuation and during the early reperfusion stage, patients are paced either atrially or have dual chamber pacing. There was no significant difference in the mean heart rate between the groups at any time point (Figure 3-7).

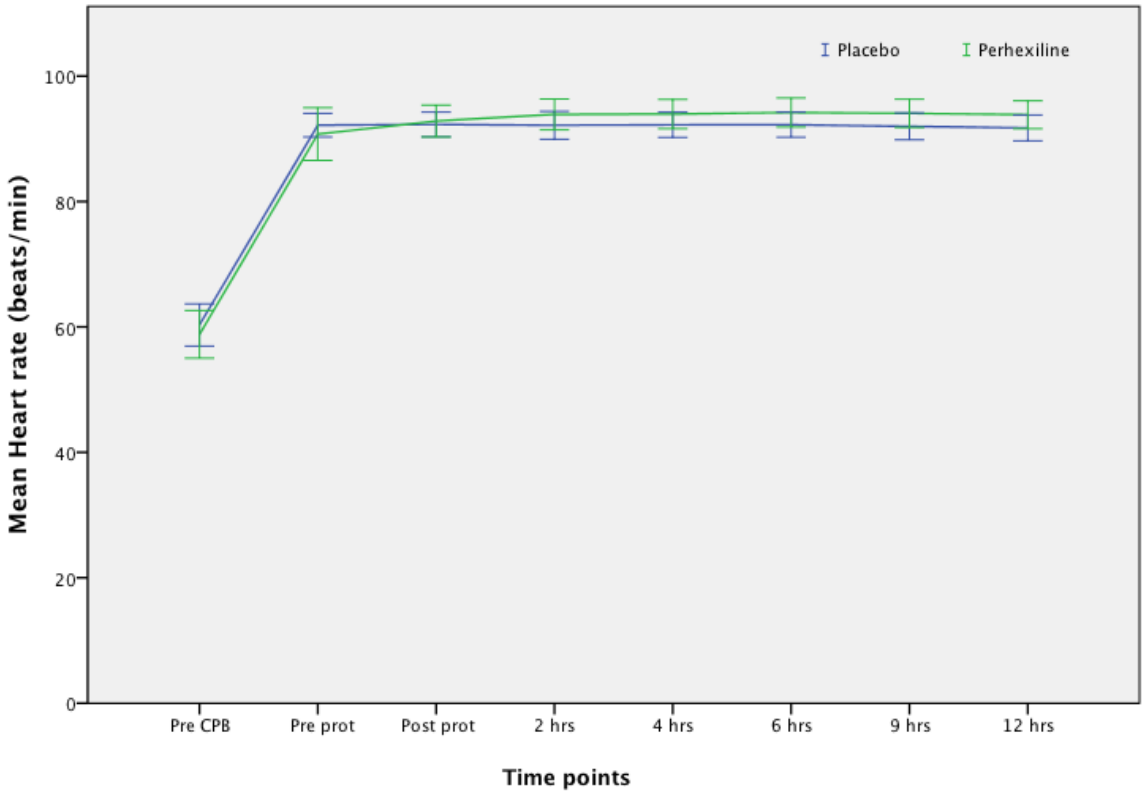


Figure 3-7 Mean heart rate between groups at each time point

Right and left heart filling pressures are assessed by the measurement of CVP and PAWP respectively and are maintained at an optimum, on an individual patient basis to achieve the optimum overall hemodynamic status.

The CVP showed no significant difference at each of the time points between groups, except at post protamine (p=0.043). Beyond 2 hours of reperfusion the filling pressures were consistently higher in the perhexiline arm (Figure 3-8). There was no significant difference in PAWP at the time point of 'post protamine' and PAWP does not follow the same trend (Figure 3-9).

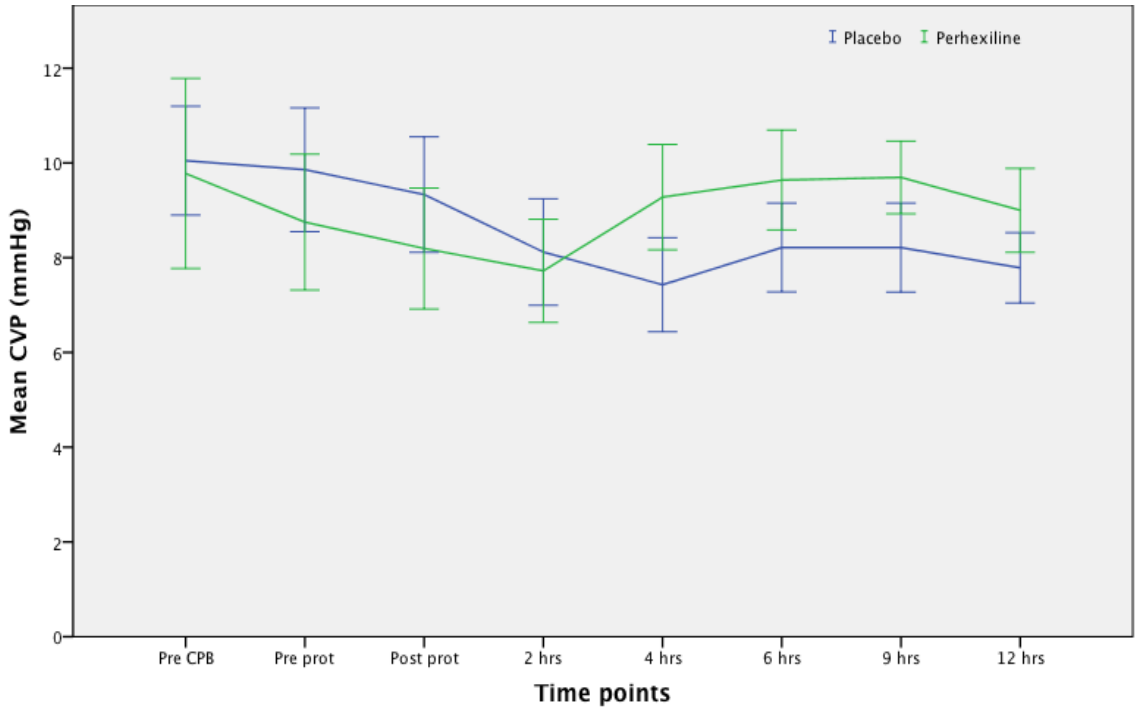


Figure 3-8 Mean CVP between groups at each time point

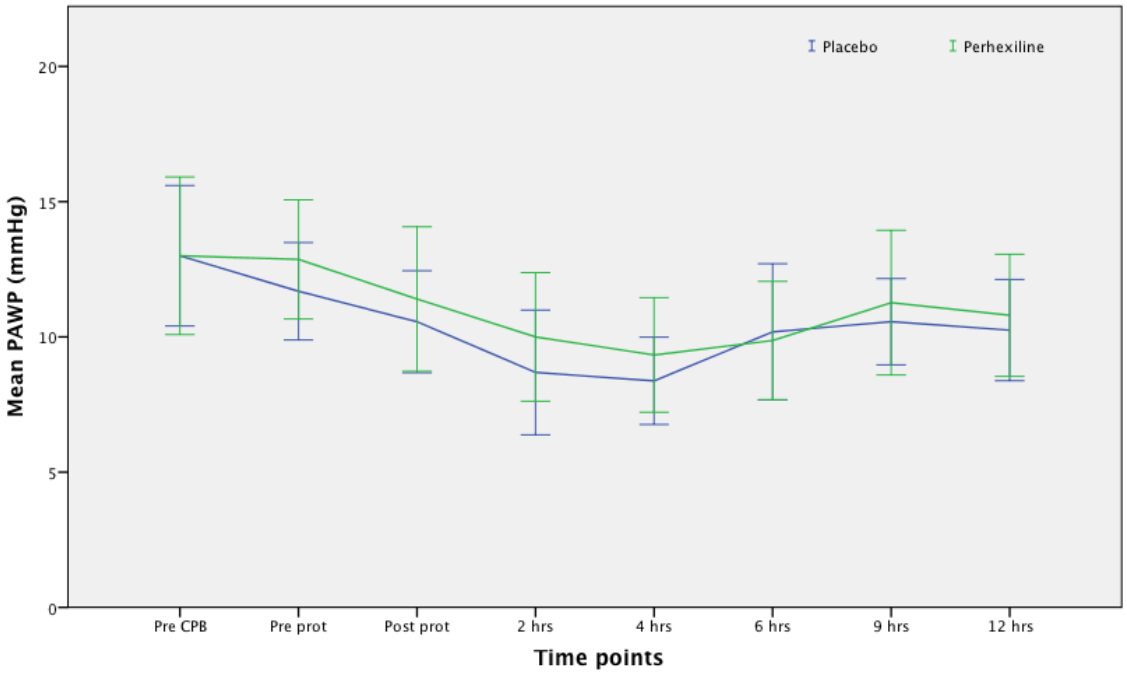


Figure 3-9 Mean PAWP between groups at each time point

3.8.2 Mean arterial pressures

There was no statistical difference in the MAP between groups at any of the time points except at 4 hours post removal of the aortic cross clamp, with marginal significance (p=0.04). The trend in MAP between groups is shown below (Figure 3-10).

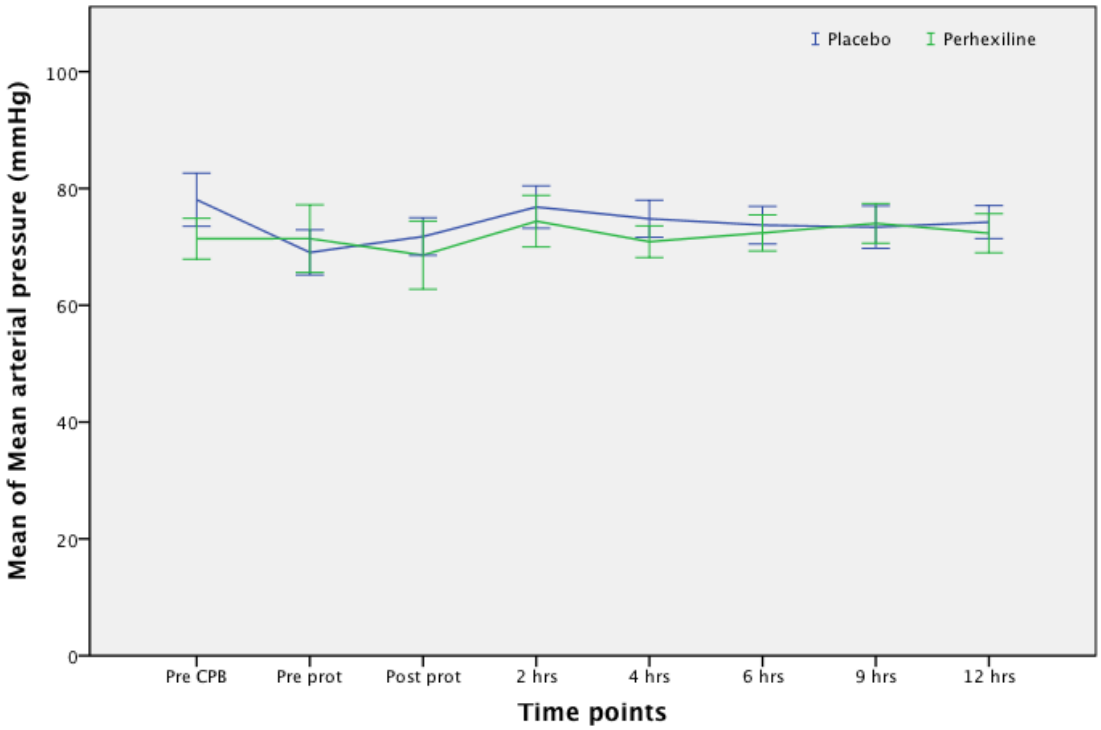


Figure 3-10 Mean arterial pressure between groups at each time point

In the assessment of mean PAP between groups, there were marginal statistical significant differences at time points; pre protamine (p=0.04), and reperfusion at 4 hours (p=0.03), 6 hours (p=0.048) and 12 hours (p=0.02). However application of the Bonferroni correction would make this not significant. Mean PAPs were higher in the perhexiline arm after 2 hours of reperfusion (Figure 3-11).

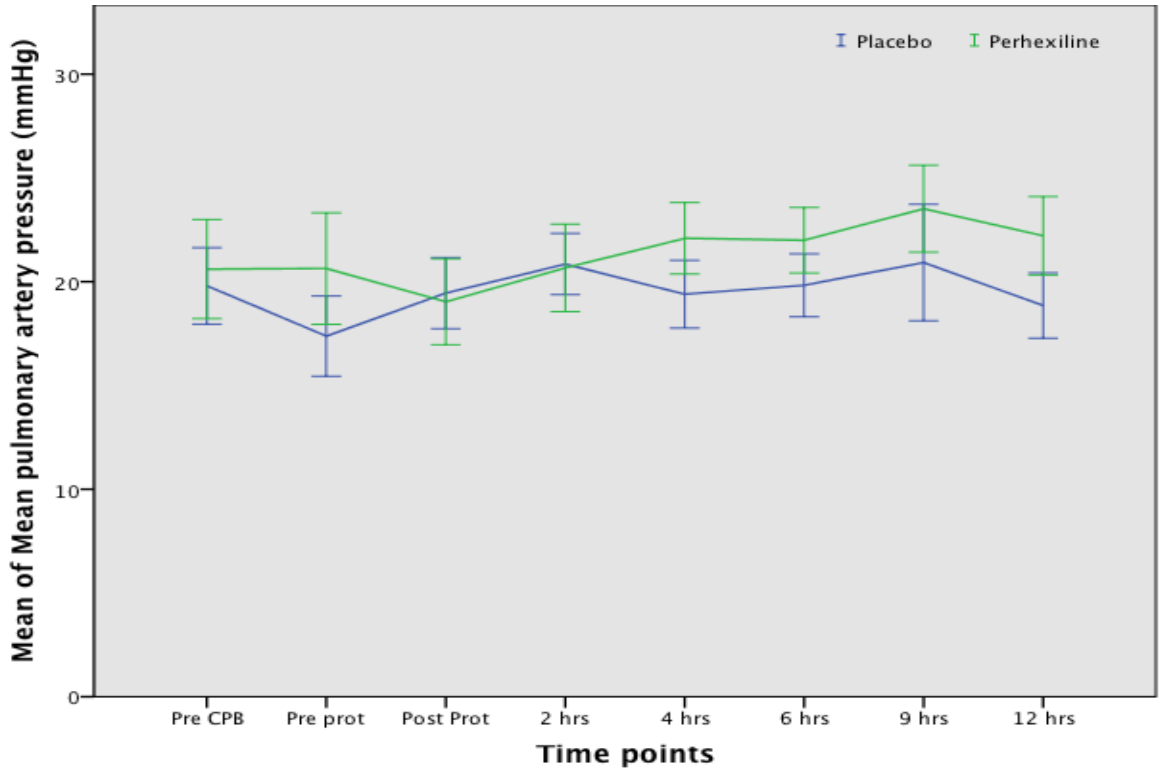


Figure 3-11 Mean pulmonary artery pressures between groups at each time point

3.8.3 Cardiac index assessments

The mean cardiac index (standard deviation) at each of the time points is depicted below (Figure 3-12) and shows no significant difference between perhexiline and placebo at all time points except at 12 hours where the placebo arm has a higher cardiac index (Table 3-6).

Measured cardiac index time point (L/min/m²)	Mean Perhexiline (SD)	Mean Placebo (SD)	Estimate (95% confidence interval)	P value
Pre CPB	1.99 (0.51)	2.15 (0.46)	0.66 (-0.35 – 0.024)	0.66
Pre protamine	2.48 (0.46)	2.51 (0.54)	0.10 (-0.23 – 0.18)	0.48
Post protamine	2.57 (0.67)	2.58 (0.56)	0.004 (-0.24 – 0.25)	0.97
2 hours	2.61 (0.69)	2.52 (0.47)	0.12 (-0.10 – 0.27)	0.27
4 hours	2.53 (0.71)	2.62 (0.54)	-0.04 (-0.27 – 0.18)	0.69
6 hours	2.57 (0.60)	2.75 (0.69)	-0.18 (-0.43 – 0.08)	0.17
9 hours	2.74 (0.56)	2.85 (0.61)	-0.11 (-0.33 – 0.12)	0.36
12 hours	2.68 (0.51)	2.90 (0.52)	-0.23 (-0.44 – -0.02)	0.03

Table 3-6 Cardiac index at each time point

There is a trend of consistently higher cardiac indices throughout the measured time within the placebo arm, particularly beyond 2 hours post reperfusion.

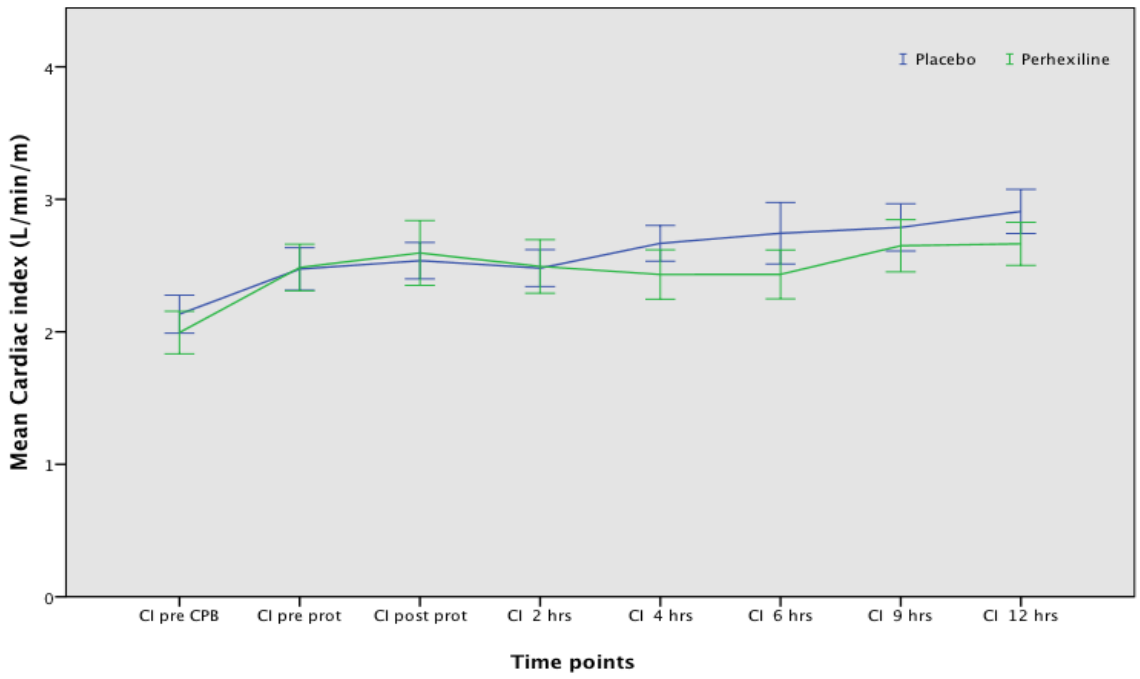


Figure 3-12 Mean cardiac index (L/min/m²) at each time point

3.9 Inotrope usage

The use of all inotropes was assessed between removal of the aortic cross clamp to 6 hours and then for the 6 – 12 hrs of reperfusion (Table 3-7). There was no difference in the usage of inotropes between groups during 0 – 6 hrs of reperfusion.

	Perhexiline (n=54)	Control (n=58)	Estimate (95% confidence interval)	P value
Inotrope use in first 6 hours	22 (40.1%)	15 (25.9%)	2.31 (0.99 – 5.74)	0.053
Inotrope use 6 - 12 hours	26 (48.1%)	15 (25.9%)	3.11 (1.34 – 7.23)	0.009

Table 3-7 Inotrope use in the 1st 6 and 6-12 hours of reperfusion

Statistical significance was present in inotrope usage 6 – 12 hours of reperfusion, with 48% of patients in the perhexiline arm requiring inotropes compared with 26% of control subjects in the same time frame.

The mean dose per kilogram of Dopamine and Adrenaline used in the 1st 6 hours and then from 6 – 12 hours of reperfusion, between the groups is depicted in Figure 3-13 and Figure 3-14 respectively, and shows no significant difference between the groups for each time point. However, there is a rise in the mean dose per kilogram of Dopamine and Adrenaline used in the perhexiline group by 6 – 12 hrs of reperfusion, reflective of the significant difference in the total incidence of inotropes used, as depicted in Table 3-7.

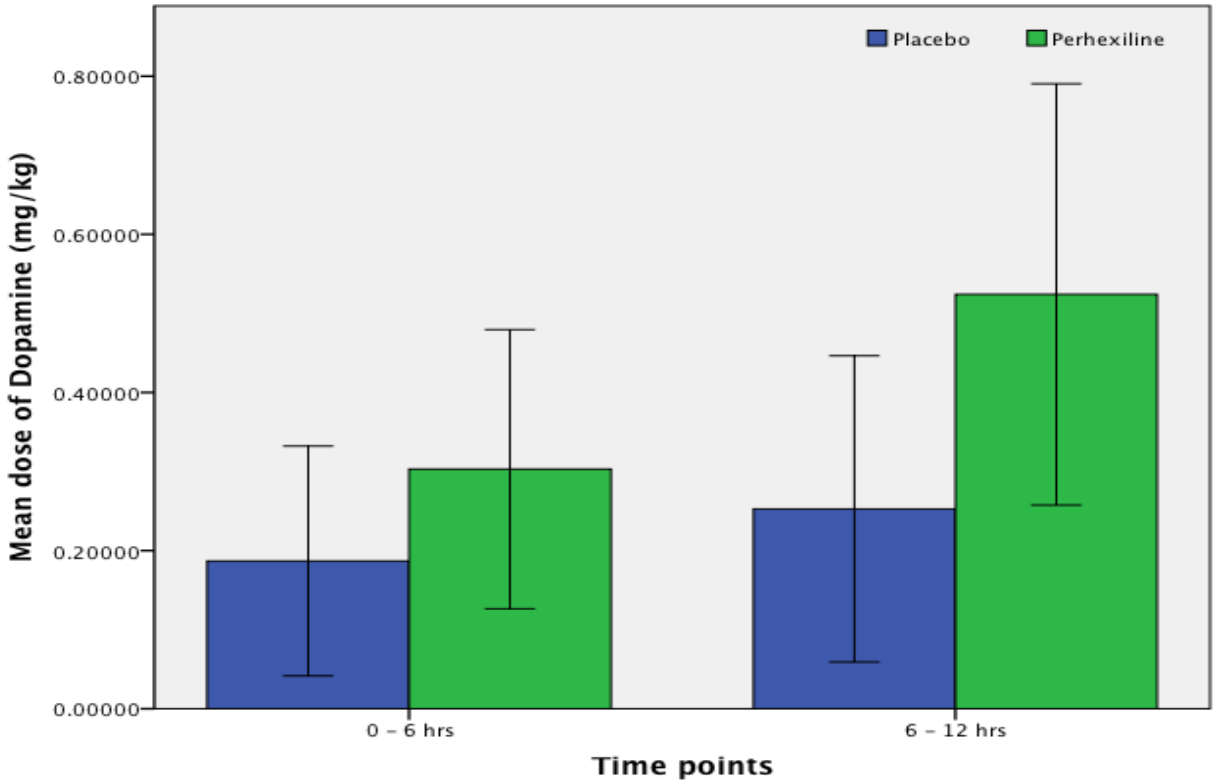


Figure 3-13 Mean dose (mg/kg) of Dopamine at each time point

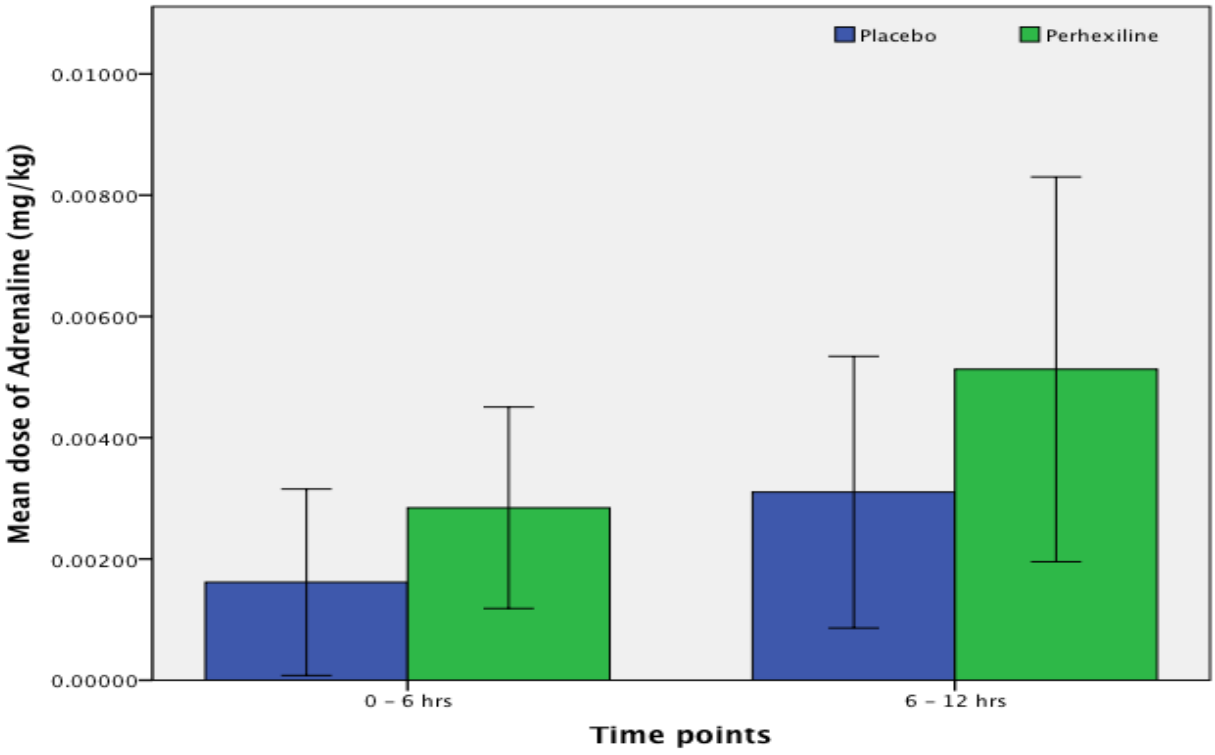


Figure 3-14 Mean dose (mg/kg) of Adrenaline at each time point

3.10 Vasoconstrictor use

Phenylephrine was the used as the 1st line vasoconstrictor to maintain haemodynamic parameters as outlined in the methods. The 2nd line vasoconstrictor was vasopressin and lastly noradrenaline at the discretion of the clinical team if the aforementioned agents did not show a benefit.

Within 0-6 hours of reperfusion overall (phenylephrine and noradrenaline) constrictor use was 53/58 in the placebo arm and 48/54 in the perhexiline arm (p=0.76). Within 6-12 hours of reperfusion overall vasoconstrictor use was 37/58 in the placebo arm and 43/54 in the perhexiline arm (p=0.09). Use of phenylephrine and noradrenaline individually at each time point was not significant between the groups (Table 3-8).

	Perhexiline (n=54)	**Control (n=58)**	**P value**
0 – 6 hours			
Phenylephrine	43 (79.6%)	52 (89.7%)	0.19
Noradrenaline	11 (20.4%)	7 (12.1%)	0.31
Both	48 (88.9)	53 (91.4%)	0.76
6 – 12 hours			
Phenylephrine	35 (64.8%)	31 (53.4%)	0.25
Noradrenaline	11 (20.4%)	10 (17.2%)	0.81
Both	43 (79.6%)	37 (63.7%)	0.09

Table 3-8 Vasoconstrictor use in the first 6 hrs and 6-12hrs of reperfusion

Dose per kilogram of phenylephrine was calculated for the time period up to 6 hours of reperfusion and then from 6 to 12 hours of reperfusion; there was no statistically significant difference between the groups at each of these time points p=0.83 and p=0.55 respectively (Figure 3-15). Similarly there was no difference in the use of noradrenaline between groups at each time point (Figure 3-16). However, within the placebo arm there was no vasopressin used as a 2nd line vasoconstrictor at either time point; compared to a mean dose per kilogram of 0.015 and 0.016 at 0-6 hrs (n=8) and 6-12 hrs (n=7) respectively in the perhexiline arm, making this statistically significant p=0.002 and 0.005 respectively.

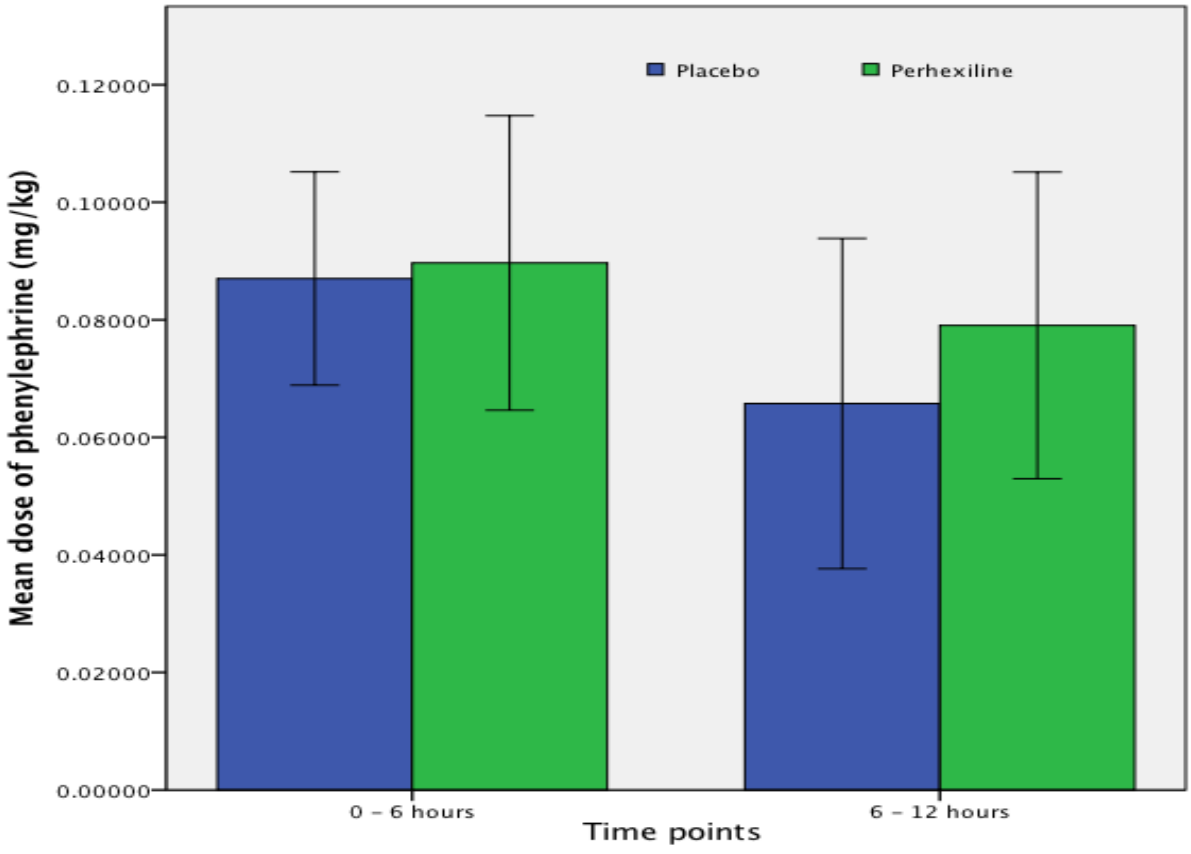


Figure 3-15 Mean dose of phenylephrine at each time point

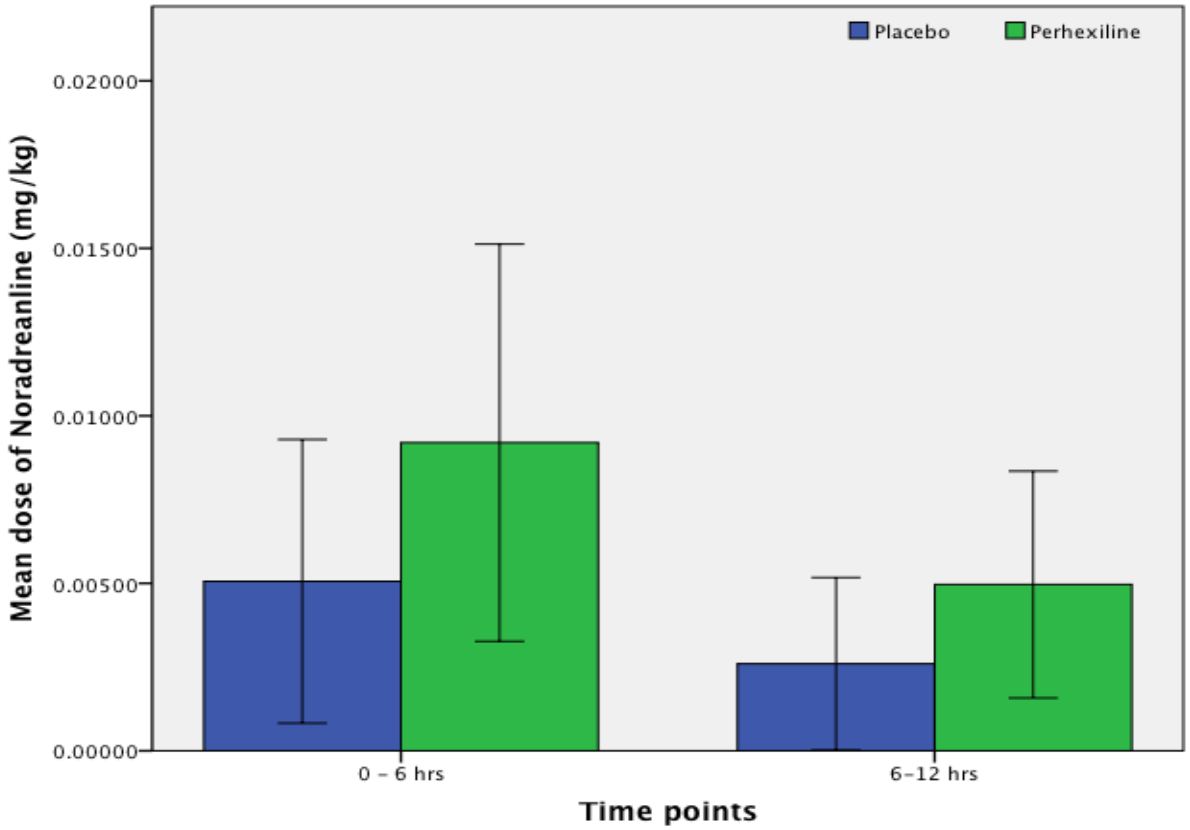


Figure 3-16 Mean dose of Noradrenaline at each time point

3.11 Myocardial injury

3.11.1 Electrocardiographic evaluation

New myocardial injury was defined as outlined in the methods and was reviewed by an independent cardiologist. New myocardial injury was identified in 2/54 patients in the perhexiline arm and 6/58 patients in the placebo arm and showed no statistical significant difference OR 0.36 (CI 0.07 – 1.97) p=0.24.

3.11.2 Cardiac troponin release

In March 2011, the method of troponin analysis within University Hospitals Birmingham changed. Therefore troponin analysis between perhexiline and placebo outlined below is as per the new and older method of troponin analysis.

The analysis of troponin between perhexiline and placebo was sub-divided into those that had the older version of troponin analysis (n=46, 23 in each arm) and those that had the newer high sensitivity troponin analysis (n=55, 24 in perhexiline and 31 in the placebo arm). Repeated measures analysis of troponin at baseline and at 6, 12, and 24 hours of reperfusion is outlined in Table 3-9, and shows no statistical difference between the groups with either method of troponin analysis.

Method of troponin analysis	Perhexiline Mean troponin (SD)	Control Mean troponin (SD)	Estimate (95% confidence interval)	P value
New method (ng/L)	1431.33 (709.30)	1114.61 (1137.44)	334.37 (-446.86 – 1115.60)	0.39
Old method (ng/ml)	0.78 (0.37)	0.85 (0.38)	-0.08 (-0.30 – 0.15)	0.50

Table 3-9 Troponin analysis

Figures 3-17 and 3-18 show the mean concentration (error bars representing the confidence interval) of troponin for each time point using both the older Troponin T measurements and the new High Sensitivity Troponin T measurement. Figure 3-18 shows the large confidence interval associated with the HS troponin particularly within the perhexiline arm.

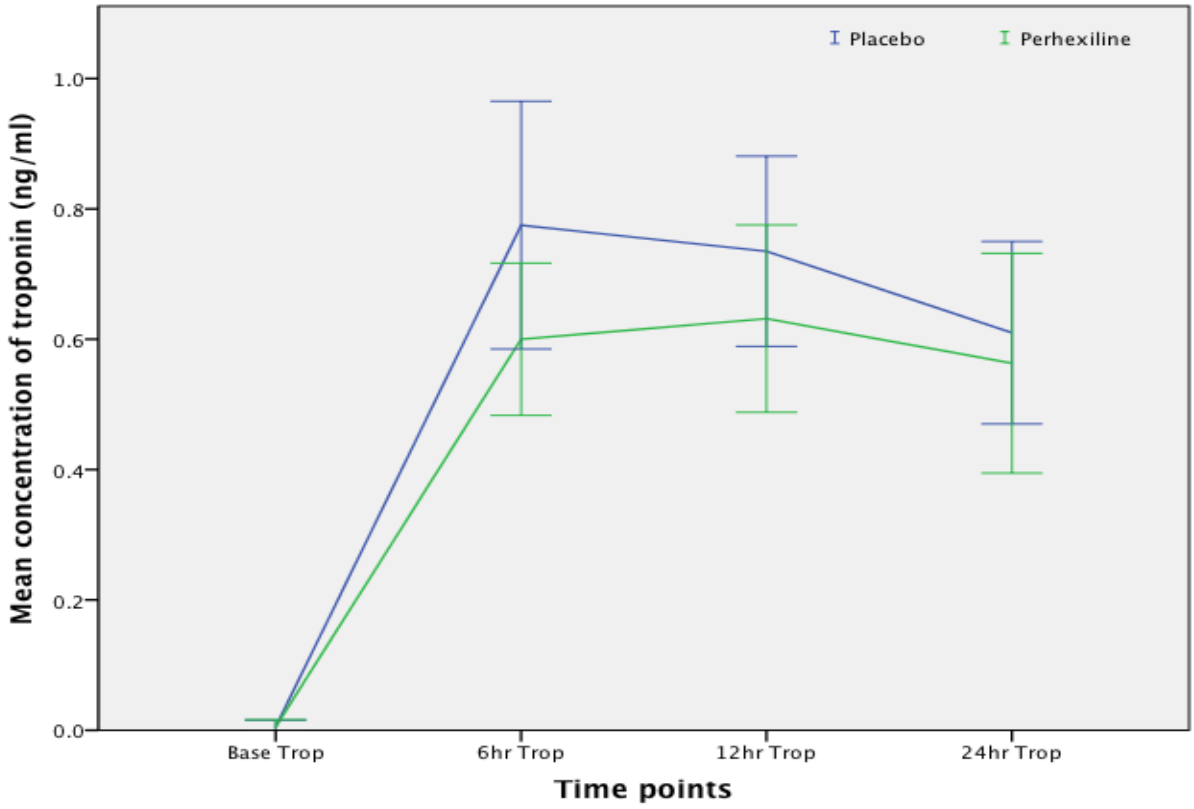


Figure 3-17 Mean Troponin concentration (old method) for each time point

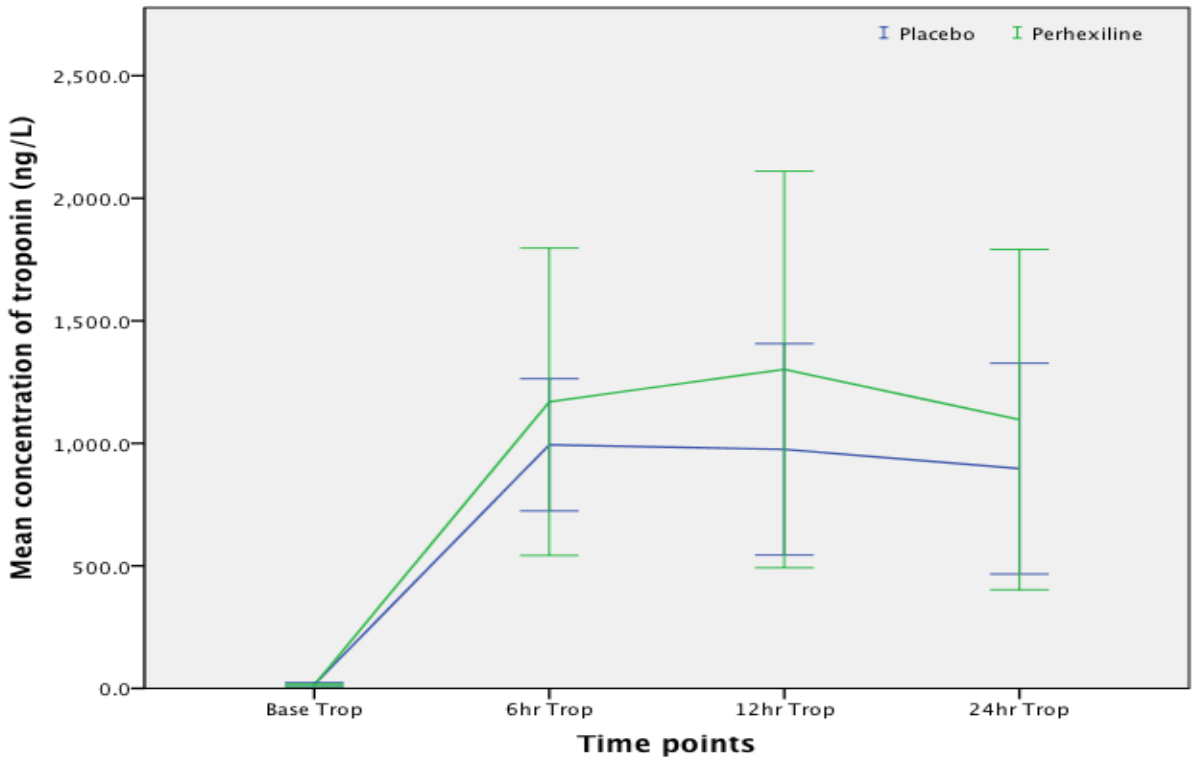


Figure 3-18 Mean Troponin concentration (new method) for each time point

3.12 Use of volume expansion

3.12.1 Administration of colloids

Volume in the form of colloids and blood were transfused to maintain the optimum parameters as outlined in the methods. The preferred colloid was Gelofusin initially and then Human Albumin Solution (HAS) once > 2L of Gelofusin had been transfused. There was no difference between the groups in the mean volume of Gelofusin or HAS administered at either 0 – 6 hrs of reperfusion or 6 – 12 hours of reperfusion (Figure 3-19 and Figure 3-20).

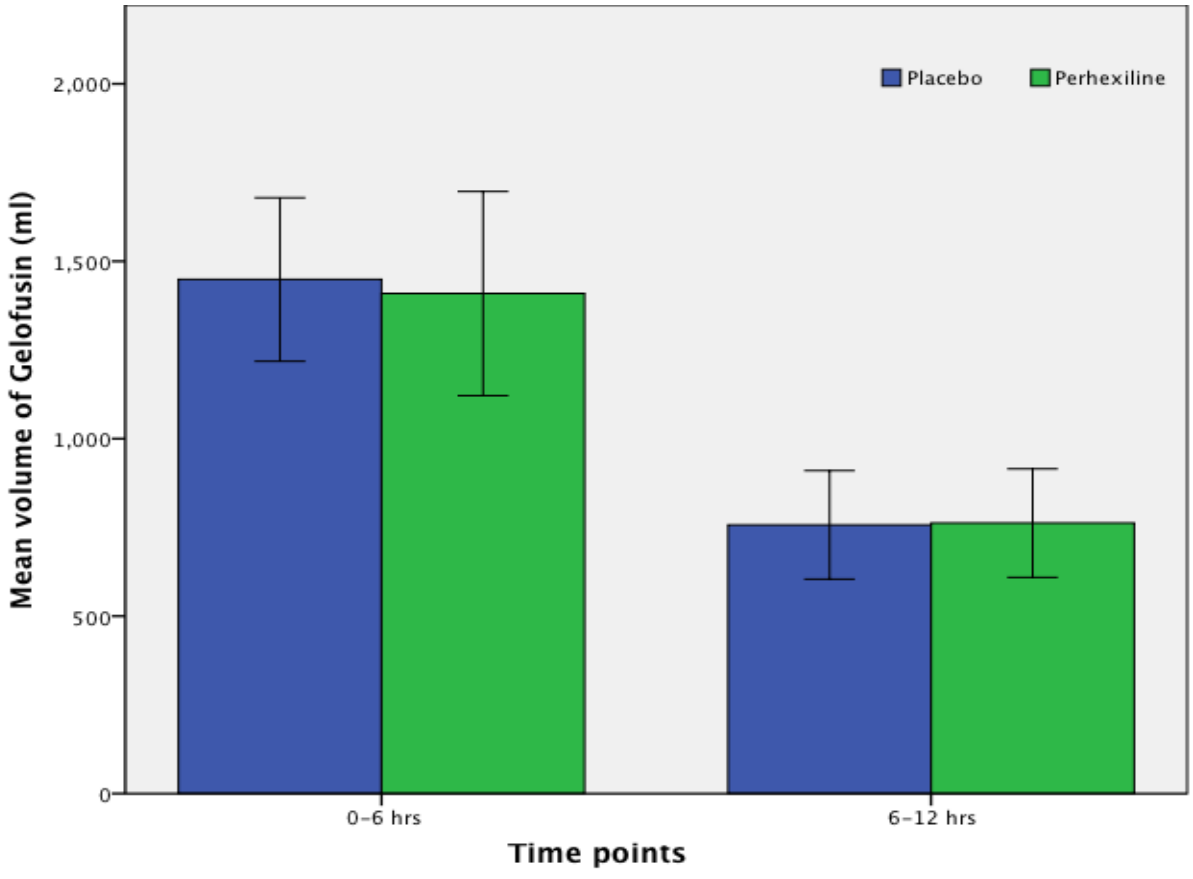


Figure 3-19 Mean volume of Gelofusin administered at each time point

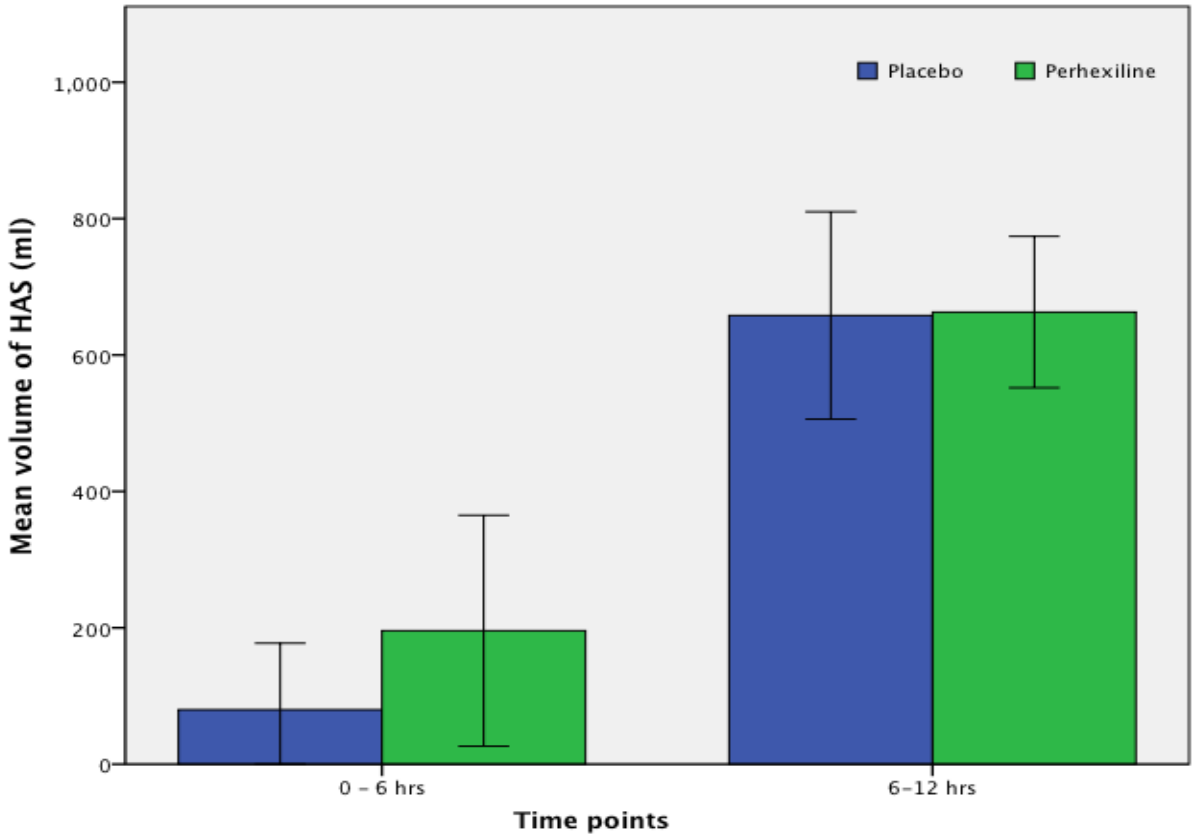


Figure 3-20 Mean volume of HAS administered for each time point

3.12.2 Administration of blood and blood products

Packed red blood cells were transfused to maintain the haemodynamics and Haemoglobin concentrations as outlined in the methods. A total of 41/58 (75%) and 34/58 (59%) of patients in the perhexiline and placebo arms respectively had received packed red cells within 0 – 6 hrs of reperfusion. However there was no difference between groups in the mean number of packed red blood cell units transfused at 0 – 6 hours (p=0.06) and 6 -12 hours (p=0.63) of reperfusion (Figure 3-21).

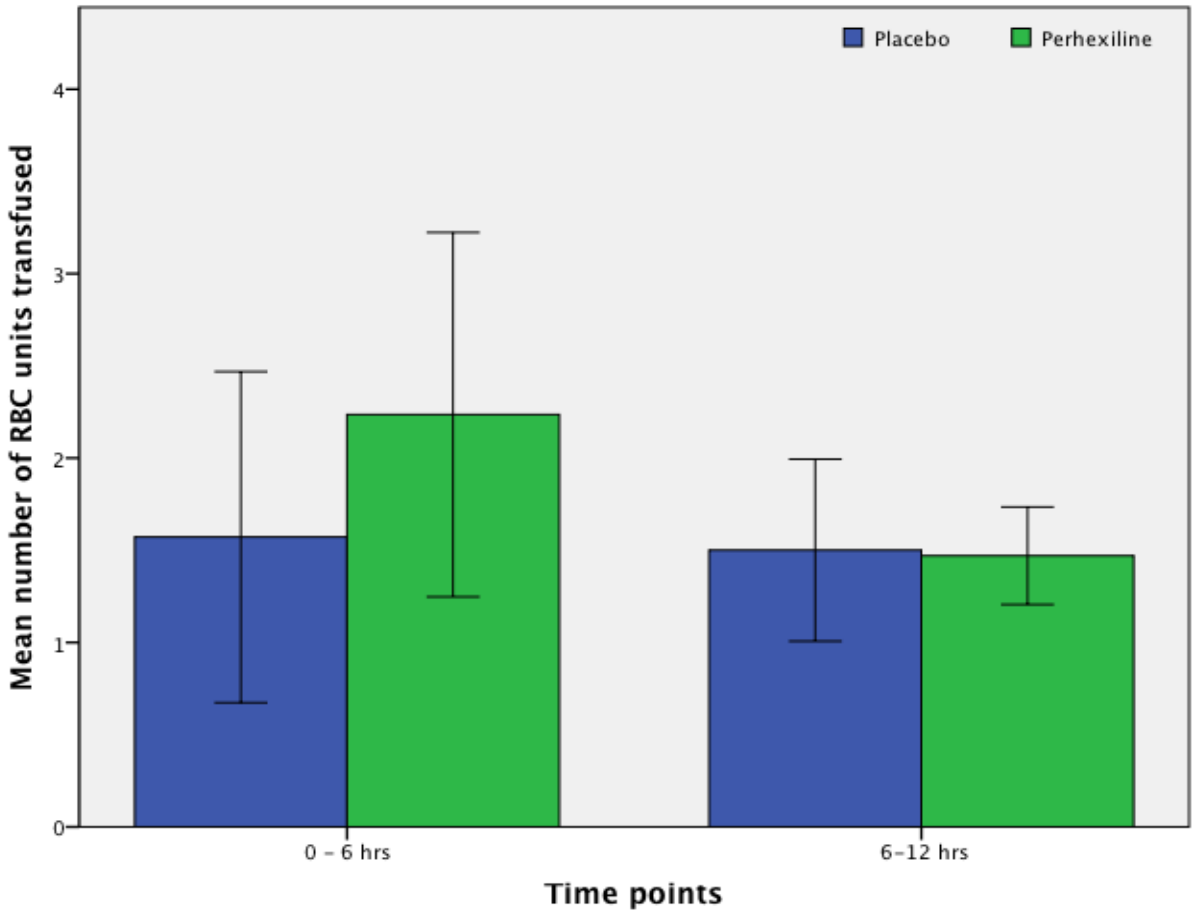


Figure 3-21 Mean packed red blood cells transfused at each time point

Blood products such as Fresh Frozen plasma (FFP), Platelets and Cryoprecipitate were transfused, if there was evidence of on-going bleeding. By 6 hrs of reperfusion a total of 36/54 (66%) and 28/58 (48%) of patients in the perhexiline and placebo arms respectively had received one of the aforementioned forms of blood products. However analysis of the mean number of units of blood products transfused, between perhexiline and placebo for each form of blood product transfused, showed no difference at 0 – 6 hrs of reperfusion (Figure 3-22).

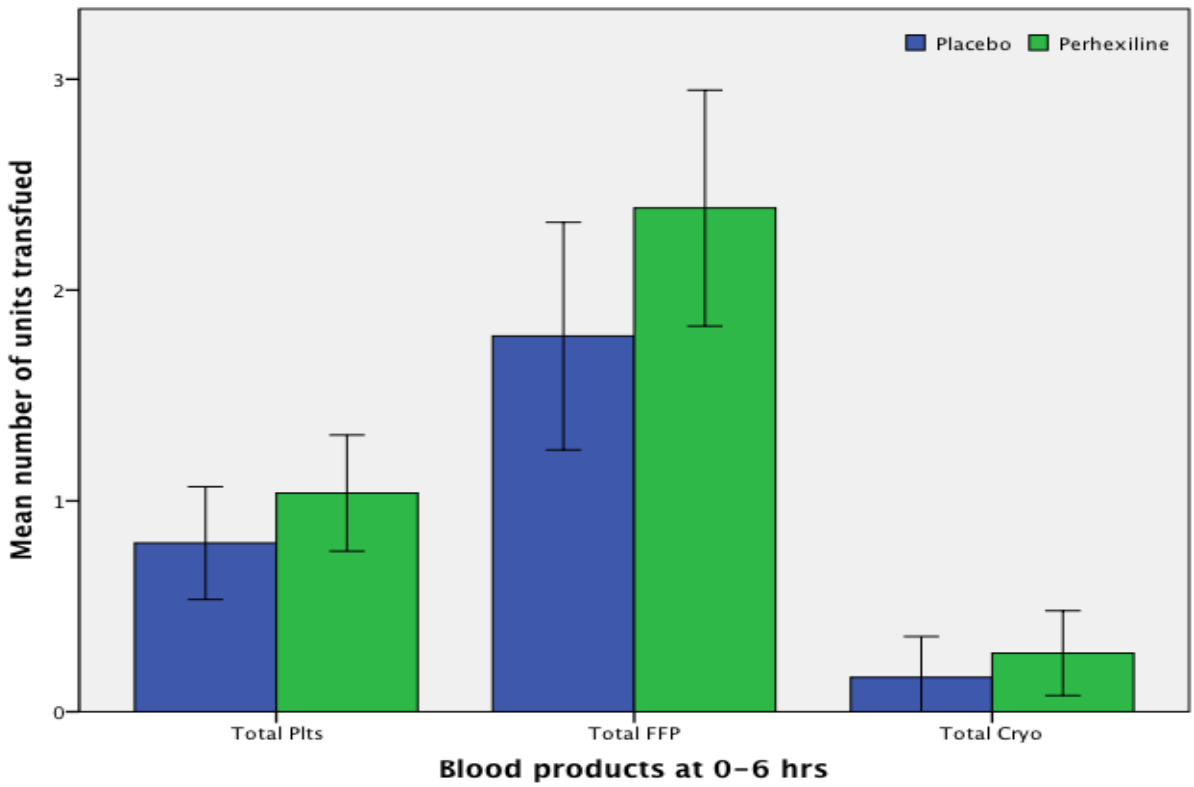


Figure 3-22 Mean number of blood products transfused 0-6 hrs of reperfusion

3.13 New reperfusion and post operative arrhythmias

Data on arrhythmias post intial reperfusion and in the post-operative period was collected and analysed. There was no significant difference in the number of arrhythmias in any of the categories (Table 3-10). There was no incidence of post-operative ventricular fibrillation in either arm. Three patients (5.2%) in the placebo arm required a permanent pace maker with none in the perhexiline arm (p=0.24).

Arrhythmias (%)	Placebo (n-58)	Perhexiline (n=54)	P value
Reperfusion VF	11 (19.0)	9 (16.7)	0.81
Reperfusion VT	0 (0)	3 (5.2)	0.11
Post op AF	28 (48.3)	33 (55.6)	0.45
Post op VT	1 (1.9)	1 (1.8)	1.0

Table 3-10 Reperfusion and post-operative arrhythmias

3.14 Time to warm, extubate and discharge

All patients were admitted to the ITU and actively warmed to 36°C. The time taken to warm to 36°C for each group, time for extubation and time to discharge from the ITU to the ward and total in-hospital stay are outlined in Table 3-11 (represented by median and IQR). There was no statistical difference between the groups in any of these outcomes. The total blood loss via the drains was evaluated at 12 hrs (median and IQR) from reperfusion and showed no difference between the groups.

An intra-aortic balloon pump was inserted in 2 and 5 patients in total in the placebo and perhexiline arm (Table 3-11). This is inclusive of those inserted in theatre as illustrated in Table 3-4.

	Placebo (n-58)	Perhexiline (n=54)	P value
Time to 36°C (hrs)	3:00 (0:56 – 4:41)	2:30 (0:00 – 5:41)	0.11
Total drain losses at 12 hrs	475 (317 – 810)	510 (345 – 940)	0.51
Intubation time (hrs)	11.39 (7:54 – 16:21)	13:29 (7:33 – 17:47)	0.51
Days on ITU	3 (2 – 5)	3 (2 – 6)	0.22
In-hospital stay	10 (7 – 14)	9 (7 – 15)	0.53
IABP (%)	2 (3.4)	5 (9.4)	0.26

Table 3-11 Duration to warm, extubate and discharge

The total mean length of hospital stay for the perhexiline and placebo arms were 15.11 ± 13.54 and 11.84 ± 6.95 days respectively and showed no statistical significance DiM 3.17 (CI -0.82 – 7.17) p=0.12.

3.15 Safety outcome measures and other postoperative complications

Safety outcome measures included postoperative mortality, stroke with residual deficit, requirement for renal replacement therapy, re-operation, and treated infective episodes. These are illustrated in Table 3-12.

Overall mortality for the trial was 1.9%; a patient who was in the perhexiline arm and died after protracted course on the ITU, secondary to multi-organ failure following a cerebral infarct. There was no statistical significant difference between groups for any of the safety outcome measures except for renal impairment (creatinine > 200); 6 patients in the perhexiline arm.

All other complications are listed in Table 3-12 and were found to have no statistical significant differences between the groups.

	Placebo (n=58)	Perhexiline (n=54)	P Value
Mortality (%)	0	1 (1.9)	0.48
Re-operation			
- Bleeding (%)	2 (3.4)	1 (1.9)	0.23
- Tamponade (%)	1 (1.7)	0	1.0
- Arrest	0	1(1.9)	0.48
Infective episodes			
- Pneumonia (%)	4 (6.9)	3 (5.5)	1.0
- Leg wound infection (%)	1 (1.7)	1 (1.9)	1.0
- Leg dehiscence (%)	0	1 (1.9)	0.48
Requirement for CPAP (%)	4 (6.9)	3 (5.5)	1.0
Re-intubation (%)	3 (5.2)	2 (3.4)	1.0
Tracheostomy (%)	2 (3.4)	6 (11.1)	0.15
Renal impairment			
- Creatine > 200 (%)	0	6 (11.1)	0.01
- Haemofiltration (%)	0	2 (3.4)	0.23
- Dialysis (%)	0	1 (1.9)	0.48
Neurological			
- Difficulty waking (%)	0	1 (1.9)	0.48
- Focal CVA	0	1 (1.9)	0.48
- Type II (%)	1 (1.7)	1 (1.9)	1.0

Table 3-12 Safety outcome measure and postoperative complications

3.16 Quality of life analysis

Quality of life was assessed using the EQ-5D (version 3L) questionnaire. This is a standard questionnaire used to assess health outcomes. The EQ-5D assessed the health state in 5 health state categories; mobility, self care, usual activities, pain/discomfort and anxiety/depression and allowed the participant to rank each on 3 levels, 1 – 3 (1 indicating no problems and 3 indicating extreme problems). In addition the questionnaire allowed the patient to rank their overall health sate on a visual analogue

scale (VAS) of 0 – 100 (100 being the best health state imaginable, and 0 being the worst).

The results from this questionnaire was analysed to assess the pre and post-operative differences. Forty-four patients completed the questionnaire pre and post surgery. The number of patients reporting ≥ 3 for each health state was small, therefore those who reported 1 are classified as having 'no problem' and those reporting > 1 are classified as having a problem (Table 3-13).

Health state	Pre surgery (%)		Post surgery (%)		P value
	Problem	No problem	Problem	No problem	
Mobility	22 (50)	22 (50	6 (13.1)	38 (86)	< 0.001
Self care	2 (5)	42 (95)	1 (2)	43 (98)	0.32
Activities	18 (41)	26 (59)	4 (9)	40 (91)	0.001
Pain	25 (57)	19 (43)	5 (11)	39 (89)	< 0.001
Anxiety	20 (46)	24 (55)	11 (25)	33 (75)	0.03
VAS (IQR)	69.5 (56 – 80)		90 (80 – 95)		< 0.001

Table 3-13 EQ-5D analysis pre and post surgery

There was a significant improvement in the mobility, ability to perform activities, pain and marginal significance with anxiety, after the operation compared to before. The VAS score also showed statistical significance with a median score of 90 after the operation compared to 60.5 before the operation.

The table below (Table 3-14) summaries the overall utility score (median and inter-quartile range) for each group at both time points of the questionnaire. The median utility score in each group is greater at follow-up as is the inter-quartile range for both groups. Analysis of the utilities between perhexiline and placebo groups showed no

statistical difference between the groups (p=0.17). Generalised liner model analysis of baseline utilities versus the end-point utility (score at FU), showed that the baseline measure does not contribute towards the follow up measure.

Health state	Pre surgery		Post surgery	
	Median	IQR	Median	IQR
Perhexiline (n=17)	0.79	0.62 – 0.92	1	0.78 - 1
Placebo (n=27)	0.72	0.69 – 0.80	1	0.85 - 1

Table 3-14 Summary of utilities at each time point

3.17 Futility analysis

The DSMB were requested to assess the trial for safety and efficacy and also assess the trial for futility based on the results (based on the clinical and metabolic findings) of the CASPER trial (using perhexiline as an adjunct to myocardial protection in patients undergoing CABG).

An Alpha Spending plan was performed to examine the effect of primary outcome including futility. The benefits are based upon the Lan DeMets plan and the harm is based upon a power family spending function. The table below (Table 3-15) shows the bounds for efficacy, futility and harm accompanied by the p value (alpha(i) – alpha(i-1)) at each planned look at 45%, 73% and 100% completed patients. This is graphically represented by Figure 3-23, that illustrates the stopping boundaries for efficacy, futility and harm for the primary outcome. The figure depicts the standard errors for each boundary (efficacy, futility and harm) versus the number of patients that would complete the trial at 45%, 73% and 100% completed patients. Harm is much less steeply pitched (enabling an earlier stop for evidence of harm).

The DSMB performed a planned observation at 45% recruitment (99 patients) and analysis of the primary outcome showed a significance of 0.823. This significance fell below the line of futility and above the line of harm, demonstrating overall futility of the trial.

If the significance at 45% fell below the line of efficacy and above the line of futility, then the trial was on target to achieve a positive outcome. Similarly, if the significance fell below the line of harm, then the trial would be halted early for the risk of harm or be modified to reduce harm to the patients. Alternatively if the significance fell above the line of efficacy, the trial would be halted early for efficacy.

Following this analysis the DSMB recommended that the trial be halted based on futility of achieving the scientific objective; there was no evidence of clinical benefit associated with the investigational treatment and therefore it was deemed futile to continue recruiting into the trial. Based on these recommendations, the trial steering committee halted the recruitment into the trial.

Efficacy

Time	Bounds	alpha(i)-alpha(i-1)	Cum alpha
0.45	3.1438	0.00083	0.00083
0.73	2.3896	0.00787	0.00871
1.00	2.0081	0.01629	0.02500

Futility

Time	Bounds	alpha(i)-alpha(i-1)
0.45	0.8724	0.383
0.73	1.6266	0.104
1.00	2.0081	

Harm

Time	Bounds	alpha(i)-alpha(i-1)	Cumulative alpha
0.45	2.4301	0.00755	0.00755
0.73	2.2813	0.00805	0.01559
1.00	2.1350	0.00941	0.02500

Table 3-15 Tables illustrating the alpha spending plan analysis

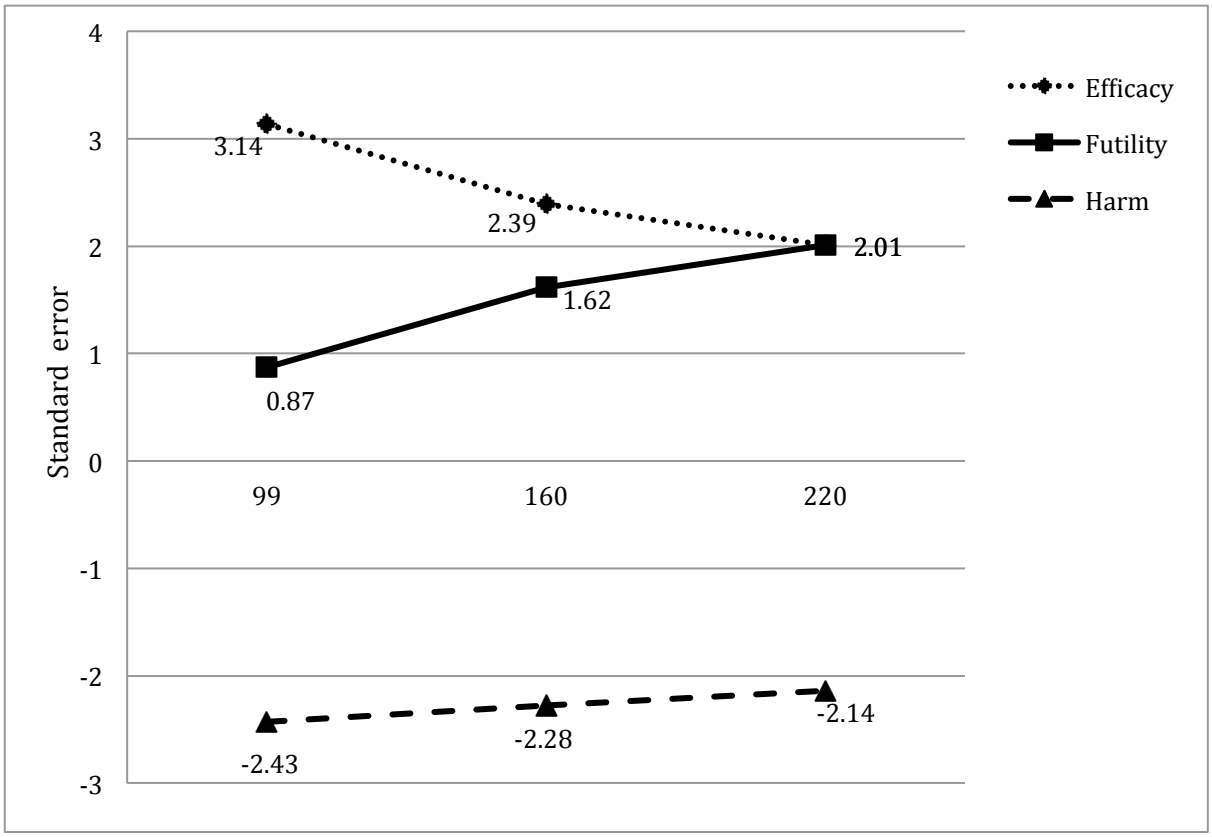


Figure 3-23 Stopping boundaries for efficacy, harm and futility

3.18 Summary of the HYPER results

The median duration of trial therapy was 8.5 days (IQR 5 – 17.5) for all trial participants in the final analysis, and was 8 days (IQR 5 – 11) and 8 days (IQR 6 – 14), in the perhexiline and placebo groups respectively (p= 0.41). In the treatment group median perhexiline concentration was 0.22mg/L (IQR 0.09 – 0.43). The majority of side effects were consistent with the known side effects of perhexiline.

The trial was halted early for futility on the advice of the DSMB. Of 110 patients who achieved the primary end point, 30 patients (16 perhexiline, 14 placebo) had inotropes started appropriately; there was no difference in the incidence of inotrope usage OR 1.65 (CI 0.67 – 4.06) p=0.28.

There was a significant increase in the overall incidence of inotrope use in the perhexiline group from six to 12 hours of reperfusion; 26 (48%) and 15 (26%) of patients in the perhexiline and placebo groups respectively requiring inotropes, OR 3.11 (1.34 – 7.23), p=0.009. All longitudinal haemodynamic measurements were comparable between the groups. Mean CI was not significant at each time point until at 12 hours of reperfusion, when the perhexiline group had a lower CI. There was no difference in myocardial injury as evidenced by electrocardiogram OR 0.36 (CI 0.07 – 1.97) p=0.24 or post-operative troponin release.

All other safety-outcome measures were comparable between the groups except for renal impairment (Creatinine > 200), with six patients developing renal impairment in the perhexiline group and none in the placebo group (p=0.01), two requiring haemofiltration and one requiring dialysis.

4 MAGNETIC RESONANCE SPECTROSCOPY TO ASSESS CARDIAC ENERGETICS – A VALIDATION STUDY

4.1 Introduction

Magnetic resonance spectroscopy is a non-invasive, radiation free investigation to acquire information about metabolites in a given tissue, using some of the standard magnetic resonance imaging techniques. Standard magnetic resonance imaging relies on the detection, signals and spin of hydrogen protons to create a two-dimensional image. However magnetic resonance spectroscopy (MRS) can detect other metabolites such as carbon, nitrogen, fluorine, sodium and also phosphorus and create a spectra of these metabolites for analysis (Holloway, Suttie et al. 2011). Evaluating ^{31}Phosporus through MRS allows the detection of phosphocreatine and ATP in cardiac tissue and thereby can be used to study cardiac energetics.

4.1.1 Cardiac magnetic resonance spectroscopy

Cardiac MRS investigates the spectrum for ^{31}Phosporus nucleus. Examination of this nucleus for a normal healthy subject should provide six resonances, which include the 3 ^{31}Phosporus atoms of ATP (alpha, beta and gamma), phosphocreatine (PCr), diphosphoglycerate (from erythrocytes) and phosphodiesters (from membrane and serum phospholipids) (Hudsmith and Neubauer 2009; Beadle and Frenneaux 2010).

Cardiac MRS was first performed in the 1980s (Bottomley 1985). In current practice a 1.5 – 3.0 T magnet is used with a ^{31}Phosporus nucleus specific coil, together with a broadband radiofrequency transmitter to excite non-proton nuclei. More recently a 3.0T field strength has been used, which provides a greater signal strength, improves

temporal resolution and spectral signal to noise ratio (Tyler, Hudsmith et al. 2008; Abozguia, Elliott et al. 2010; Shivu, Abozguia et al. 2010) In addition to this, MRS acquisition sequence post processing data analysis software is also required (Hudsmith and Neubauer 2009). The setup for data acquisition therefore consists of a superconducting magnet, interfaced with a computer and a radiofrequency (RF) transmitter and receiver. The transmitter and receiver is combined into a single disk shaped device that is placed onto the chest wall, with the patient in supine or prone position, to allow close approximation of the coil to the heart. The RF generator and workstation create a RF impulse, which is sent to the ^{31}Phosporus nucleus specific coil. This impulse creates the nuclear spin excitation in the heart. The response to this impulse (the resulting magnetic resonance signal) is also detected by the ^{31}Phosporus nucleus specific coil and is relayed to the computer. The data that is relayed is stored as free induction decay (FID), represented as the relationship between signal intensity and time. The FID is then converted to a magnetic resonance spectrum by Fourier transformation. The spectrum relates resonance frequency (on the x axis) to intensity (on the y axis). The area under each resonance frequency is proportional to the amount of each ^{31}Phosporus nucleus species in the heart and hence the relative concentrations of each metabolite can be calculated and quantified (Arad, Moskowitz et al. 2003). The acquisitions are performed with ECG gating (surface ECG), which allows for the acquisition to be obtained during diastole when the heart motion is least. To obtain absolute quantification of the metabolites, further calibration of the ^{31}Phosporus signal is required to accommodate for the ^{1}Hydrogen water signal and this process is more complex.

For spectral acquisition, a specific area of the heart is identified using ^{1}Hydrogen magnetic resonance imaging. This allows confirmation of coil position and identification of an anatomical voxel selection for spectral acquisition and under ideal circumstances includes the myocardial inter-ventricular septum. There are several localisation sequences to position the voxel. These include depth resolves surface coil spectroscopy, image-selected in vivo spectroscopy or 3-dimentional chemical shift imaging (CSI) using a selection of multiple voxel in 3 dimensions (Bottomley 1994). Care should be taken to exclude signal from overlapping skeletal muscle.

Analysis of the acquired spectra can provide the phosphocreatine to ATP ratio and through this the energetic state of the heart can be assessed. The analysis of the acquired spectra can be performed offline.

4.1.2 MRS spectral analysis

Spectral analysis can be performed using the Java based Magnetic Resonance User Interface (JMRUI), a software package which is a graphical user interface and allows for time-domain analysis of the MRS data (Beadle and Frenneaux 2010). This software allows accurate analysis of the frequency peaks and hence calculation of the PCr:ATP ratios. To help with this, the Advanced Method for Accurate, Robust and Efficient Spectral (AMARES) fitting can be performed, which allows non-linear fitting in the time domain. In addition, JMRUI includes algorithms for frequency selective filtering of signals. In combination and adapting these methods, accurate quantification of the area under the peaks can be calculated and PCr:ATP ratio obtained (Hudsmith and Neubauer 2009; Beadle and Frenneaux 2010).

For further accuracy, blood contamination can be minimized. Blood contains ATP but not PCr. Therefore calculation of the 2,3-diphosphoglycerate peak and exclusion of this can avoid under estimation of the PCr:ATP ratio, particularly when the 2,3-DPG peak is greater than the γ-ATP peak.

4.1.3 Cardiac energetics and metabolism

Cardiac energetics refers to the study of high-energy phosphates and their metabolism in the myocardium. High-energy phosphates such as phosphocreatine allow energy storage and rapid utilisation when required. Phosphocreatine functions as a reserve of energy and provides ATP when required for contraction and other ATP dependent enzyme reactions and shuttles. The process of PCr to ATP conversion is outlined in Figure 4-1 below. Therefore the real end product of oxidative phosphorylation is the formation of PCr.

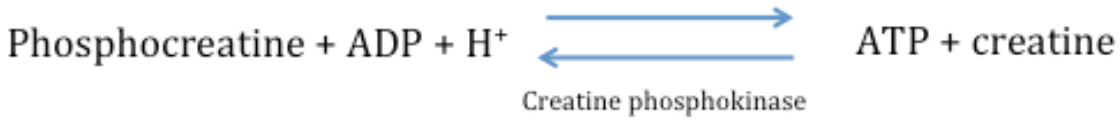


Figure 4-1 Phosphocreatine and ATP energy transfer

The equilibrium shown above favours the production of ATP by approximately 50 times. The amount of ATP is maintained for utilisation and therefore during times of greater demand PCr is converted to ATP and therefore the overall PCr:ATP ratio falls. ATP is subsequently hydrolysed to form ADP to create energy and then reformed from the interaction with PCr. Therefore ATP levels may eventually fall, only when PCr is substantially depleted. A total decrease in the creatine pool may also eventually reduce the PCr:ATP ratio.

The creatine phosphokinase (CK) shuttle is involved in providing ATP to the sites that most require it. The mitochondrial CK isoenzyme is situated between the inner and outer mitochondrial membrane and therefore able to form PCr from creatine. The PCr formed this way, is transferred to the cytosol, where it can be transferred to other sites within the cytosol and utilised. The outer mitochondrial membrane is freely permeable to PCr. The CK shuttle provides ATP to sites that require ATP other than the mitochondrion, such as the myosin ATPase, sarcoplasmic reticulum Ca-ATPase and the Na-K exchanger and therefore plays a role in functional compartmentalisation of ATP. To allow this process, CK is present in different extra mitochondrial sites, with a larger proportion of the MB isoenzyme in the cytosol and MM isoenzyme in the myofibrils. This forms the CK shuttle, where energy is transferred from one site in cytosol to another.

The ratio of PCr:ATP is an indicator of the energetic state of the heart and can be affected in certain heart disorders. It is this ratio that is commonly measured by ^{31}Phosporus MRS with a normal ratio in a healthy individual measured at approximately 1.8 (Bottomley 1994), with a range of 1.1 to 2.5 (Hudsmith and Neubauer 2009) and a likely decrease in PCr:ATP ratio with increasing age (Kostler, Landschutz et al. 2006). This large variability is mostly due to methodological variation, due to differences in acquisition protocols, localisation techniques, spectral analysis, spectral selection criteria and correction calculations.

The aim of this study was to validate the method and techniques in acquiring a magnetic resonance spectrum, using ^{31}Phospurous MRS on a 3-Tesla Philips Achieva whole-body magnet, to measure high-energy phosphates and evaluate the ratio of phosphocreatine to ATP in healthy cardiac tissue. Thereby assess the reproducibility of the technique,

acquisition protocols and spectral analysis in acquiring phosphocreatine to ATP ratios by our group, and hence its applicability to other research areas within the department.

4.2 Methods

4.2.1 Patient selection

Ethical approval was sought and obtained from the University of Birmingham. All work was carried out according to the principles of the declaration of Helsinki. Healthy volunteers with no history of cardiovascular disease over the age of 18 were recruited into the study. Each participant gave written informed consent. Following consent, all participants underwent a detailed history to review and confirm they had no cardiovascular disease, confirm their drug history and smoking status. All patients' height and weight were measured.

4.2.2 Equipment and MRS scanning

All ^{31}Phosphorous MRS scans were performed on a on a 3-Tesla Philips (Philips Healthcare, Reigate, Surrey) Achieva whole-body magnet, using a commercially available 'all purpose' linearly polarized transmit and receive ^{31}Phospurous coil with a diameter of 14 cm.

The scan was performed with the participants in the supine position, with the coil directly over the precordium (ideally over the left ventricle). All scans were performed between 08:00 and 10:00 after an overnight fast. In this validation study, two cardiac MRS scans were performed per participant in one sitting. The second scan was performed after a period of rest following their 1st scan, but after repositioning the participant and coil.

4.2.2.1 Acquisition protocol

The coil was secured in place by straps around the upper body and coil. The participants were then positioned inside the magnet with the centre of the coil at the isocenter of the magnet. The coil has a small disk buried in the centre containing both water and methylphosphonic acid that can be seen with the use of proton localisation images. Localization was achieved by image selected in-vivo spectroscopy (ISIS) volume selection. The position of the coil was checked with a survey image. The subjects and/or the coil was repositioned if required to ensure that the distance between coil to the cardiac septum and apex of the heart is minimized to allow for maximize signal strength and to maintain consistency. These surveys were repeated to achieve an optimum placement.

Localized iterative 1st order shimming was performed with an image guided shim volume that included the entire heart, using the unsuppressed water signal acquired with the body coil as reference. The shimming process involves an automated Hydrogen-1 spectral acquisition to test the quality of the shim that is expressed as a full width at half maximum (FWHM). This is repeated until a FWHM of less than 40 Hz is achieved to maintain consistency and achieve a good quality shim. The process of shimming reduces the infield inhomogeneties in the region of interest and the narrower the FWHM peak the better the achieved shim in the region of interest.

A short axis cine scan was acquired to calculate the trigger delay for ECG triggering and to check quality of shimming and F_0 determination. The trigger delay was calculated such that the spectrum was acquired in the diastolic period when the heart is as still as possible, hence spectral acquisition was ECG gated. To allow for an acquisition time of 170ms, 250-300ms were deducted from the R-R interval of the cardiac cycle. The 3-

dimensional ISIS voxel of acquisition was planned to include most of the septum and apex of the heart within the shimmed area. Care was taken to minimize blood contamination from the right ventricle, liver and skeletal muscle as much as possible. The voxel size was kept constant at 89.54ml (44x55x37mm^3) to allow comparisons between different subjects and scans. A screen shot was taken of the voxel during the 1st scan, which helped with accurate re-positioning of the voxel during the 2nd scan.

Following this the ^{31}Phospurous spectrum was acquired with a repetition time of 10,000ms, 136 averages and 512 samples. A repetition time of 10,000ms was found to be optimal for adequate reduction of saturation effects without increasing the scan time greatly. The total scan time was approximately 23 minutes. The total time the patient was in the scanner including set up time was approximately 48 minutes.

A standard phosphorus spectroscopy sequence provided by the manufacturer was used. It was based on hyperbolic secant pulses for slice selective inversion and adiabatic half passage radio frequency (RF) pulses for non-selective excitation. In order to minimize increased chemical shift artefacts at 3T, slice selective inversion for ISIS encoding was based on adiabatic hyperbolic secant pulses which achieved a pulse bandwidth between 1300Hz (at a distance of 9cm from the surface coil) and 2000Hz (at a distance of 3cm from the surface coil). This corresponds to a chemical shift displacement of 6-10% for the investigated metabolites PCr and γ-ATP for volumes of interest that were between 3 and 9cm from the coil (Shivu, Abozguia et al. 2010).

4.2.2.2 Analysis of the spectra

The spectra were analysed and quantified on the Java based Magnetic Resonance User Interface (JMRUI) software. Post-processing of the acquired spectra was performed

with 15Hz Gaussian line broadening filter and subsequent Fourier transformation. Phase correction was performed with PCr peak as the reference peak. Quantification was performed with Advanced Method for Accurate, Robust and Efficient Spectral (AMARES) fitting time domain fitting program. This allows for and involves selecting peaks and defining their line width (Vanhamme, Sundin et al. 2001). The concentrations of PCr, ATP (γ, α and β) and 2,3-Diphosphoglycerate (2,3-DPG) were calculated as the area under the peaks. The PCr/ATP ratio was determined after correcting the γ ATP peak for blood contamination as described previously based on the quantity of 2,3-DPG in the derived spectrum (Conway, Bottomley et al. 1998).

Cramer Rao lower bounds (CRLBs) were then calculated to assess the quality of the spectral fit. The CRLB is the lower bound on the variance of any unbiased estimator. In other terms it is the lowest possible standard deviation of all unbiased model parameter estimates obtained from the data. CRLBs are widely used as a measure of attainable precision of parameter estimates (Cavassila, Deval et al. 2001). The software used for the analysis (JMRUI) generates a standard deviation for each measured peak. Using this, participants that had a CRLB greater than 20% for the PCr peak were excluded from further analysis. The 20% threshold has been previously used with this technique in clinical studies and in published validation reports (Lamb, Doornbos et al. 1996; Abozguia, Elliott et al. 2010).

4.2.3 Analyses for validation

Analysis of the PCr:ATP ratio prior knowledge, which is an automated peak identification process (an option that is available within JMURI and AMARES software), was compared to manual peak selection (manual identification of the peak by the

operator). The PCr:ATP ratio was measured in two forms; using an average of the ATP peaks (γ, α and β) and using the γ-ATP peak alone to calculate PCr:γ-ATP ratio, for the 1st scan only.

Intra-subject variability was assessed by the absolute difference between the value of the PCr:ATP ratio measured at the 1st and 2nd scans for each subject. The co-efficient of repeatability was calculated and the data plotted in the form of a Bland and Altman plot. Inter-subject variability was assessed by calculating the co-efficient of variation of PCr:ATP values across the 1st scans of all subjects. Intra-observer variability was assessed by one of the observers assessing all scans twice, with each analysis separated by more than one week.

4.2.4 Statistical analysis

Analysis was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL). Continuous data was assessed for normal distribution and presented as mean ± standard deviation of the mean or median and inter-quartile range. Student's t-test was used to analyse normally distributed data. Difference between measurements between the same participant was analysed using the paired students t-test. Skewed data was analysed by Mann-Whitney U-test. Categorical data was analysed by Fisher's exact test. Paired non-normal data was analysed using Wilcoxon signed rank test. Statistical significance was defined as a $p < 0.05$.

4.3 Results

4.3.1 Basic demographics

The study recruited 16 subjects, of which 4 were excluded due to poor spectral quality. A poor spectral quality prevented accurate quantification of spectral analysis. These patients were also identified by CRLBs greater than 20%. Therefore 24 scans from 12 participants were suitable for analysis. Patient characteristics are outlined in Table 4-1.

Patient characteristics	**N=12**
Age (mean, SD)	28 ± 10
Weight (mean, SD)	83 ± 9.7
Height (mean, SD)	178.5 ± 7.1
BMI (mean, SD)	24.2 ± 8.2

Table 4-1 Patient characteristics for MRS validation

The mean CRLBs for the PCr and γ –ATP peak for the included participants for the 1st and 2nd scans are outline in Table 4-2.

	CRLBs Scan 1	**CRLBs Scan 2**
PCr peak	7.66 ± 6.79	5.96 ± 1.63
γ ATP	10.68 ± 5.49	9.34 ± 2.51

Table 4-2 CRLBs for PCr and ATP peaks

Figure 4-2 shows a typical spectrum obtained through MRS using the acquisition sequence outlined above.

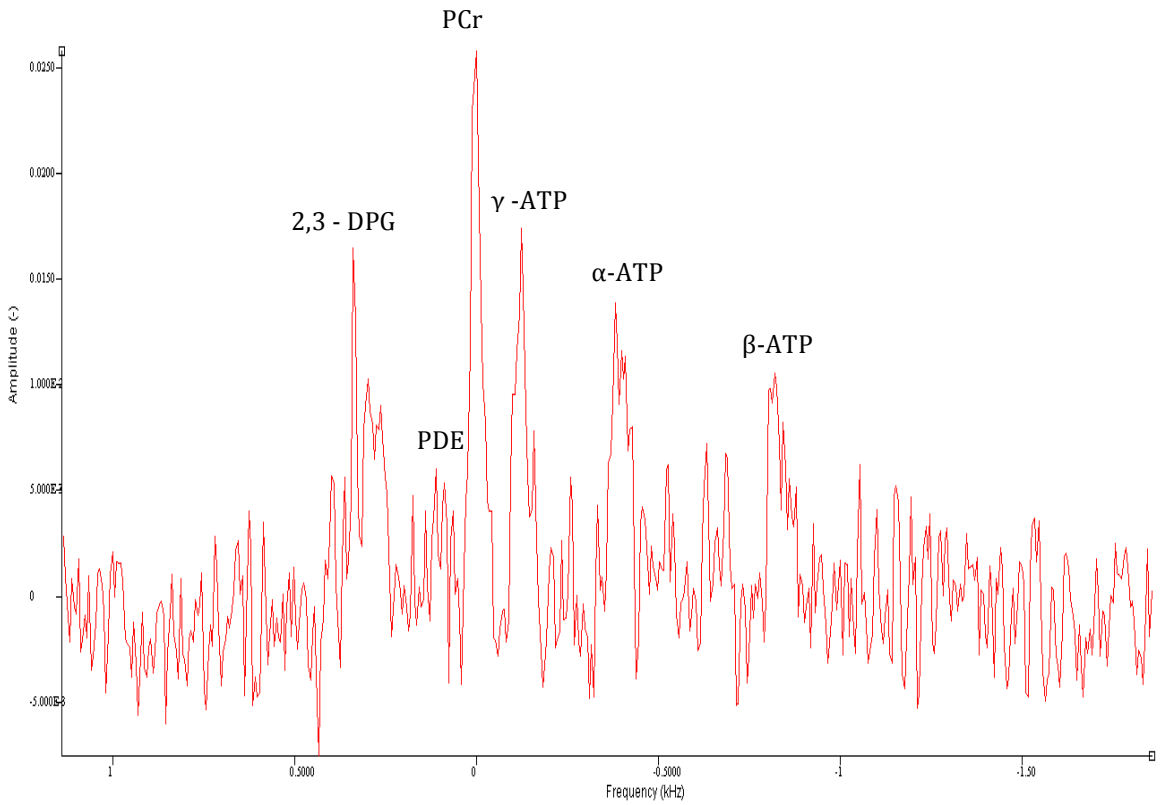


Figure 4-2 Typical MRS spectra with measured peaks labelled

DPG, diphosphoglycerate; PDE, Phosphodiesters, PCr, phosphocreatine; ATP, adenosine triphosphate

4.3.2 Validation analysis

4.3.2.1 Assessment of analysis method

Analysis of the PCr: ATP ratio using prior knowledge gave a higher PCr:ATP ratio for both the 1st and 2nd scans compared to the manual peak defining approach as shown in Figure 4-3. This was statistically significant for both the 1st (p=0.004) and 2nd scans (p=0.002). The standard deviation was higher with prior knowledge compared to manual selection for the 1st and 2nd scans, 0.50 and 0.34 versus 0.33 and 0.28 respectively.

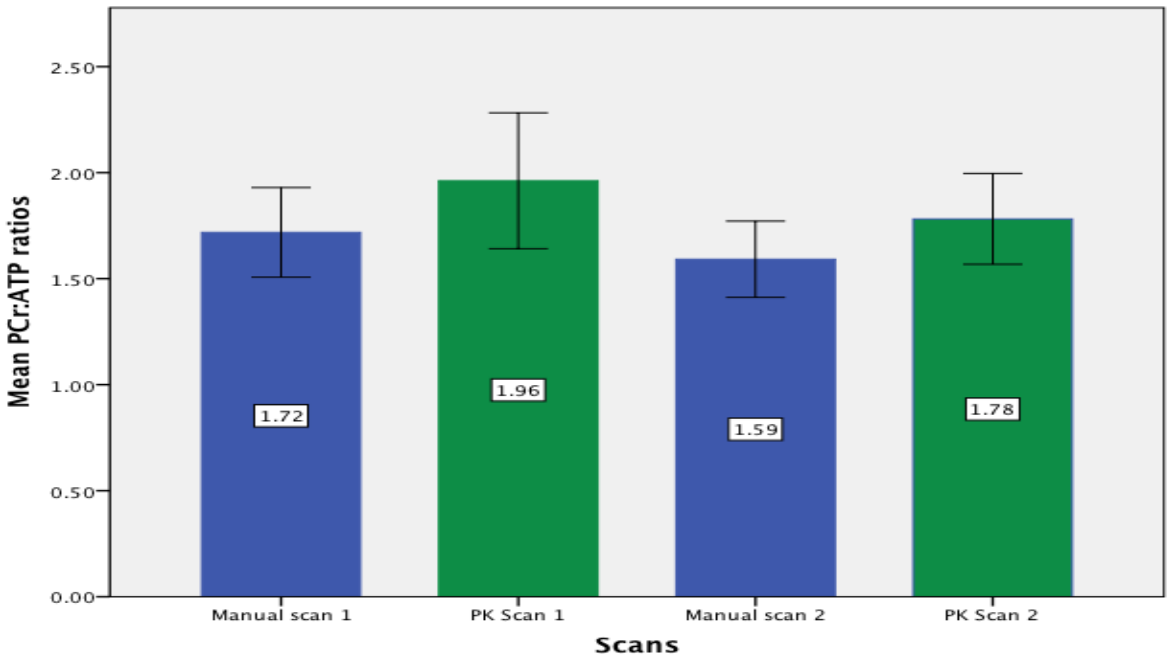


Figure 4-3 PCr:ATP ratios between manual defined and prior knowledge defined peak identification

Analysis of the PCr:ATP ratio by the average of the ATP peaks gave a lower PCr:ATP ratio compared to using the γ-ATP alone and was statistically significant (p=0.005) as shown in Figure 4-4.

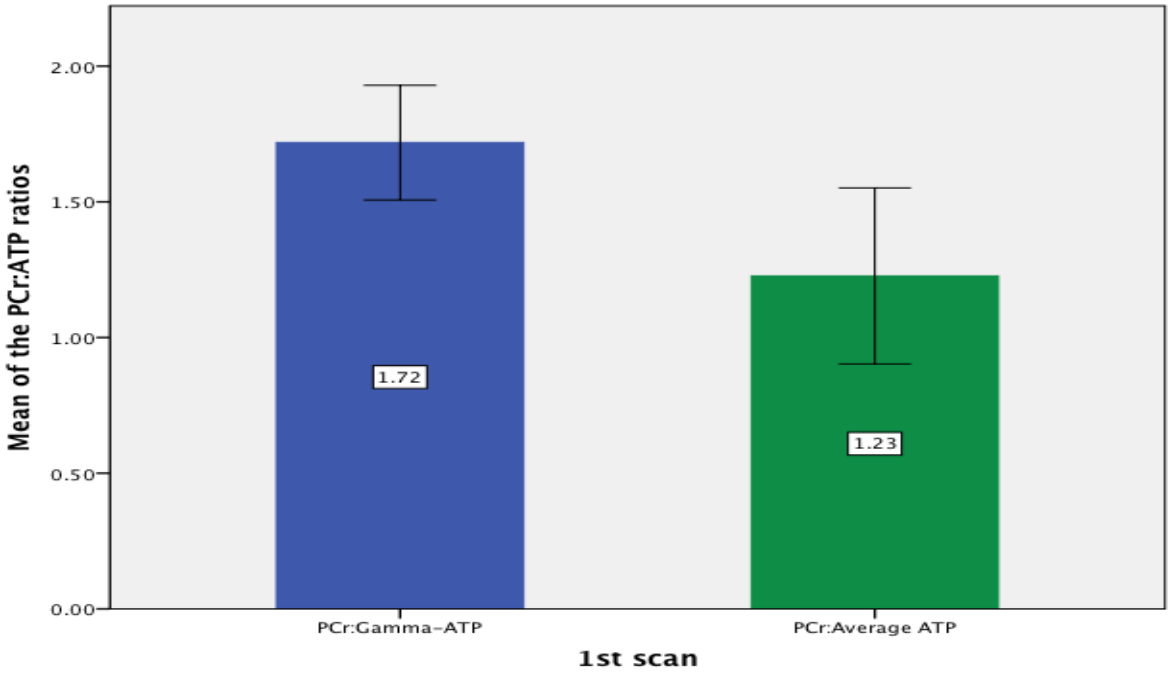


Figure 4-4 PCr:ATP ratios using Gamma ATP versus Average ATP measurements

4.3.2.2 Assessment of reproducibility

The following assessments of reproducibility were performed using a manually selected peak technique and using the γ-ATP alone for calculating the PCr:ATP ratio.

The intra-subject variability was the absolute difference between the 1st and 2nd scans for each subject. The mean PCr:ATP ratio for the 1st and 2nd scans were 1.72 ± 0.33 and 1.59 ± 0.28 respectively, producing a mean difference of 0.13 with a percentage difference of 7.6%. The mean difference between the measurements was 0.13 ± 0.26 (95% confidence interval -0.4 – 0.29, p=0.12). This is graphically represented by the Bland-Altman plot of the data (Figure 4-5). The intraclass correlation coefficient was 0.71 (p=0.10). This correlation is further represented by Figure 4-6.

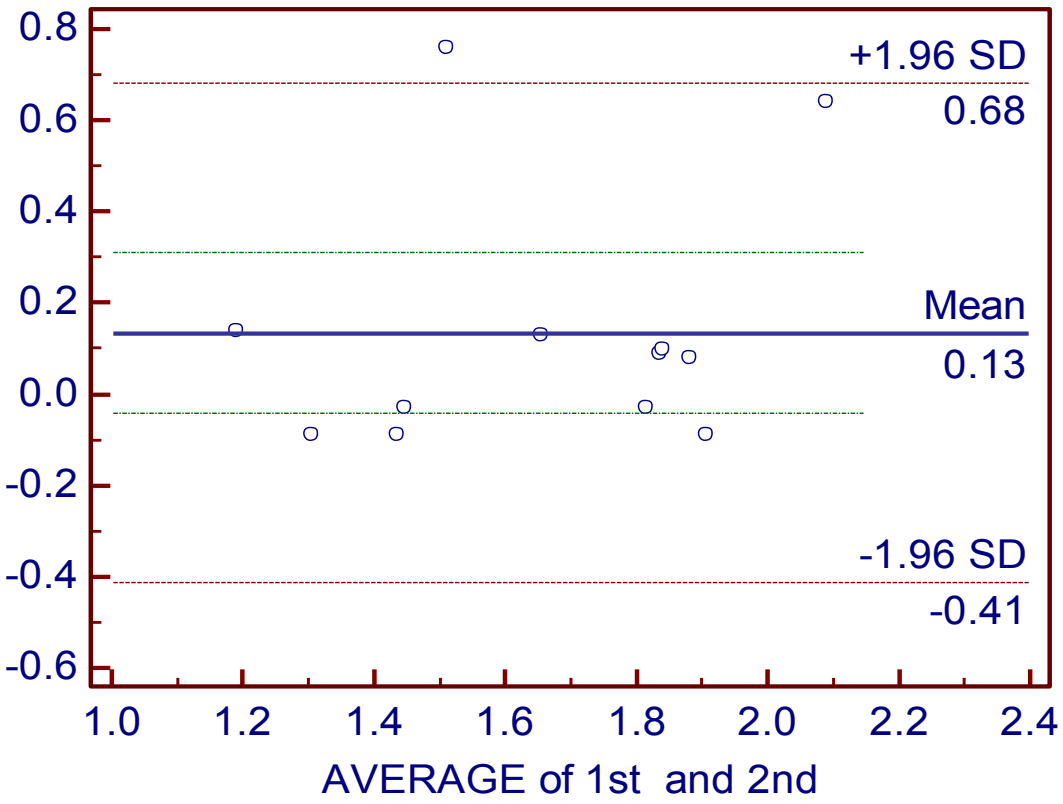


Figure 4-5 Bland-Altman plot of the intra-subject variability

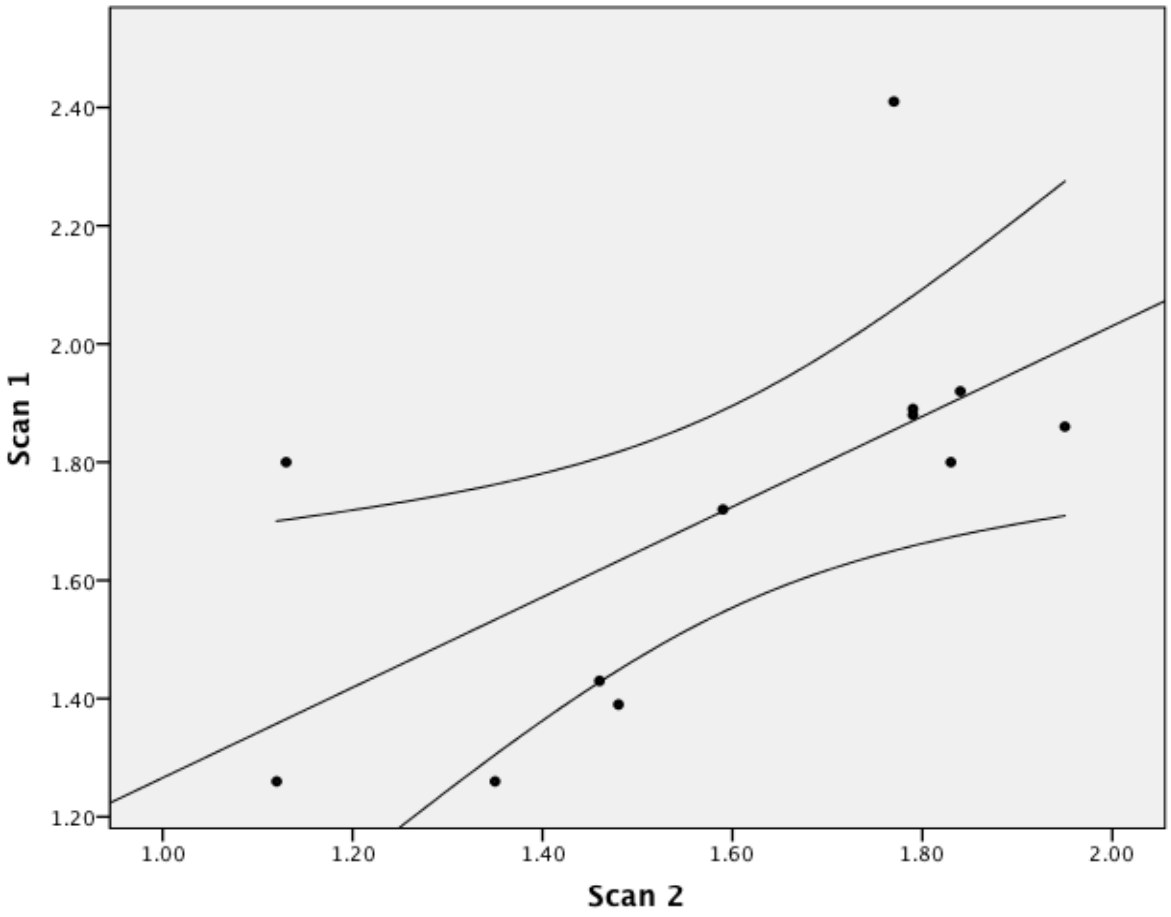


Figure 4-6 Scatter plot of Scan 1 versus Scan 2 with a regression line and 95% confidence intervals

The inter-subject variability was assessed using the average of the 1st scans and was 1.72 ± 0.33 and showed an inter-subject coefficient of variation of 19.3%. The intra-observer variability was calculated by assessing the absolute difference between each scan analysed twice and was 0.001 ± 0.005, showing no significant difference (p=0.34).

4.4 Discussion

This study illustrates the feasibility of performing ^{31}P cardiac Magnetic Resonance Spectroscopy. Using the methodology and spectral analysis techniques described above, this study has demonstrated a normal PCr: ATP ratio of 1.72 ± 0.33 using the manual selected peak technique and using the γ-ATP peak alone, to calculate the ratio.

The acquisition protocol and spectral analysis has proven to be both reproducible and reliable. The CRLBs for this cohort was relatively low at < 11% for both PCr and γ-ATP measurements on both scan sittings. Intra-subject variability was low with a mean difference and percentage difference of 0.13 and 7.6% respectively. In addition the inter-subject variability and intra-observer variability were also low with no significant differences, confirming reproducibility and hence affirming the validity of this methodology.

A number of other groups have performed validation studies of ^{31}P cardiac MRS, however each groups' acquisition protocols, techniques and methods of spectral analysis remains different, and is governed by departmental procedures and type of scanning equipment available (Lamb, Doornbos et al. 1996; Tyler, Emmanuel et al. 2009). This study concentrated on reproducibility of ^{31}P Cardiac MRS at 3-Tesla using a Philips magnet. The PCr: ATP ratios obtained in this study are as expected different to those reported by other validation studies. Tyler et al report a ratio of 2.07 – 2.14 in a group of healthy young males; key differences being their subjects were positioned prone lying on the surface coil, a Siemens magnet and multi-voxel chemical shift imaging acquisition protocol (Tyler, Emmanuel et al. 2009). In contrast Lamb et al report a PCr: ATP ratio of between 1.41 – 1.31 using either 3D image selected in-vivo spectroscopy (ISIS) or a combination of 1D spectroscopic imaging and 2D ISIS (Lamb, Doornbos et al. 1996). This underscores the importance of validating your own MRS protocol particularly when these protocols are used for other cardiovascular research within the department, given that the differing techniques and complexity of the measurement may result in highly variable high-energy phosphate ratios.

The acquisition and analysis protocols used in this study have been developed and optimised over the years. This study has demonstrated that using prior knowledge during spectral analysis gave higher PCr:ATP ratio with larger standard deviation, than if it was measured using manual peak selection. Prior knowledge can make an inaccurate assessment of the 2,3 DPG peak leading to an over correction for blood, resulting in an over estimation of the PCr:ATP ratio. In addition using prior knowledge resulted in greater CRLBs and at times some peaks were missed entirely leading to inconsistency during spectral analysis. When the multiple splitting of ATP into its relative components does not occur due to poor shim quality or it is less obvious, the prior knowledge algorithm fails to define some of these peaks and underestimates the γ-ATP peak, once again leading to over estimation of the PCr: ATP ratio.

In addition this study demonstrated that using the γ-ATP peak alone to measure PCr:ATP ratios resulted in a higher ratio in comparison to using the average of the ATP peaks in combination. The use of the β-ATP peak can lead to errors due to an inhomogeneous RF excitation pulse profile. The use of γ-ATP peak alone can be limited by spectral overlap with the PCr peak (Tyler, Emmanuel et al. 2009). At 3-Tesla the issue of spectral overlap should be reduced due to the field dependent increase in spectral separation. The higher ratios obtained in this study using the γ-ATP alone can be attributed to the decreased intensity of the β-ATP and α-ATP peaks. The band with of the excitation pulse may have been limited and insufficient to excite the whole frequency range of interest; the β-ATP and α-ATP peaks have a large frequency offset compared to the PCr peak therefore the intensity of these peaks are decreased or lost in some spectra. This is in contrast to the study by Tyler at el where an average ATP measurement resulted in greater PCr:ATP ratios than using the γ-ATP alone.

Other validation studies have shown similar levels of reproducibility. Lamb et al demonstrated PCr:ATP ratio percentage difference of 18-21% using either 1D-CSI with 2D ISIS or 3D-CSI acquisition methods. (Lamb, Doornbos et al. 1996). Tyler et al demonstrated a percentage difference of 20% in PCr:ATP ratio using 3D-CSI (Tyler, Emmanuel et al. 2009). This study shows an improved level of reproducibility with a percentage difference of 7.6%. Inter-subject variability reported here is 19.3% comparable to other studies, ranging between 14 – 18% (Lamb, Doornbos et al. 1996; Tyler, Emmanuel et al. 2009).

The sequence used in this study is one that has been optimized following preliminary work carried out by our group (Shivu, Abozguia et al. 2010), which obtained a percentage difference of 12% in PCr:ATP ratios in one subject measured eight times. During this preliminary work, the use of volume suppression bands to avoid contamination from adjacent structures, proton decoupling and the use of Nuclear Overhauser Enhancement (NOE) had been addressed and found no added advantages to the quality of the spectra using the acquisition sequence described here. NOE can increase the SNR, but this can impart different amounts of energy to ATP and PCr and hence alter the PCr:ATP ratio which would need additional correction (Shivu, Abozguia et al. 2010). Therefore the additional techniques that may improve spectral quality were not evaluated in this validation study.

In this validation study four participants were excluded due to poor spectra quality and CRLBs being >20%. These participants were recruited at the initial stages of the study and at that stage the shimming process had just been optimised. Poor shimming leads to broader and ill-defined peaks, which are difficult to analyse and hence less accurate. The ISIS process used in this study uses a larger shimming volume of interest, using a

large voxel that covers most of the myocardium. This larger area increases the heterogeneity (significant blood contamination) and the increased movement of the myocardium makes it more difficult to achieve satisfactory results. The 3D CSI technique using a smaller voxel may improve these results (Tyler, Emmanuel et al. 2009)

The cohort of subjects consisted of a younger aged population and this can be considered a limitation of this study, in particular for the assessment of normal PCr: ATP ratio. However, we intended to assess the reproducibility of our techniques and therefore it is accepted that the PCr: ATP ratio of this study may reflect that of a younger aged population. Other limitations of this study remain those that exist of any study performing cardiac MRS. These include high signal to noise ratio, large voxel size (greater with ISIS; technique used here) and the distance of the transmitter/receiver coil placed on the chest wall to the targeted area of acquisition (variable depending on body habitus). The limitation in signal to noise ratio (SNR) impacts on the temporal and spatial resolution and spectral quality, such that there is a compromise between spectral quality and length of scanning time. The advent of the 3-Tesla MRI scanning is thought to improve SNR and hence temporal and spatial resolution (Tyler, Emmanuel et al. 2009).

Spectroscopy has an inherent limitation, where the magnetic resonance has a low sensitivity to the phosphorus signal. This is made worse by motion of the heart and during respiration. Furthermore, although absolute measurement of the metabolites is preferable, this is difficult to perform and requires calibration of the ^{31}P signal to the ^{1}H water signal of the voxel (Bottomley, Atalar et al. 1996).

In conclusion, despite the known limitation of MRS this validation study has shown that the techniques and protocol for ^{31}P cardiac MRS acquisition at 3-Tesla and the subsequent spectral analysis used by our group, is both reproducible and reliable in measuring high-energy phosphates in cardiac myocardium. Therefore these techniques can reliably be used in other cardiovascular research within the department.

5 A STUDY TO ASSESS CARDIAC ENERGETICS AND FUNCTIONAL STATUS IN PATIENTS WITH LEFT VENTRICULAR HYPERTROPHY TREATED WITH PERHEXILINE

5.1 Introduction

Left ventricular hypertrophy is an adaptive response to physiological stressors placed on the left ventricle and therefore can initially be a normal physiological response (Lorell and Carabello 2000). However, over time in patients with aortic stenosis this initial adaptive response to pressure overload results in an abnormal myocardium. As the degenerative aortic stenosis worsens, the left ventricle is subjected to a progressive increase in pressure overload. Therefore the initial adaptive response of the myocardium to become hypertrophied, renders itself to a sub-normal state with deleterious consequences; diastolic dysfunction, impaired energetic state, gene reprogramming and metabolic derangement. These changes and consequences have been discussed in detail in the preceding introductory chapters. If LVH secondary to AS is left untreated by eliminating the cause for pathological LVH (aortic stenosis), the heart begins to fail and patients succumb to heart failure. It is suspected that the transition into heart failure in patients with LVH is dependent on the underlying energetic state (Neubauer 2007) and that in LVH there is reduced high-energy phosphates to supplement cardiac function (Conway, Allis et al. 1991; Beer, Seyfarth et al. 2002).

5.1.1 Left ventricular hypertrophy and cardiac energetics

Measurement of cardiac energetics as described in the previous chapter can be used to evaluate the ratio of Phosphocreatine to ATP, which is an index of the energetic state of the heart (Neubauer 2007). ^{31}P MRS has been used in a number of clinical studies to investigate the cardiac energetic state (Hudsmith and Neubauer 2009). In patients with heart failure there is a reduction in PCr:ATP with an overall reduction in creatine levels, which reduces the ratio of PCr:ATP further (Neubauer, Krahe et al. 1992). This reduced PCr:ATP ratio is associated with other markers of functional status such as the New York Heart Association classes of dyspnoea (Neubauer, Krahe et al. 1992) and echocardiographic assessment of ventricular function: associated with systolic and diastolic dysfunction (Neubauer, Horn et al. 1995; Lamb, Beyerbacht et al. 1999).

There is ample evidence to suggest that the cardiac energetic state is diminished in patients with hypertrophic cardiomyopathy (Jung, Sieverding et al. 1998; Crilley, Boehm et al. 2003). It is further suggested that this energetic derangement is independent of the degree of hypertrophy in patients with hypertrophic cardiomyopathy (Crilley, Boehm et al. 2003). However hypertrophic cardiomyopathy is an inherited disorder and although a decreased energetic state exists it is unclear how much the hypertrophy *per se* contributes towards this. In LVH there appears to be a down regulation of both free fatty acid and carbohydrate utilisation. This is reflected as a low energy state reflected by impaired cardiac energetics, in the presence of increased cardiac energy utilisation and reduced oxygen delivery.

A study by Neubauer and colleagues demonstrated that in patients with aortic stenosis the PCr: ATP ratio was reduced at 1.55 ± 0.12 and the ratio was particularly reduced

further to 1.13 ± 0.03 in a select group of patients who had a left ventricular end-diastolic pressure of > 15 mmHg or when LV diastolic stress was > 20 kdyne/cm^2. This led Neubauer et al to conclude that pressure overload induces significant impairment in cardiac energetics and this is related to the degree of heart failure (Neubauer, Horn et al. 1997). In another study Conway et al demonstrated a PCr:ATP ratio of 1.56 in patients with LVH and in those that had heart failure, the ratio was reduced further to 1.1 (Conway, Allis et al. 1991). This deranged energetic state seems to improve in patients that undergo an aortic valve replacement for aortic stenosis, from 1.28 ± 0.17 to 1.47 ± 0.14 at 9 months post surgery (Beyerbacht, Lamb et al. 2001). Lamb et al demonstrated that with myocardial hypertrophy there is a reduced energetic state without heart failure with a PCr: ATP ratio of 1.2 ± 0.18 and further showed that this energetic deficiency correlated with a diastolic dysfunction (Lamb, Beyerbacht et al. 1999). Furthermore Smith et al showed that there is a 35% reduction in PCr levels in patients with pressure overload LVH and that there is a further 65% reduction in the net ATP flux through CK in patients with LVH and heart failure, compared to those with LVH alone (30% reduction in ATP flux) when compared to normal subjects; therefore stipulates that it is the ATP turnover that distinguishes between the failing and non-failing hypertrophic hearts (Smith, Bottomley et al. 2006).

5.1.2 Metabolic modulation to improve cardiac energetics

The role and use of metabolic modulation using metabolic therapies such as GIK has been extensively discussed in the preceding introductory chapters. In LVH, metabolic therapies to improve substrate utilisation may prove an added benefit especially in the early post ischaemic period following cardiac surgery. There is further evidence that

metabolic modulation through manipulating substrate utilisation, can improve cardiac energetics as measured by ^{31}P MRS.

Perhexiline is an anti-anginal agent and thought to modulate metabolism by promoting glucose substrate utilisation (Ashrafian, Horowitz et al. 2007). This is achieved through inhibition of mitochondrial free fatty acid uptake enzyme carnitine palmitoyl transferase-1 (CPT-1) and CPT-2 (Kennedy, Unger et al. 1996; Yellon and Hausenloy 2007). A recent study by Lee and colleagues demonstrated that treatment with perhexiline therapy shortened phosphocreatine recovery in skeletal muscle after exercise by 34% compared to a placebo group that remained unchanged (Lee, Campbell et al. 2005). This impact on energetics has subsequently been tested in cardiac muscle. Abozguia et al demonstrated that perhexiline therapy improved cardiac energetics (PCr:ATP ratios) from 1.23 ± 0.02 to 1.73 ± 0.02 in patients with hypertrophic cardiomyopathy compared to controls (Abozguia, Elliott et al. 2010).

5.1.3 Functional assessment of metabolic modulation

In addition to improvements in cardiac energetics, Abozguia and colleagues showed a significant improvement in exercise capacity and oxygen consumption measured by peak VO_2 and improvement in symptomatic status in NYHA class (Abozguia, Elliott et al. 2010). These findings corroborate a study by Lee et al showing similar functional improvements with perhexiline therapy in patients with moderate to severe congestive heart failure; VO_2 max increased by 17% and improvement in left ventricular ejection fraction (LVEF) by 42%, long axis systolic function by 20% and myocardial function at rest by 15% (Lee, Campbell et al. 2005). Furthermore Lee et al demonstrated a 24%

reduction in E/E' ratio (a non invasive marker of LV end-diastolic pressure) with perhexiline therapy.

Speckle tracking using standard transthoracic echocardiography can provide another dimension to the assessment of ventricular function in longitudinal, circumferential and radial planes (Amundsen, Helle-Valle et al. 2006; Bertini, Nucifora et al. 2009; Chang, Kim et al. 2010; Kearney, Lu et al. 2012). There is evidence to suggest that LV twist is preferable to LVEF in assessing ventricular function. A study by Bertini et al showed that LV twist and untwisting rates determined by speckle tracking are strongly related to the systolic and diastolic functions respectively with a strong correlation of LVEF to LV twist (Bertini, Nucifora et al. 2009). Torsion is a measure of systolic function and untwisting is a measure of diastolic function. In patients with hypertrophy secondary to hypertension there is a delay in early diastolic untwisting and untwisting rate, which is reduced in parallel to the degree of LVH (Takeuchi, Borden et al. 2007), and may contribute towards LV relaxation abnormalities. In patients with apical hypertrophy secondary to hypertrophic cardiomyopathy, there is a marked reduction in overall twist due to a reduction in apical rotation (Chang, Kim et al. 2010). A further study by Takamura et al has shown a reduced peak systolic strain and peak relaxation rate in patients with hypertensive LVH (Takamura, Dohi et al. 2010).

It is believed that longitudinal strain is governed by the subendocardial layer and is most sensitive to myocardial disease (Bertini, Nucifora et al. 2009). In patients with aortic stenosis, there is a stepwise progressive impairment in longitudinal, circumferential and radial strain and strain rate with increasing AS severity despite normal ejection fractions (EF > 50%) (Ng, Delgado et al. 2011). Furthermore this study showed that the dysfunction started in the subendocardium and progressed to

transmural dysfunction with increasing AS severity. The most useful measurement as a prognostic indicator is global longitudinal strain (GLS) (Mignot, Donal et al. 2010). Kousa et al demonstrated reduced longitudinal strain in patients with concentric and eccentric hypertrophy compared to control (Kouzu, Yuda et al. 2011). Miyazaki et al showed that GLS progressively decreased with an increase in aortic stenosis and there is a strong correlation between GLS and aortic valve area, mean pressure gradient, LV mass index and early diastolic mitral annular velocity (e') (Miyazaki, Daimon et al. 2011). The extent and impact of LVH in these patients warrants further investigation and examination of these parameters may help elucidate the impact of metabolic therapy on this vulnerable group of patients.

The role of perhexiline therapy as a metabolic modulator on myocardial energetics and ventricular function on LVH secondary to AS has not been previously examined. The aim of this study is to measure the cardiac energetic status using magnetic resonance spectroscopy, in patients with only left ventricular hypertrophy (without concomitant IHD) secondary to aortic stenosis and evaluate the impact of metabolic modulation with perhexiline on their high energy phosphates and energetic status. In addition this study aimed to look at the effect of longer perhexiline therapy on left ventricular function using speckle tracking and evaluate the functional capacity of these individuals.

5.2 Methods

5.2.1 Study design

This study constituted a sub-study to the main HYPER trial (Methodology outlined earlier in Chapter 2). Therefore this sub-study was consistent with a double-blind placebo controlled randomised controlled trial design. Study participant received pre-

operative oral loading of perhexiline or placebo. Recruitment into this sub-study was at a single centre (University Hospitals Birmingham).

All ethics and approvals were as per the main HYPER trial outlined in Chapter 2 section 2.2 and were approved as a substantial amendment to the main trial ethics and MHRA approvals.

5.2.2 Patient selection, recruitment and randomisation

Patients listed for an isolated AVR only, were included into this study. Those with concomitant IHD needing a concomitant CABG were not eligible, in addition to the inclusion/exclusion criteria outlined in Table 2-1.

Patient screening, enrolment and randomisation were as per the main HYPER trial procedures outlined in sections 2.9.1 to 2.9.3. Those patients who were willing to undertake a prolonged period of trial therapy as outlined below and were clinically able to await surgery until follow-up investigations were completed, were recruited into this trial. Those who didn't want to undertake a prolonged course of IMP but agreed to participate in the main trial were managed along the main trial protocols.

5.2.3 Sample size and primary outcome measure

The primary outcome is to detect a 20% improvement in PCr:ATP ratio with metabolic manipulation from baseline. PCr:ATP ratio in patients with LVH is 1.3±0.3, with a difference between means of 0.26; SD 0.3 (Beer, Seyfarth et al. 2002). To achieve an improvement of 20%, with a α 0.05 and β 0.9 would require 34 patients. An improvement of 20% in PCr:ATP ratio would mean a near normalisation of this index.

5.2.4 Baseline studies

All patients had their height and weight recorded on arrival before their baseline investigations. Venous blood was taken for baseline full blood count, urea and electrolytes and liver function tests. Additional venous blood was taken into Z Serum Sep Clot Activator, K3E K3EDTA and Z Serum clot Activator (plain) vacuette bottles and immediately centrifuged at 3000g for 5 mins. The supernatant was pipetted out into NUNC cryotubes and frozen at -80 °C for later analysis.

In addition to the above baseline blood samples a transthoracic echocardiography, Cardiac MRS and a 6-minute walk test were also performed in that sequence at baseline, before administration of trial therapy. These tests were then repeated 4 weeks after trial therapy (placebo/perhexiline), before their cardiac operation.

5.2.5 Transthoracic echocardiography

5.2.5.1 2-Dimentional echocardiography

A standard TTE (Vivid 7, GE Vingmed Ultrasound, Horten, Norway) was performed with subjects in the left lateral decubitus position by an experienced blinded echocardiographer using second harmonic imaging and an M3S multi-frequency transducer. All parameters were measured in triplicate and averaged as per the recommendations of the American Society of Echocardiography (Schiller, Shah et al. 1989).

Analysis was performed offline by a single blinded observer using an EchoPAC workstation (GE Vingmed Ultrasound, Horten, Norway). Ventricular dimensions, wall thickness, chamber volume, stroke volume and ejection fraction were determined by

standard methods (Lang, Bierig et al. 2005). Resting left ventricular diastolic function was determined using standard techniques (Nagueh, Appleton et al. 2009). Tissue Doppler assessment of ventricular function was measured using peak systolic (S'), early diastolic (E') and late diastolic (A') mitral annular velocities at end expiration at the anterolateral, inferioseptal, inferior and anterior left ventricular walls with real time pulsed wave tissue Doppler (Alam, Wardell et al. 1999).

5.2.5.2 Speckle tracking echocardiography

Greyscale images for 2-dimensional left ventricular strain was acquired in cine-loop format in triplicate from the apical 4-, 2- and 3-chamber views and parasternal short axis views at basal, papillary and apical ventricular levels at end expiration at frame rates >70Hz for offline analysis using commercially available software (Speqle Tracking, GE Healthcare, United Kingdom).

The endocardial border was manually tracked at end-systole and the software generated a region of interest over the myocardium. This generated region was then adjusted and optimised manually. This enables frame-to-frame tracking of ultrasonic speckles that change position according to surrounding tissue motion throughout the length of the cardiac cycle. Peak systolic velocities, strain, strain rate, rotation and twist were measured for each myocardial segment in triplicate and averaged. Peak systolic longitudinal strain was calculated by averaging the peak systolic values of the six segments in the four-chamber view and expressed as Global Longitudinal Strain (GLS) and integrated from time to give global longitudinal strain rate (GLSR).

Rotation of the heart refers to the rotation of the short axis segments of the left ventricle. The left ventricle has a spiral architecture formed by the myofibrils. Therefore

when viewed from the apex, the base of the LV rotates in a clockwise direction and the apex rotates in a counter-clockwise direction. This opposing motion leads to a left ventricular wringing motion during systole and is referred to as twist. Left ventricular twist is the net difference at isochronal time points between apex and base along the left ventricular longitudinal axis. Left ventricular torsion is the twist corrected by the distance between the apex and the base. These results are integrated over time to give torsion and untwist rates.

5.2.6 Cardiac Magnetic Resonance Spectroscopy

Cardiac MRS was performed according to the acquisition and analysis protocols outlined in the chapter sections 4.2.2.1 and 4.2.2.2 respectively. This technique was subjected to a validation study performed to assess the reproducibility and reliability of cardiac MRS outlined in Chapter 4. Therefore the techniques used in this study follow the principles outlined in the preceding chapter.

5.2.7 6-minutue walk test

All patients performed a 6-mintue walk test as per the American Thoracic Society guidelines (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories 2002) This involved walking between 2 set points, 54 meters apart. The total distance walked in 6 minutes was calculated. The resting and post 6-minute walk symptoms of dyspnoea and fatigue were recorded as per the Borg scale (Borg 1982), outlined in Appendix 9.13.

5.2.8 Trial therapy dose adjustment

Patients who entered this sub-study were given an extended period of trial therapy for 4 weeks. Hence these patients did not have an initial loading dose, but were started on 100mg bd of perhexiline/placebo after the base line investigation.

Trial therapy levels were monitored between 1-2 weeks after starting the IMP to avoid toxicity related to perhexiline therapy; a venous blood sample was taken and sent for perhexiline level analysis. An unblinded contact was informed of the perhexiline levels and they individually contacted the participant to optimise the dosage requirements. Dose adjustments were made according to the dosing regime in Appendix 9.14.

5.2.9 Study management protocols

After undergoing the above studies at baseline and four weeks post randomisation and trial therapy administration, these patients were included into the main trial and followed the methodology as outlined in Chapter 2. Therefore patients enrolled into this sub-study were operated when they were on the 4^{th} week of their trial therapy. All methodology and trial protocols as per the main HYPER trial were followed thereafter as outlined in sections 2.11 to 2.15.

5.2.10 Statistical analysis

Analysis was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL). Continuous data was assessed for normal distribution and presented as mean ± standard deviation of the mean or median and inter-quartile range. Student's t-test was used to analyse normally distributed data. Difference in measurements between the same participants was analysed using the paired students t-test. Skewed data was

analysed by Mann-Whitney U-test. Categorical data was analysed by Fisher's exact test. Paired non-normal data was analysed using Wilcoxon signed rank test. Statistical significance was defined as $p < 0.05$.

5.3 Results

5.3.1 Consort flow diagram

Recruitment into this sub-study started in October 2010. The consort flow diagram (Figure 5-1) outlines the number of patients screened for eligibility, the reasons for their exclusion and pathway of patients post randomisation as based on the locked database. Based on the exclusion criteria as per the main HYPER trial, the majority of patients (n=20) had aortic regurgitation and the remainder had diabetes or atrial fibrillation. Of those eligible to enter this sub-study, 18 patients did not want to take a prolonged period of IMP or have the additional baseline and follow-up investigations. These patient, were enrolled into the main HYPER trial.

Following randomisation into this sub-study, one patient in the control group was excluded from the sub-study; a baseline magnetic resonance spectroscopy was of inadequate quality for subsequent analysis despite multiple attempts to acquire an analysable spectrum. This patient therefore didn't undergo any other baseline investigations and entered the main HYPER trial instead. Two patients in the control group after randomisation into the sub-study were brought forward for early surgery on clinical grounds of urgency. These patients therefore did not complete baseline or FU tests for this sub-study and hence entered the main HYPER trial.

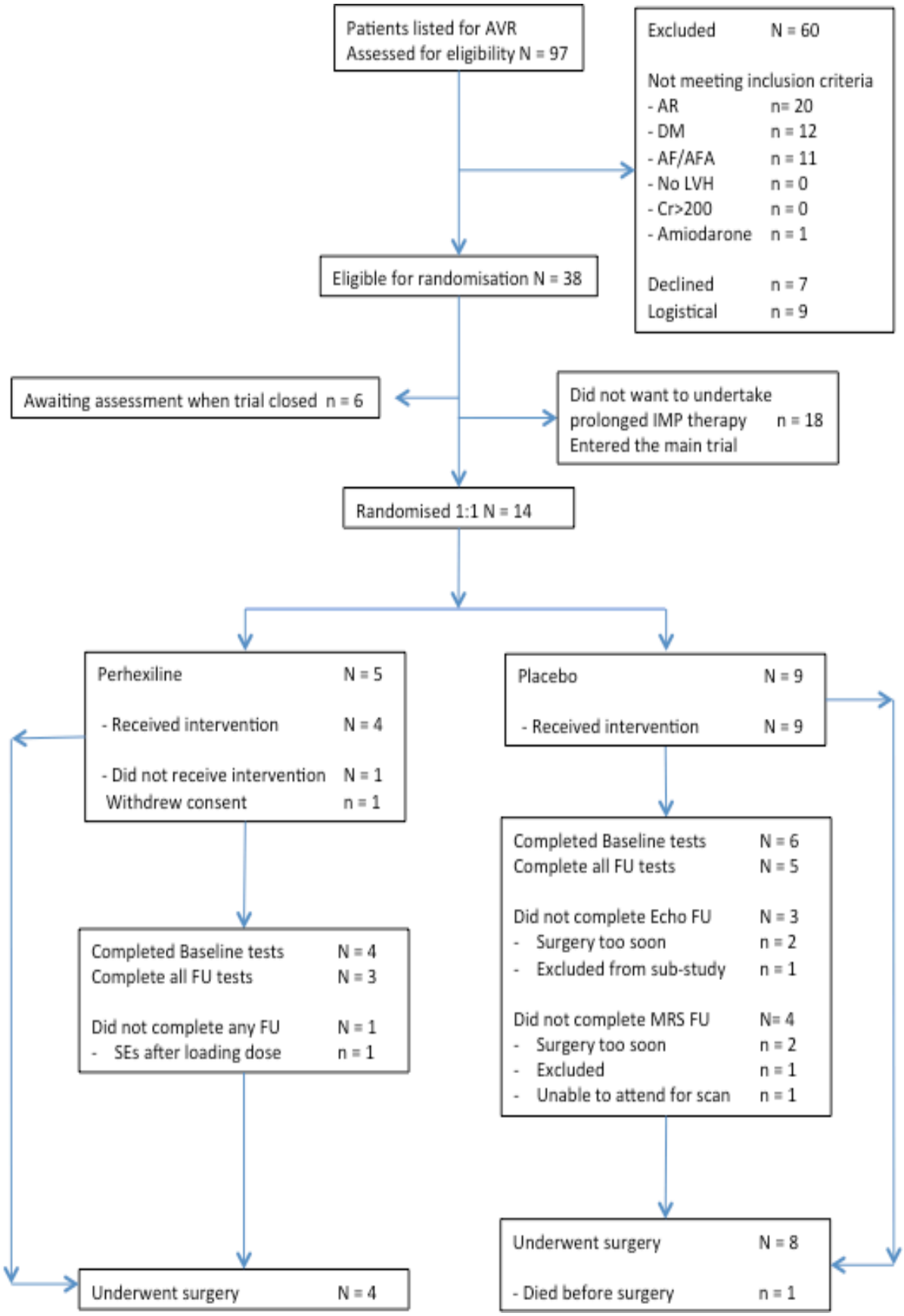


Figure 5-1 Consort flow diagram for patients included into the MRS sub-study

5.3.2 Sample group

The patient demographics and pre-operative echocardiographic data and are outlined in Table 5-1 and Table 5-2 respectively. The echocardiographic data represents the measurements at the time of randomisation prior to trial administration of IMP. Ten patients entered this study.

Variable (Median, IQR)/(%)	Control (n=6)	Perhexiline (n=4)
Age	64.2 (50 – 77)	79 (73 – 82)
BSA	1.81 (1.28 – 2.20)	1.82 (1.59 – 2.06)
Male: Female	4:2 (67:33)	2:2 (50:50)
Caucasian	6 (100)	5 (85)
Status		
- Elective	6 (100)	4 (100)
EuroSCORE	3 (2 – 7)	7 (6.00 – 8.75)
Logistic EuroSCORE	2.12 (1.67 – 7.50)	7.29 (4.80 – 12.66)
CCS		
- 1	3 (50)	3 (75)
- 2	3 (40)	0
- 3	0	1 (25)
NYHA		
- 1	0	1 (25)
- 2	4 (67)	1 (25)
- 3	2 (33)	2 (50)
- 4	0	0
Previous MI	0	0
Risk factors		
- Hypercholesterolemia	0	1 (25)
- Hypertension	1 (17)	2 (50)
Medication		
- ACE	0	0

- A2	1 (17)	0
- Statin	3 (50)	3 (65)
- β blocker	1 (17)	1 (20)
IABP pre surgery	0	0
Coronary artery disease	0	0

Table 5-1 Baseline demographics characteristics of the patient sample

Variable (Median, IQR/%)	Control (n=6)	Perhexiline (n=4)
Ejection fraction (%)	57 (46 – 61)	59 (52 – 64)
LVID (cm)	4.45 (2.70 – 4.72)	4.7 (3.75 – 5.36)
IVSd (cm)	1.65 (1.50 – 1.72)	1.23 (1.01 – 1.70)
PWd (cm)	1.46 (1.10 – 1.55)	1.4 (1.2 – 1.4)
Aortic valve Vmax (m/s)	4.30 (3.81 – 5.30)	4.55 (4.17- 4.85)
Valve area (m^2)	0.70 (0.58 – 0.95)	0.50 (0.40 – 0.50)
Peak Aortic Valve Gradient (m/s)	74.0 (65.3 – 112.5)	82 (63.3 – 101.5)
Mean Aortic Valve Gradient (m/s)	46.0 (38.8 – 62.8)	47.5 (35.5 – 53.5)

Table 5-2 Baseline echocardiographic data of the patient cohort

5.3.3 Perhexiline concentrations

Of the 10 patients entered into the study, four patients were in the perhexiline arm at the time of the baseline investigations. After administration of the investigation medicinal product (IMP), only three completed the follow-up investigations; one patient developed side effects (diarrhoea and vomiting) after the initial 3 days and declined to continue with IMP despite halving the regime. The mean perhexiline and hydroxyl-perhexiline concentrations of those in the treatment group (n=3) were 0.20 ± 0.07 and 1.31 ±0.73 (μg/L) respectively. Of the three, one patient was sub-therapeutic with a perhexiline concentration of 0.14 at follow-up.

5.3.4 Echocardiographic analysis

Of the 10 patients who entered this study, echocardiographic data was available for nine patients (one declined follow-up testing after stopping IMP for SEs) at follow-up (4 weeks after intervention, before their cardiac surgery). The data presented below applies to those that completed the follow-up investigations.

Due to the small number of patients in the treatment arm, normal distribution was not possible even after log transformation. Therefore all analyses are performed using non-parametric testing as outlined above.

5.3.4.1 2-Dimentional echocardiography analysis

The two-dimensional echocardiography data is outline in Table 5-3 for this patient cohort at baseline and at follow-up, between the groups. There was no significant difference in any of the ventricular dimensions, wall thickness, chamber volumes or function at baseline or at follow-up with the IMP. Furthermore, there was no difference in tissue Doppler assessments of ventricular function at either of the measured points with the IMP.

	Baseline			Follow-up		
	Control (n=6)	Perhexiline (n=3)	P	Control (n=6)	Perhexiline (n=3)	P
EF	69.5 (52.3 –77.8)	71.0 (60 – 71.0)	0.71	67.5 (58.8 – 76.3)	77.0 (71.0 – 77.0)	0.26
IVSd	1.25 (1.0 – 1.78)	1.5 (0.7 – 1.5)	1.0	1.3 (1.3 – 1.4)	1.4 (1.1 – 1.4)	0.71
PWd	0.95 (0.8 – 1.2)	1.3 (1.1 – 1.3)	0.10	1.4 (1.4 – 2.0)	1.5 (1.1 – 1.5)	0.90
LVIDd	4.9 (4.5 – 5.5)	4.8 (4.7 – 4.8)	0.91	5.35 (4.72 – 6.2)	4.9 (4.6 – 4.9)	0.17
LVEDV	113 (96 – 153)	107 (102 – 107)	0.91	139 (106 – 195)	114 (98 – 114)	0.17
SV	66 (58 – 93)	73 (72 – 73)	0.38	100 (63 – 128)	85 (48 -85)	0.38
MV E	0.81 (0.57 – 1.02)	0.83 (0.79 – 0.83)	1.0	0.83 (0.65 – 0.94)	0.68 (0.65 – 0.68)	0.38
MV A	0.90 (0.71 – 0.96)	1.12 (0.81 – 1.12)	0.26	0.92 (0.60 – 1.03)	0.88 (0.66 – 0.88)	0.74
E/A	0.89 (0.67 – 1.19)	0.74 (0.59 0.74)	0.91	0.94 (0.73 – 1.33)	0.75 (0.53 – 0.75)	0.38
MV DT	423.8 (375.5 – 490.9)	457 (393.0 – 457.0)	1.0	403.3 (357.3 – 478.7)	411.5 (387.5 – 411.5)	1.0
IVRT	87.3 (59.9 – 109.1)	109.3 (67.0 – 109.3)	0.26	119.2 (89.3 – 139.4)	145.0 (84.3 – 145.0)	0.71
CI	2.41(2.07 – 3.18)	3.87 (0.93 – 3.87)	0.55	2.49 (1.2– 2.6)	3.01 (3.01 – 3.01)	0.29
E/E[1]	12.3 (7.6 – 15.6)	13.7 (11.3 – 13.7)	0.38	13.7 (9.2 – 15.4)	9.62 (9.28 – 9.62)	0.26
TDi AL S	6 (5 – 7)	6 (3 – 6)	0.91	7 (4.75 – 8.25)	7 (5 – 7)	1.0
TDi AL E	6.5 (5.75 – 8.25)	6 (4 – 6)	0.38	6.5 (5.75 – 7.25)	7 (6 – 7)	0.55
TDi AL A	9.5 (8 – 10.25)	8 (8 – 8)	0.26	9.5 (6.75 – 12.0)	8 (8 – 8)	1.0
TDi IS S	6 (4.75 – 7)	5 (5 – 5)	0.55	6 (4.75 – 8)	6 (5 – 6)	0.71
TDi IS E	4.5 (4 – 6)	5 (4 – 5)	0.71	5.5 (4.75 – 10.25)	5 (5 – 5)	0.71
TDi IS A	7 (5.5 – 8.25)	8 (7 – 8)	0.38	8.5 (7.75 – 12.0)	9 (7 – 9)	1.0
TDi A S	6.5 (5 – 7)	8 (5 – 8)	0.38	6.5 (5.75 – 7.75)	7 (5 – 7)	1.0
TDi A E	6.5 (4.75 – 8.25)	6 (6 – 6)	0.91	6.5 (5.75 – 8.25)	5 (4 – 5)	0.10
TDi A A	9 (8.75 – 11)	10 (9 – 10)	0.38	10.5 (6.5 – 14)	8 (8 – 8)	0.55
TDi IN S	6 (5 – 6.5)	7 (7 – 7)	0.38	7 (5.75 – 9)	6 (6 -6)	0.71
TDi IN E	6 (5- 7.25)	6 (5 -6)	0.17	5.5 (4 – 6)	6 (5 – 6)	0.55
TDi IN A	10.5 (8.75- 11.5)	10 (8 -10)	0.71	10 (9.5 – 12.25)	8 (8 – 8)	0.55

Table 5-3 2D Echocardiographic data at baseline and follow-up

EF, ejection fraction; IVSd, Inter-ventricular septum diameter in diastole; PWd, Posterior wall diameter in diastole; LVID, Left Ventricular Internal Diameter in diastole; LVEDV, left ventricular diastolic volume (mL); SV, Stroke volume; MV E, mitral valve E wave velocity (cm/s); MV A, mitral valve A wave velocity (cm/s); E/A, mitral valve E to A ratio; MV DT, mitral E wave deceleration time (ms); IVRT, isovolumetric relaxation time (ms); CI, cardiac index (l/min/m^2); TDi, tissue Doppler imaging during systolic (S), and passive (E) and active (A) relaxation from AL (anterolateral), IS (inferoseptal), A (anterior) and IN (inferior) wall peak annular velocities.

5.3.4.2 Speckle tracking echocardiography

The speckle tracking echocardiography data is outline in Table 5-4. The baseline characteristics between the groups are not comparable. Through non-parametric testing, characteristics of basal rotation, twist, torsion, peak twist, systolic twist rates and untwist rates were not comparable albeit with small statistical significance. However post IMP therapy speckle tracking echocardiography failed to demonstrate a change in any of the measurements including twist, twist rates, torsion or in left ventricular strain between the groups.

	Baseline			Follow-up		
	Control (n=6)	Perhexiline (n=3)	P value	Control (n=6)	Perhexiline (n=3)	P value
Apical rotation	14.0 (10.2 – 117.5)	15.0 (14.3 – 15.0)	0.55	15.0 (7.8 – 18.4)	14.8 (8.1 – 14.8)	1.0
Basal Rotation	-6.5 (-7.8 – -3.8)	-11.9 (-16.9 – -11.9)	**0.02**	-9.8 (-11.3 – -3.3)	-8.4 (-8.7 – -8.4)	0.55
Twist	19.3 (14.6 – 25.7)	30.1 (26.8 – 30.1)	**0.02**	22.4 (12.3 – 27.6)	23.3 (5.4 – 23.3)	1.0
Torsion	2.3 (1.7 – 3.2)	4.2 (3.8 – 4.2)	**0.02**	2.9 (1.6 – 3.7)	3.5 (0.8 – 3.5)	0.71
Peak Twist	16.5 (12.0 – 24.0)	27.3 (25.6 – 27.3)	**0.02**	20.9 (12.8 – 26.8)	21.9 (12.1 – 21.9)	1.0
Time to peak twist	559 (519 – 597)	508 (413 – 508)	0.17	510 (454 – 540)	466 (78 – 466)	0.26
Peak Untwist	4.0 (1.1 – 5.8)	7.6 (-0.02 – 7.6)	0.39	4.9 (-1.0 – 108.7)	95.4 (6.6 – 95.4)	0.26
Time to peak untwist	843 (708 – 932)	778 (704 – 778)	0.79	740 (524 – 875)	551 (300 – 551)	0.71
Systolic twist rate S	97.5 (69.5 – 116.6)	169.2 (145.1 – 169.2)	**0.02**	64.4 (-63.7 – 117)	131.5 (-61.3 – 131)	0.71
Untwist rate E	-91.2 (-144.5 – -76.6)	-120.5 (-180.4 – -120.5)	0.91	- 125.0 (-170.0 – -92.1)	-148.5 (-207.8 – 148.5)	0.91
Untwist rate A	-59.1 (-84.1 – -45.8)	-120.1 (-127.4 – -120.1)	**0.05**	-79.8 (-95.8 – -74.8)	-83.3 (-107.0 – -83.3)	1.0
GLS	-13.1 (15.8 – -10.8)	-14.4 (-17.2 – -14.4)	0.55	-14.1 (-15.2 – -9.6)	-14.5 (-15.5 – -14.5)	0.38
GLSR S	-0.73 (-1.03 – -0.63)	-1.04 (-1.04 – -1.04)	0.10	-1.01 (-1.18 – -0.63)	-1.3 (-1.58 – -1.3)	0.17
GLSR E	0.88 (0.78 – 1.13)	1.22 (1.0 – 1.22)	0.17	1.21 (0.65 – 1.31)	1.28 (1.14 – 1.28)	0.71
GLSR A	1.11 (0.87 – 1.43)	1.06 (0.95 – 1.06)	1.0	1.23 (0.61 – 1.39)	1.46 (1.42 – 1.46)	0.17

Table 5-4 Speckle tracking echocardiography data

Rotation, Peak twist and Peak untwist expressed in degrees (°); Torsion (°/cm); Time to peak twist and untwist (s); Twist rate (°/s); GLS, Global Longitudinal Strain (%); GLSR, Global Longitudinal Strain Rate (%/s) at systolic (S), passive (E) and active (A) relaxation.

5.3.5 MRS analysis

Of the 10 patients who entered this study, all patients underwent baseline MRS assessment. Of these, eight patients had both baseline and follow-up scans performed, prior to their cardiac surgery for subsequent analysis. One patient was unable to attend for their second scan in time, and the other discontinued follow-up testing (stopped IMP due to side-effects). The PCr:ATP ratio for each of the eight participants is outlined in Figure 5-2. Subject number 7 shows a relatively greater increase in PCr:ATP ratio on the follow-up scan. The other subjects show a consistent difference in ratios between the baseline and follow-up scans.

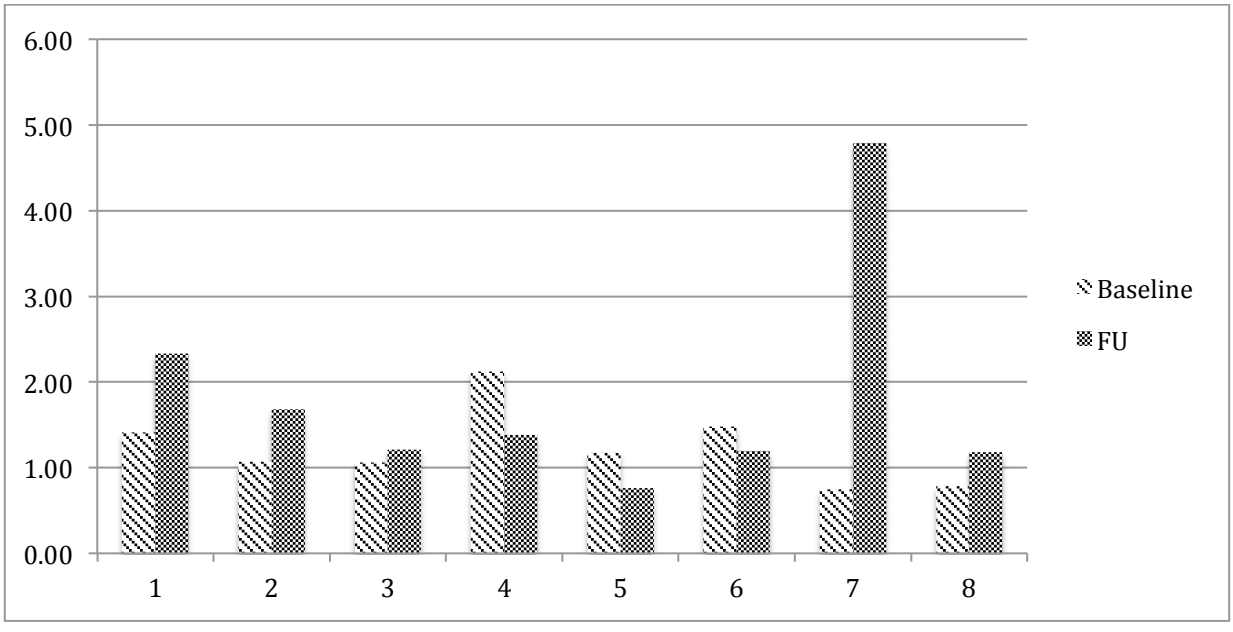


Figure 5-2 PCr:ATP ratio for each of the participants at baseline and follow up

The PCr:ATP ratios for the baseline and follow-up scans for each treatment group did not show a normal distribution and this was consistent even after log transformation. Therefore analysis was performed using non-parametric tests as outlined above. The median and interquartile range for PCr:ATP ratios at baseline and at follow-up are outlined in Table 5-5.

	Control (n=5)	Perhexiline (n=3)	P value
Baseline (median/IQR)	1.17 (1.07 – 1.45)	0.78 (0.74 – 0.78)	0.571
Follow-up (median/IQR)	1.21 (0.99 – 2.01)	1.28 (1.18 – 1.38)	0.786

Table 5-5 PCr:ATP ratios at baseline and follow-up

Statistical analyses showed no difference in PCr:ATP ratios at baseline or at follow up between the groups. Subject 7 may have had a spurious result in their PCr:ATP ratios however, their spectra was within the parameters of CRLBs and hence analysable.

5.3.6 Six minute walk test

Of the 10 patients who entered this study, the six-minute walk test was performed on 9 participants at baseline. One patient required a walking stick for daily mobilisation due to osteoarthritis and hence did not undertake this test; this patient was in the treatment arm of the study. Of those that undertook this test at baseline only 6 completed the test at follow-up. One patient failed to attend follow-up testing (discontinued IMP after side-effects), and a further two patients were unable to attend FU tests. These results are outline in Table 5-6.

	Baseline		Follow-up	
	Control (n=6)	Perhexiline (n=3)	Control (n=5)	Perhexiline (n=1)
Baseline Dyspnoea	0 (0 – 3)	0 (0 – 0)	0 (0 – 1.25)	1
End dyspnoea	1.75 (0 – 3.25)	5 (0.5 – 5)	3 (1 – 3.5)	4
Baseline Fatigue	0 (0 – 3.25)	0 (0 – 2)	0 (0 – 1)	2
End fatigue	1 (0 – 3.25)	6 (0 – 6)	0 (0 – 3.5)	3
Baseline HR	66 (60 – 77)	80 (68 – 80)	80 (65 – 80)	92
End HR	89 (75 – 109)	104 (88 – 104)	88 (76 – 102)	92
Total distance	450 (396 – 468)	324 (324 – 324)	403 (350 – 438)	364

Table 5-6 Baseline and follow-up 6-mintue walk test

Statistical analysis showed no difference between the treatment and control groups at baseline. An expected increase in HR at the end of the 6-mintue-walk test is evident as is a slight increase in the dyspnoea and fatigue status (measured using the Borg scale) at the end of the test compared to these findings before starting the walk test. Statistical analysis at follow-up was futile due to the small sample size in the treatment arm at follow-up.

5.4 Discussion

This study was designed to assess the impact of metabolic modulation with perhexiline on a homogenous group of patients with LVH secondary to AS by evaluation of their cardiac energetic status and functional capacity after an extended duration of IMP therapy. Perhexiline did not improve the cardiac energetic status and therefore showed no added benefit. Furthermore, through standard echocardiographic assessment, tissue Doppler imaging and speckle tracking echocardiography, perhexiline therapy failed to

demonstrate an improvement in overall systolic and diastolic ventricular function. Functional exercise capacity assessment was inadequate to comment on the value of perhexiline therapy in this cohort.

In this study, the sample groups were disproportionate from the outset dictated by clinical urgency. There was limited tolerance to purse clinical experimentation when a subjects' clinical condition necessitated early surgery; symptomatic severe aortic stenosis which would otherwise be operated on urgently without delaying 4 weeks i.e. for trial therapy. However, this reflects the demands of a real world trial. These real-world difficulties continued throughout this sub-study; patients reluctant to attend follow-up investigations, unwilling to continue taking IMP due to side effects and the inability to acquire an analysable spectrum despite multiple attempts. Recruitment was made more challenging as most subjects although being suitable, preferred to enter the main HYPER trial, which negated the need to take a prolonged duration of IMP (4 weeks) or undergo any further experimental investigations. Furthermore the main HYPER trial was intrinsically linked to this sub-study due to the same IMP and dosing regimes; therefore under the recommendation of the DSMB of the HYPER trial, this sub-study too had to be halted due to the futility of the IMP being tested. This futility analysis is discussed in greater detail in the discussion chapter later in this thesis.

The three subjects in the treatment group that completed the FU investigations had a mean perhexiline concentration of 0.20 ± 0.07, which fell within the therapeutic range albeit by a marginal 0.05 md/dL. It is possible that despite therapeutic levels, the duration of trial therapy was insufficient to maintain the patients within therapeutic levels for long enough for perhexiline to impart any change on the energetic or functional status. Lee at al who showed an improvement in functional status

administered perhexiline for up to 8 weeks (Lee, Campbell et al. 2005). Similarly Abozguia et al administered perhexiline for a prolonged duration with a mean follow-up duration of 4.6 months and showed significant improvements in energetics and functional status in hypertrophic cardiomyopathy patients (Abozguia, Elliott et al. 2010).

The GLS for patients with severe aortic stenosis in this study was calculated at between -13.1 and -14.4, which is comparable to an earlier study assessing GLS in patients with aortic stenosis (Lafitte, Perlant et al. 2009) and is also comparable to a more recent study examining GLS in asymptomatic AS patients (Donal, Thebault et al. 2011). Kearney et al demonstrated that GLS deteriorated with increasing severity in aortic stenosis in patients with normal LV ejection fraction and correlated with LV mass index and symptom class. Furthermore, GLS was a strong independent predictor of all-cause mortality (HR: 1.38, $p<0.001$), supporting the value of GLS in identifying subclinical dysfunction (Kearney, Lu et al. 2012). This may help identify optimal timing in operative intervention, not just in patients with aortic stenosis but even other heart pathologies. However the value of GLS and GLS rate to evaluate the change in ventricular function towards metabolic therapies is still novel and although perhexiline modulation in this study has not shown any change in this subtle marker of myocardial function, further experimental studies using GLS is warranted.

Previous work that has shown improvements in myocardial energetics and functional status using perhexiline as a metabolic modulator have been on subjects either in chronic heart failure or those with hypertrophic cardiomyopathy (Lee, Campbell et al. 2005; Abozguia, Elliott et al. 2010). In the study by Lee et al the baseline ejection fraction in patients with chronic heart failure was between 20 – 30%, hence these

patients may benefit most from metabolic modulation to improve their energetics, compared to the subjects in this cohort who despite having severe AS had near normal ventricular function. In a RCT conducted in our department, evaluating the role of perhexiline in patients with dilated cardiomyopathy (known to have severe metabolic derangement) with moderate to severe LV impairment, perhexiline showed an improvement in cardiac energetics; myocardial PCr/γATP ratio increased following treatment in the perhexiline group versus placebo from 1.16±0.08 to 1.51±0.11 versus 1.38±0.07 to 1.34±0.07 in the control $P<0.0005$ (Beadle 2013). However the anticipated improvements in cardiac function using 2D and STE, including cardiac efficiency by invasive monitoring were not demonstrated. This study too followed a one-month regime of perhexiline therapy, and this may have dictated the efficacy of perhexiline in showing benefit towards cardiac energetics.

This study further demonstrates the correlation between the metabolic and functional status of the heart to the clinical outcomes as evident from the main HYPER trial. The clinical outcomes as outlined in the main results chapter demonstrate no added clinical benefit with perhexiline in patients with LVH secondary to AS (discussed in further detail in the main discussion chapter). Similarly, given the limitations of this sub-study, the energetic and functional assessments of these patients substantiate the clinical outcomes of perhexiline therapy.

This study is inherently limited by the sample size of the cohort studied. The recruitment target was 34 participants randomised 1:1 to perhexiline and control. This poor recruitment is in part, due to participants' being reluctant to undertake prolonged administration of trial therapy (4 weeks) and attend baseline and follow-up investigations prior to their surgery and also due to the main HYPER trial being halted

due to futility, which prevented any further recruitment into this sub-study due to the same IMP being used in both studies. Therefore this study lacks statistical power to infer strong conclusions on these end-points.

In conclusion, MRS is a feasible mode of experimental investigation to assess myocardial energetics; in particular for those patients who are vulnerable and are known to have a diminished myocardial metabolic state and warrant metabolic modulation. Speckle tracking echocardiography remains a feasible and subtle mode of functional assessment. Perhexiline may remain a potential metabolic modulator in patients who are in heart failure refractory to standard medical therapy, and only in this group would it show maximum metabolic and clinical benefit. Cardiac energetic studies will help extrapolate cardiac function based on other clinical outcomes, in patients treated with an investigational medicinal product during clinical trials.

6 A STUDY LOOKING AT METABOLOMIC ANALYSIS OF THE MYOCARDIUM IN LEFT VENTRICULAR HYPERTROPHY SECONDARY TO AORTIC STENOSIS

6.1 Introduction

The heart is a unique arrangement of muscular tissue that creates four chambers: 2 atria and 2 ventricles that are separated by the atrioventricular valves. The ventricles empty blood into the ventricular outflow tracts via the outflow tract valves. The pathophysiology of left ventricular hypertrophy is discussed in detail in the introductory chapter. In brief, obstruction of the left ventricular outflow tract by aortic stenosis exerts pressure overload on the left ventricle. In order to combat the excessive pressure the LV responds by hypertrophy, thereby changing the normal muscular structure of the heart.

6.1.1 Muscular structure of the heart

The muscular structure of the heart consists of three distinct layers: epicardium, myocardium and endocardium (Stevens and Lowe 2001). The epicardium is the visceral pericardium of the heart and is a thin layer covered with flat mesothelial cells that produce a smooth outer surface. These cells lie on a stroma of fibrocollagenous support tissue that contains elastic fibres. In addition this layer contains the coronary arteries that provide blood to the heart wall and the venous tributaries that carry blood from the heart wall. The coronary arteries send branches deep into the myocardium.

The myocardium makes up the bulk of the heart and is the contractile component composed of specialised striated skeletal muscle, which form a interconnecting network of myocardial fibres separated by loose fibrocollagenous tissue. The left ventricle deals with the majority of the workload, pumping blood into the high-pressure systemic circulation and therefore the myocardial diameter of the LV is greater than other chambers of the heart. The outer surface of the myocardium beneath the epicardium is smooth however the internal surface beneath the endocardium is trebeculated and is most marked in the ventricle. These trabeculations are covered by the smooth endocardium.

The endocardium lines the internal surface of the heart and is composed of three separate layers; layer in direct contact with the myocardium, the middle layer and the innermost layer. The layer in contact with the myocardium is composed of irregularly arranged collagen fibres that merge with the collagen surrounding the muscle fibres within the myocardium. The middle layer is the thickest layer and is composed of more regularly arranged collagen fibres containing variable number of elastic fibres (compact and arranged in parallel). The innermost layer is composed of flat endothelial cells. The endocardium is the thinnest layer in the ventricle.

In hypertrophy there is an increase in the thickness of the myocardium. This occurs as a result of an increase in the number of sarcomeres in the cardiomyocyte. A sarcomere consists of a number of myofibrils that run in parallel, held in place by plates of accessory proteins; sarcomeres are the force generating units in the myocyte. Myofibrils are the contracting elements within the cardiomyocyte and are composed of overlapping thick myosin and thin actin filaments.

6.1.2 Left ventricular hypertrophy and clinical implications

The clinical implications of LVH have been described extensively in the introductory chapter. LVH and concentric remodelling are well known risk factors for peri-operative and midterm mortality (Orsinelli, Aurigemma et al. 1993; Mehta, Bruckman et al. 2001). In an editorial on LVH in aortic stenosis, Yotti and Bermejo conclude the LVH should no longer be considered only as a favourable adaptive mechanism to AS (Yotti and Bermejo 2011). This editorial raises the controversial question on how and if the degree of hypertrophy should influence the timing of surgery and if so how should this be best measured. In a study by Cioffi et al, multivariate analysis proved that inappropriate increase in left ventricular mass was an independent predictor of outcome in asymptomatic patients with severe AS (Cioffi, Faggiano et al. 2011) and this consolidates previous findings of a maladaptive response with systolic dysfunction in patients with LVH in AS (Kupari, Turto et al. 2005).

In recent years LVH has been studied at a cellular level and Selvetella et al describe separate intracellular pathways for an adaptive and maladaptive response to hypertrophy (Selvetella, Hirsch et al. 2004). Furthermore, there is evidence to suggest that in hypertrophy there is a less dense capillary bed (Tomanek, Palmer et al. 1986; Weber and Brilla 1991) and an increased diffusion distance for oxygen and metabolites from the capillaries to the myocyte (Bache, Vrobel et al. 1981; Gosse and Clementy 1995). These changes may in part, increase the susceptibility of the hypertrophic ventricle to the ischaemic insults during cardiac surgery. Fundamentally there is an increase in myocardial muscle mass with a thicker myocardium which is capable of dealing with pressure overload (Lorell and Carabello 2000).

Despite numerous evidence associating LVH to poor clinical consequences and outcomes (prior to or after cardiac surgery) there has yet been no study evaluating the molecular basis or metabolome of the hypertrophied heart muscle per se. Given the extent of hypertrophy and the pathophysiological changes the ventricle undergoes, it may be plausible that the metabolome may differ within the ventricle, from the epicardial to the endocardial surface.

6.1.3 Metabolomics

Metabolomics is the scientific study of the chemical processes that involve the metabolites of a cell or tissue and allows the study of the fingerprint that specific cellular processes leave behind (Griffin, Atherton et al. 2011). A metabolome refers to the collection of all the metabolites within a specific tissue. Detection and analysis of these complex metabolites requires specific research tools. Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy (NMR) based metabolomics are the two main forms of metabolomic study.

NMR relies on the spin properties of the nuclei within a magnetic field, and the frequency at which a nucleus resonates is used to identify a particular chemical structure. Despite its advantages of being fast and reproducible mass spectrometry (MS) is more sensitive at identifying metabolites wth low biological concentrations.

There are two main forms of mass spectrometer based metabolomic analysis: Fournier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer and the Orbitrap mass spectrometer analysis. Mass spectrometry provides a spectrum of the metabolites within a tissue sample by identifying the mass to charge ratio (m/z) of positively or negatively charged ions and measures the abundance of these metabolites (Figure 6-1);

each peak representative of a metabolite at a given m/z and the height of each peak reflective of the abundance of the metabolite (Southam, Payne et al. 2007).

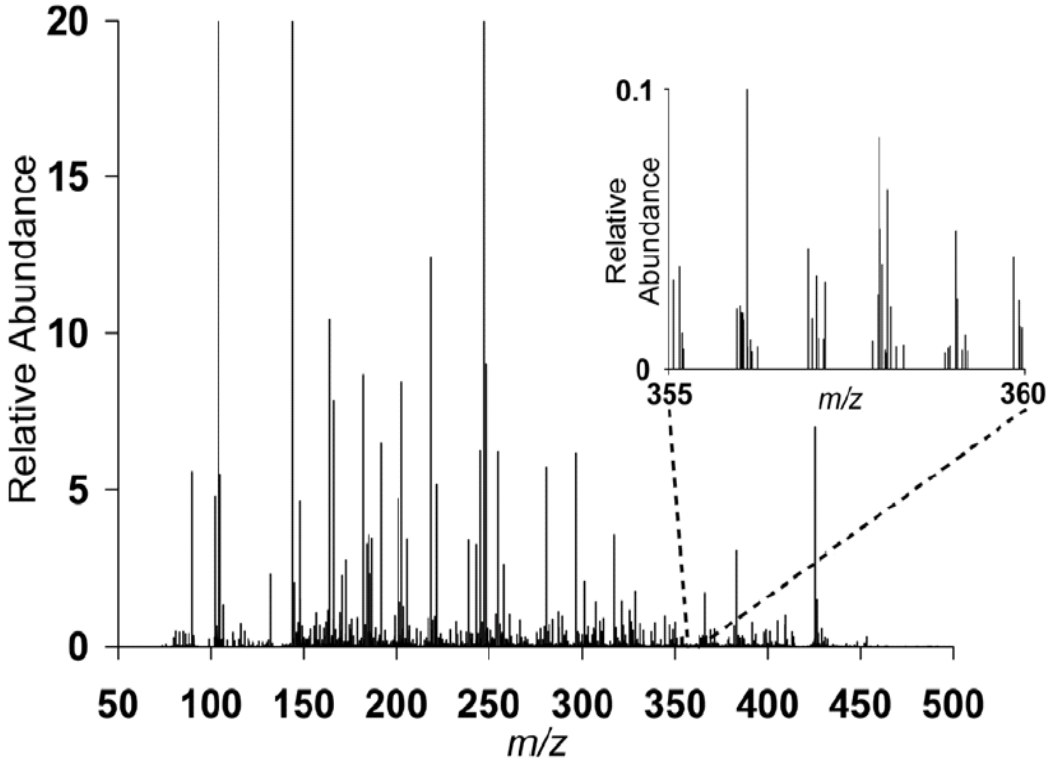


Figure 6-1 An example spectrum using FT-ICR MS

The principle of achieving an optimum metabolomic analysis relies on high mass resolution and high mass accuracy; mass resolution refers to the ability to distinguish specific metabolites with a specific mass to charge ratio and mass accuracy refers to the approximation of the mass measured to the actual mass of the metabolite (Southam, Payne et al. 2007). FT-ICR offers a greater resolution of metabolites and an accuracy of < 1ppm, hence superior to other forms of metabolomic analyses. This therefore narrows down the number of metabolites that can be identified at a given m/z, thereby increasing the metabolite identification rate.

With FT-ICR mass spectrometry, mass is measured by detecting the image current produced by negatively or positively charged metabolites, cyclotroning within a magnetic field. Detectors at fixed locations measure the electrical signals of ions that pass near them over time, forming a periodic signal. The m/z ratio of an ion determines the frequency of the ion's cycling. This allows each ion to be counted more than once and this increases the resolution of FT-ICR MS. Extensive data processing is then required to analyse the spectra obtained.

With complex biological samples there are a large number of ions to be analysed, hence mass accuracy can be reduced. An analysis technique that uses selected ion monitoring (SIM), which records a series of spectrum with small mass ranges and then stiches these multiple SIM spectra, to generate a SIM stitched spectrum improves the overall sensitivity (Southam, Payne et al. 2007). Further data processing and analysis takes place and this includes noise filtration, normalisation, generalized log-transformation and application of false discovery rate during statistical analysis.

6.1.4 Metabolomics with cardiac tissue

Our group have conducted a number of preliminary unpublished studies to optimise the methodology and the technical aspects in tissue preparation of small cardiac tissue samples, and the subsequent data analysis. Following this, our group studied the metabolomic profile of LVH compared to non-LVH cardiac tissue in humans and have shown a significant difference in the metabolomic profile between these groups p=7.91 x 10^5 and 0.0053 in the positive and negative ion modes respectively (Howell 2010). A subsequent study evaluated the metabolomic profile of human left ventricular myocardium and the impact on the metabolome after metabolic modulation with

perhexiline. This latter study showed no difference in the metabolome following perhexiline therapy and concluded that perhexiline has no effect on the myocardial metabolome (Drury 2012)

The aim of this study was to examine the metabolomic profile of the myocardium in LVH secondary to aortic stenosis by comparing the epicardial and endocardial halves of the left ventricle. Thereby distinguishing if there is an inherent metabolomic difference within the myocardium of the left ventricle, which could contribute towards the susceptibility to injury following cardiac surgery and its associated clinical outcomes.

6.2 Methods

6.2.1 Patient sample

Patients recruited into the HYPER trial were the subjects of this study and the patient cohort used in this study was a randomly selected sample. These patients underwent aortic valve replacement ± CABG for aortic stenosis and/or coronary artery disease. Left ventricular hypertrophy was a co-existing pathophysiological response in these patients to long-standing aortic stenosis.

6.2.2 Tissue extraction and preparation

Left ventricle (LV) free wall full thickness biopsies were obtained from non-fibrotic areas between the left anterior descending artery and the first diagonal artery. This was performed using a Trucut biopsy needle (Allegiance Healthcare, McGaw Park, IL), immediately after institution of cardiopulmonary bypass and before the onset of ischaemia (placement of the aortic cross clamp). These biopsies were immediately split into equal endocardial and epicardial halves using a scalpel and placed into individual

NUNC cryotubes and immediately snap frozen in liquid nitrogen and then stored in a -80°C freezer until analysis.

6.2.3 Laboratory methodology

A detailed protocol for sample preparation and subsequent mass spectroscopy is outlined in Appendix 9.15**.** This protocol has been optimised for extraction of metabolites from small LV biopsies (< 10mg) and is a result of a number of preliminary studies designed to extract such metabolites. This methodology has been used in previous studies from our group for mass spectrometry based metabolomic analysis (Howell 2010; Drury 2012).

6.2.3.1 Sample preparation

Metabolites were extracted from the cardiac tissue biopsies using a methanol:water:chloroform solvent system (Wu, Southam et al. 2008). The polar fractions from each biphasic mixture were removed, dried using a centrifugal concentrator and stored at -80°C for analysis. Subsequently, each dried polar extract was re-dissolved in 80:20 methanol:water containing 20mM ammonium acetate (for negative ion analysis), vortexed and centrifuged at 10,000g prior to mass spectrometry (MS). Quality control (QC) samples were prepared by pooling an aliquot of each sample.

6.2.3.2 Mass spectroscopy

MS analyses were conducted using a hybrid 7-Tesla linear ion trap Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (LTQ FT Ultra, Thermo Fisher Scientific, Germany) equipped with a Triversa chip-based nanoelectrospray ion source (Advion Biosciences, NY) using conditions as described previously (Weber, Southam et al. 2011).

Three mass spectra for each sample were collected using a selected-ion-monitoring (SIM) stitching method from mass-to-charge ratio (*m/z)* 70-740 in negative ion mode (Southam, Payne et al. 2007; Weber, Southam et al. 2011) processed, normalized and generalized-log transformed as reported previously (Payne, Southam et al. 2009; Hrydziuszko and Viant 2011). This produced a peak intensity matrix representing the metabolic profile of each extracted biopsy. Using MI-Pack software, *m/z* measurements were putatively annotated (Weber and Viant 2010).

6.2.4 Statistical analysis

Student's t-tests were conducted on the non-generalized log-transformed peak intensity matrix, using a false discovery rate (FDR) of 5%, to determine if individual peaks changed significantly between groups (Benjamini and Hochberg 1995). In addition, principal components analyses (PCA) were conducted to discover metabolic differences between the sample groups.

6.3 Results

6.3.1 Sample group

Pre-ischaemic left ventricular biopsies from 10 randomly selected patients were included in this study. The demographics and echocardiographic data of this patient cohort are outline in Table 6-1 and Table 6-2 respectively. Of the 10 patients, three patients were in the perhexiline group with perhexiline levels of 0, 0.08 and 0.27 and hydroxy-perhexiline levels of 0, 1.59 and 1.6 respectively. Hence just one patient had therapeutic levels of perhexiline in their serum. Two patients had coronary artery disease and underwent concomitant AVR + CABG surgery.

Variable (IQR)/(%)	Sample size (n=10)
Age	73 (69 – 75)
BSA	1.83 (1.79 – 1.93)
Male: Female	5:5 (50:50)
Caucasian	8 (80)
Status	
- Elective	10 (100)
EuroSCORE	6 (5.3 – 6.8)
Logistic EuroSCORE	4.5 (4.1 – 75.9)
CCS	
- 1	6 (60)
- 2	3 (30)
- 3	1 (10)
NYHA	
- 1	2(20)
- 2	4 (40)
- 3	3 (30)
- 4	1 (10)
Previous MI	0 (0)
Risk factors	
- Hypercholesterolemia	2 (20)
- Hypertension	4 (40)
Medication	
- ACE	1 (10)
- A2	1 (10)
- Statin	5 (50)
- β blocker	0 (0)
IABP pre surgery	0 (0)
Coronary artery disease	2 (20)

Table 6-1 Demographic characteristics of the patient sample

Data are median (interquartile range) or N (%). BSA, Body surface area; CCS, Canadian Cardiovascular Society; NYHA, New York Heart Association; MI, Myocardial Infarction; ACE, Angiotensin Converting enzyme inhibitor; A2, Angiotensin II antagonist; IABP, Intra-aortic Balloon Pump

Variable (IQR/%)	N = 10
Ejection fraction	63 (60 – 68)
LVID	3.6 (3.5 – 4.1)
IVSd	1.45 (1.30 – 1.70)
PWd	1.37 (1.31 – 1.48)
Aortic valve Vmax	4.43 (4.11 – 5.08)
Valve area	0.7 (0.60- 0.79)
Peak Aortic Valve Gradient	94 (72 – 103)
Mean Aortic Valve Gradient	45 (40- 67)

Table 6-2 Echocardiographic characteristics of the patient sample

Data are median (interquartile range) or N (%). LVEDV, Left ventricular end-diastolic volume; LVID, Left ventricular internal diameter; IVSd, Intra-ventricular septum thickness in diastole; PWd, Posterior wall diameter thickness in diastole; Vmax, Maximum velocity

Each epicardial and endocardial halve was normalised to achieve the least mass difference between them. This was achieved by shaving fractions of tissue to achieve an optimal mass. The mass of each epicardial and endocardial half, pre and post normalisation is outlined in Table 6-3.

	LV mass Before normalisation			LV mass After normalisation		
	Epi	**Endo**	**Delta**	**Epi**	**Endo**	**Delta**
1	4.80	3.00	**1.80**	4.80	3.00	1.80
2	4.00	3.10	0.90	4.00	3.10	0.90
3	2.50	2.80	-0.30	2.50	2.80	-0.30
4	4.30	4.20	0.10	4.30	4.20	0.10
5	5.80	**7.30**	**-1.50**	5.80	**5.48**	**0.33**
6	4.40	**7.20**	**-2.80**	4.40	**4.80**	**-0.40**
7	2.80	3.00	-0.20	2.80	3.00	-0.20
8	2.50	**7.00**	**-4.50**	2.50	**2.31**	**0.19**
9	4.00	**9.00**	**-5.00**	4.00	**4.50**	**-0.50**
10	2.00*	2.20	-0.20	2.00*	2.20	-0.20
Min	2.00	2.20	-5.00	2.00	2.20	-0.50
Max	5.80	9.00	1.80	5.80	5.48	1.80
Mean	3.71	4.88	-1.17	3.71	3.54	0.17
Median	4.00	3.65	-0.25	4.00	3.05	-0.05

*Mass spectrometry analysis failed

Table 6-3 LV mass of epicardial and endocardial halves before and after normalisation

6.3.2 Metabolomic analysis

Polar extracts from pre-ischemia left ventricular biopsies were analysed from 10 patients. Epicardial and endocardial halves suitable for analysis were available in 9 and 10 patients respectively.

The final intensity matrix of the complete spectrum after data processing consisted of 2020 peak intensity measurements for each sample having incorporated a 80% samples filter.

All peaks were examined using univariate statistics to determine if any changed intensity significantly in response to perhexiline treatment. No significant peak intensity changes were found (FDR 5%). In addition, there was no significant peak intensity changes found between the endocardial and epicardial halves with either un-paired or paired student's t-tests with a 5% FDR correction, as outlined in Table 6-4 and Table 6-5 respectively.

	P-value criteria		
Number of *m/z* measurements	< 0.01	< 0.05	< 0.5
Before FDR correction at 5%	1	12	55
After FDR correction at 5%	0	0	0

Table 6-4 Summary of *m/z* measurements changing significantly following unpaired Student's t-tests

	P-value criteria		
Number of *m/z* measurements	< 0.01	< 0.05	< 0.5
Before FDR correction at 5%	3	30	145
After FDR correction at 5%	0	0	0

Table 6-5 Summary of *m/z* measurements changing significantly following paired Student's t-tests

m/z indicates mass-to-charge ratio; FDR, false discovery rate.

Multivariate PCA was used to reduce the dimensionality of the data and visualize the metabolic similarities and differences between the two groups. The PCA scores plot did not show any separation due to the difference in tissue location as illustrated in Figure 6-2**.** More than 500 *m/z* measurements in the FT-ICR MS dataset were assigned (within ≤ 1.5 ppm) and the following ion forms were used: $[M-H]^-$, $[M+K-H_2]^-$, $[M+Na-H_2]^-$, $[M+^{35}Cl]^-$, $[M+^{37}Cl]^-$) to at least one putative named metabolite.

6.3.3 Quality control

The median spectral relative standard deviation (RSD), which is a benchmark to assess the reproducibility in metabolomics (Tian, Musi et al. 2001) was assessed. Each sample was analysed in triplicate by MS and the median spectral RSD was relatively small and consistent across all samples (mean 10.8%, SD 1.3%). The clustering of the QC samples in the PCA plot (Figure 6-2) demonstrates the consistency in instrument performance over time.

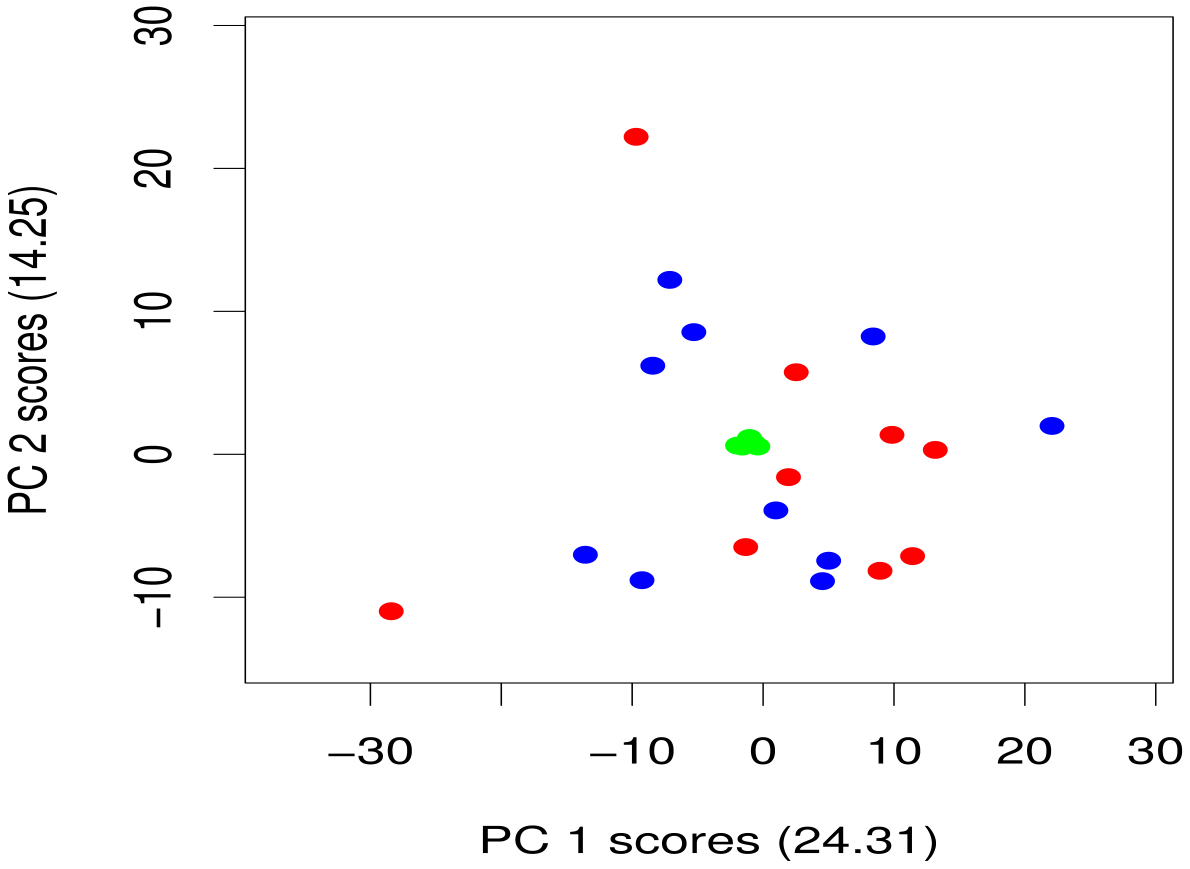


Figure 6-2 PCA scores plot from analysis of negative ion FT-ICR mass spectra of left ventricular extracts

9 epicardial samples (●) and 10 endocardial samples samples (●), show no separation along PC1 and PC2 axes. All five QC samples (●) are in a narrow cluster, confirming the consistency in MS instrument performance over time.

6.4 Discussion

This study is a novel metabolomics analysis of the human myocardium that has been subjected to pressure overload hypertrophy secondary to aortic stenosis, and is the only study to evaluate the human myocardium using metabolomics to assess a change in metabolites based on location. This study has demonstrated that there is no difference in the metabolome of the hypertrophied myocardium between the epicardial and endocardial halves hence there is no difference in metabolome in the subendocardium.

Hypertrophy initially is a normal physiological response to deal with pressure overload i.e. allows for a reduction in systolic wall stress and maintains ejection fraction (Sasayama, Ross et al. 1976; Grossman 1980). However there is ample evidence to implicate hypertrophy with poor clinical outcomes. Poor clinical outcomes include greater risk of cardiac morbidity and mortality (Levy D, Salomon M et al. 1994), increased ventricular ectopic activity (Messerli, Ventura et al. 1984; McLenachan, Henderson et al. 1987), and increased infarct size and greater mortality post myocardial infarction (Koyanagi, Eastham et al. 1982). The underlying molecular basis for poor prognosis associated with LVH is unclear.

More pertinent to cardiac surgery, is the increased susceptibility of hypertrophic hearts to ischaemic injury and reperfusion (Kohya, Kimura et al. 1988; Zhang and Xu 1995; Minami, Gohra et al. 2000). This might be in part due to hypertrophied hearts exhibiting a limited vasodilatory coronary reserve (Marcus, Doty et al. 1982) and reduced subendocardial blood flow resulting in a transmural maldistribution of blood flow causing subendocardial ischaemia (Bache, Vrobel et al. 1981; Bache, Vrobel et al. 1981). Therefore the hypertrophic myocardium with these inherent limitations consequently

has an altered metabolism (Sambandam, Lopaschuk et al. 2002) with a reduced fatty acid metabolism, uncoupling of glycolysis and glucose metabolism and accelerated glycogen metabolism during ischaemia.

With hypertrophy, there is not only an increase in the number of sarcomeres per cardiomyocyte but also an increase in the extracellular matrix and surrounding architecture including the connective tissue, capillary and nerve networks (Lorell and Carabello 2000). The connective tissue is mainly composed of collagen and this collagen network between the cardiomyocytes is responsible for the force generation between cardiomyocytes resulting in ventricular contraction and is also responsible for the passive diastolic stiffness (Weber, Sun et al. 1994). However despite the changes that occur within the myocardium, coronary blood supply arises from the coronary arteries, which sit on the epicardium, with its branches and capillary networks flowing towards the endocardium. Canine studies have shown a reduction in both capillary numeric density and capillary volume density towards the endocardium (Tomanek, Palmer et al. 1986). Moreover there is evidence for raised minimal coronary resistance with reduction in vascular density, reduced lumen size due to concentric vessel wall hypertrophy and extrinsic compression due to LVH (Gosse and Clementy 1995) particularly affecting the endocardium. These features support the hypothesis that there is reduced oxygen delivery hence an impaired metabolism towards the endocardium and therefore the endocardial metabolome should be distinctly different from the epicardial metabolome.

A preliminary review on coronary blood flow in LVH, concluded that the transmural coronary blood flow was diminished particularly during exercise (Wicker and Tarazi 1982) and these findings may be consist with subendocardial ischaemia on ECG, evident

in patients with LVH exposed to stress. This alone may explain the neutral findings reported in this study, as the tissue analysed was obtained prior to the insult of ischaemia and without imposing any additional stress on the myocardium. Therefore the metabolism within the myocardial halves may not have changed enough to project a difference in their metabolome. Furthermore this cohort of patients had no previous myocardial infarction and had good ventricular function, hence was less vulnerable even prior to ischaemia.

Another paradigm in LVH is the extent and impact of ventricular fibrosis (Weber, Janicki et al. 1990; Weber and Brilla 1991), a feature that is part of the pathological process of hypertrophy and implicated in the failing hypertrophic heart (Villari, Campbell et al. 1993). This is evident initially as diastolic dysfunction and subsequently manifests as systolic dysfunction (Lorell and Carabello 2000; Hein, Arnon et al. 2003) and eventually heart failure. Although these features may well have been present in this cohort, assessment of myocardial fibrosis was not conducted; hence its impact on the epicardial and endocardial metabolome cannot be assessed.

This sample group was selected randomly to avoid any selection bias towards the degree of hypertrophy. Echocardiographic quantification of this cohort shows a moderate degree of LVH. Due to the random selection, there were three patients from within the treatment arm of the HYPER trial and had received perhexiline therapy, however only one patient was marginally within the therapeutic range. Drury *et al* demonstrated in patients with ischaemic heart disease, that perhexiline therapy has no impact on the metabolome (Drury NE, Howell NJ et al. 2014), hence perhexiline therapy should not impact on the evaluation of the metabolome between epicardial and endocardial halves. Furthermore this cohort had two patients with IHD who underwent

concomitant AVR+CABG surgery. Patients with IHD in the setting of LVH should theoretically have a more deranged metabolism in comparison to LVH alone; these patients have made the epicardial and endocardial metabolome heterogeneous and its impact is difficult to quantify through this study alone.

This study is limited by the small sample size and although there is no statistical test or power calculation to infer an adequate sample size for such a study (Nyamundanda, Gormley et al. 2013), further evaluation with a larger sample size may prove more reassuring. The laboratory methodology used in the study to deal with small ventricular biopsies and tissue samples had been previously optimised. However, this is an inherent limitation and dealing with such small tissue samples can prove to be technically challenging albeit the quality control analysis shows consistency. Although the intension is to obtain a full thickness left ventricular biopsy this too can be technically challenging on a beating heart pre ischaemia and as a consequence the epicardial and endocardial portions may not be split exactly in half.

This study examined the hypertrophic myocardium prior to an ischaemic insult (placement of the aortic cross clamp). Further assessment of the myocardium after a period of ischaemia and subsequent reperfusion with comparison between these time points may give insight into the metabolomic changes that take place with ischaemia/reperfusion and could help elucidate the susceptibility of the hypertrophic heart to these insults. Furthermore metabolomic studies on different heart chambers and other pathological settings i.e. volume overload hypertrophy can help create a metabolome for the spectrum of heart disease.

In conclusion metabolomic assessment of the human pressure overload hypertrophic myocardium does not show a change in metabolome between the epicardial and endocardial portions. The metabolomic differences that may exist are subtle hence further studies are warranted to examine the metabolome of the hypertrophic myocardium.

7 A STUDY OF KEY REGULATORS INVOLVED IN CARDIAC METABOLISM AND THEIR ROLE IN LOW CARDIAC OUTPUT EPISODES DURING CARDIAC SURGERY

7.1 Introduction

Cardiac metabolism is controlled by a host of different mechanisms of which some are affected by the process of ischaemia and reperfusion. During cardiac surgery the insults of ischaemia-reperfusion are inevitable due to the nature of the procedural requirements such as elective myocardial arrest. This renders some individuals susceptible to myocardial injury that is clinically projected as a low cardiac output episode. Key regulators of myocardial metabolism may be involved in this clinical outcome.

7.1.1 Acetyl CoA carboxylase

Acetyl CoA Carboxylase (ACC) is a critical enzyme that regulates fatty acid synthesis and degradation (Berg, Tymoczko et al. 2012) and is subjected to both local and hormonal regulation. Phosphorylation by AMP-activated protein kinase (AMPK) inactivates ACC, while dephosphorylation by protein phosphatase 1 (PP1) activates it (Figure 7-1). As the activity of AMPK is modulated by AMP and ATP, cellular energy levels can directly influence the activity of ACC (Figure 7-2). The extent of ACC phosphorylation can therefore act as a surrogate marker for AMPK activity and therefore the cellular response to energy stress.

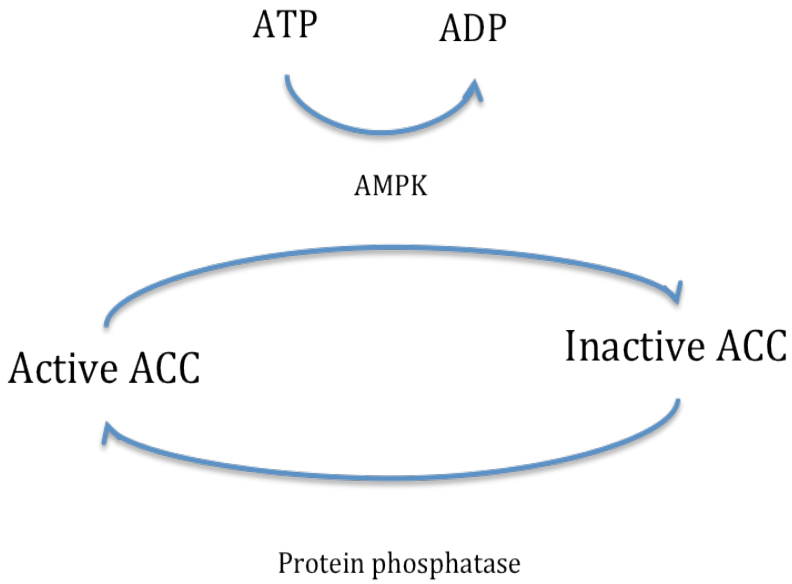


Figure 7-1 ACC activation and inactivation

The acetyl-CoA carboxylase reaction forms malonyl-CoA through the carboxylation of acetyl-coA. Malonyl-CoA is a potent endogenous inhibitor of carnitine palmitoyl transferase (CPT), a key regulator of free fatty acid (FFA) uptake into the mitochondrion (Stanley, Recchia et al. 2005). It follows therefore that, through the production of malonyl-CoA, ACC acts as a regulator of mitochondrial FFA degradation. Hence when energy levels are high, FFA oxidation is inhibited by malonyl-CoA. ACC activity is also stimulated by insulin and inhibited by glucagon and adrenaline. The latter hormonal control is a response to allow fatty acid degradation rather than synthesis in times of need.

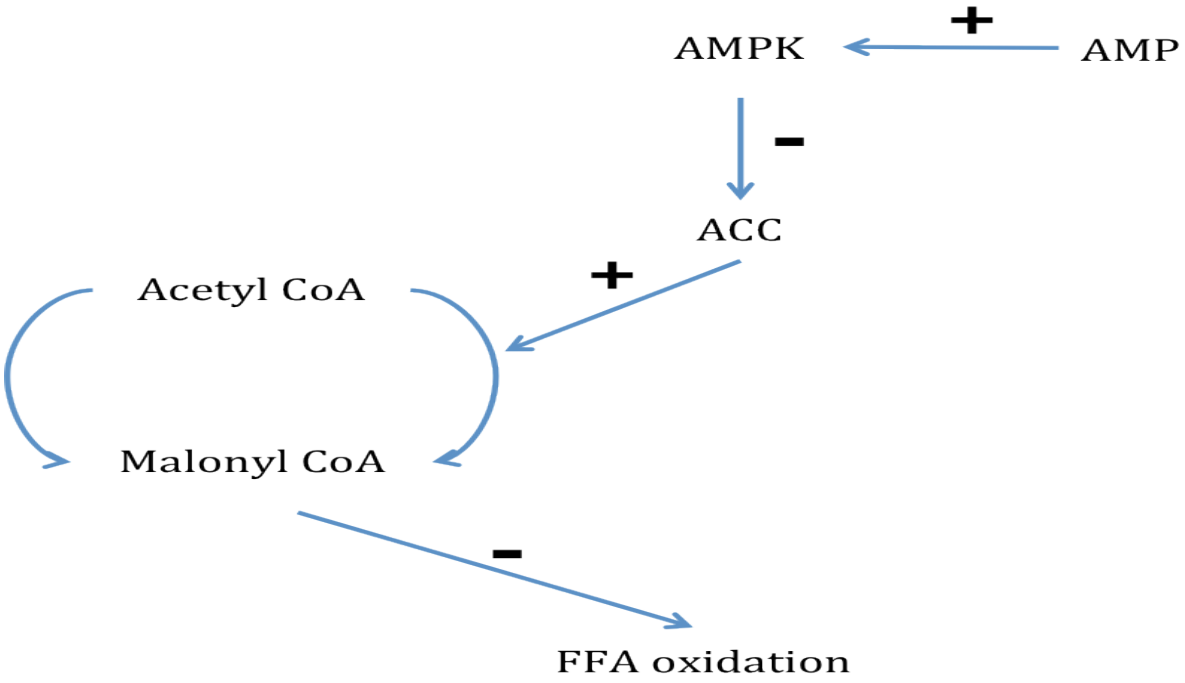


Figure 7-2 Control mechanisms of ACC

Activation of AMPK will inhibit ACC, in turn reducing malonyl-CoA production, which results in increased FFA oxidation (Stanley, Recchia et al. 2005). A rat animal model has shown a chronic increase in AMPK activity with LV hypertrophy produced by aortic banding (Tian, Musi et al. 2001) and suggested that AMPK activity may play an important role in respiratory substrate utilisation. Furthermore, mutations in AMPK that lead to its constitutive activation has been shown to result in abnormalities in carbohydrate utilisation causing glycogen storage, consistent with hypertrophic cardiomyopathy (Arad, Benson et al. 2002), making the myocardium more prone to arrhythmias such as Wolff-Parkinson-White syndrome (Arad, Moskowitz et al. 2003). AMPK activity is also known to influence glucose transporter translocation and glucose uptake (Stanley, Recchia et al. 2005), therefore giving it a central role through its ability to control both carbohydrate and FFA metabolism in order to meet the metabolic demands of the heart.

7.1.2 Glycogen synthase kinase-3

Glycogen synthase kinase-3 (GSK-3) belongs to a family of serine/threonine kinases and in humans 2 isoforms of GSK-3 exist: GSK3α and GSK3β (Hardt and Sadoshima 2002). GSK3β is ubiquitously expressed, and is therefore present in cardiomyocytes, and has been more extensively studied. This enzyme was initially identified as a regulator of glycogen synthesis by phosphorylating glycogen synthase, thereby inhibiting glycogen synthesis (Embi, Rylatt et al. 1980; Parker, Caudwell et al. 1983). However more recently there is evidence for the multifaceted roles of GSK3β which include other areas of metabolism, as well as the regulation of gene expression and cytoskeletal integrity (Grimes and Jope 2001)

In general, the result of GSK3β phosphorylation of its substrates is their inhibition. Therefore inactivation of GSK3β results in upregulation of the downstream signalling mechanisms (Hardt and Sadoshima 2002). Inactivation of GSK3β is through phosphorylation by upstream protein kinases, of which protein kinase B (AKT) is one that is most thoroughly studied. AKT is a major protein kinase downstream from phosphatidylinositol 3-kinase (PI3K). Therefore any stimuli that activate PI3K will inactivate GSK3β through AKT (Figure 7-3).

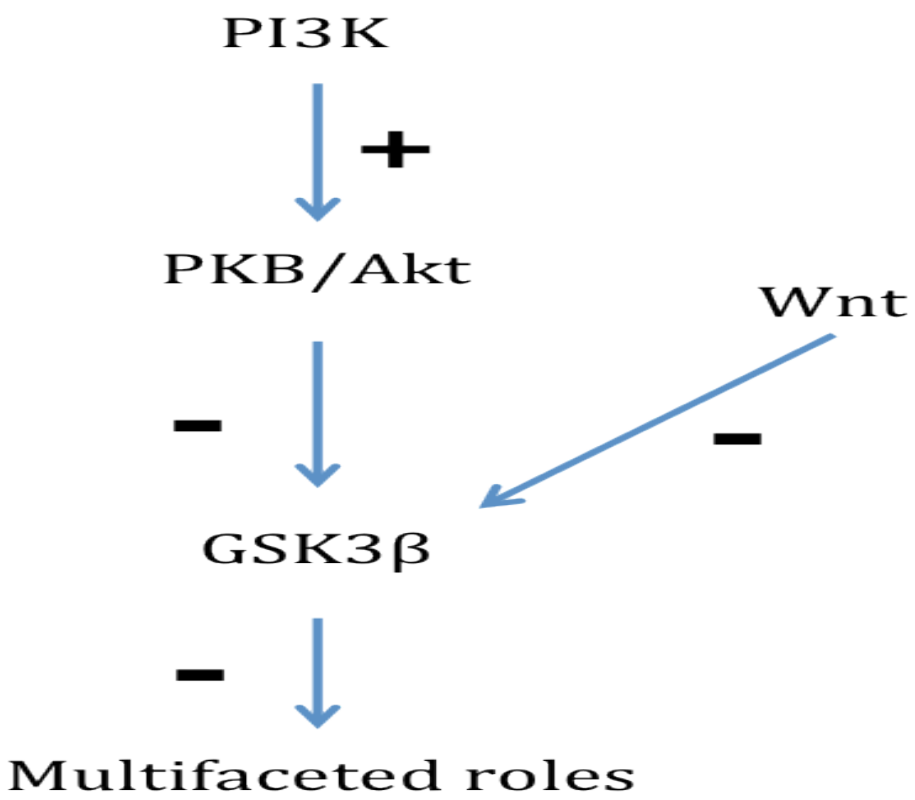


Figure 7-3 Mechanism for GSKβ inactivation

Stimuli known to activate the PI3K-AKT pathway include β-adrenergic stimulation (Morisco, Zebrowski et al. 2000) and other cardiac hypertrophic stimuli (endothelin-1, Fas, pressure overload) (Haq, Choukroun et al. 2000; Badorff, Ruetten et al. 2002). Apart from the PI3K-AKT pathway, other pathways known to inactivate GSK3β include the Wnt signalling pathway (Hardt and Sadoshima 2002). In addition insulin signalling through the PI3K pathway is also thought to inactive GSK3β (Jonassen, Sack et al. 2001) and inactivation via the Wnt pathway is thought to act distinctly differently to inactivation by insulin.

Furthermore GSK3β has shown to play a crucial role in the development of cardiac hypertrophy by imparting a negative influence towards hypertrophy. (Haq, Choukroun et al. 2000; Antos, McKinsey et al. 2002; Badorff, Ruetten et al. 2002). Inactivation of

GSK3β by phosphorylation of Ser9 via the PI3K/AKT pathway due to activation of the hypertrophic program (stimulation of β-adrenergic receptors, G_q-coupled receptors and Fas receptors) leads to hypertrophy. Hardt and Sadoshima in their review of GSK3β speculate on the evidence of transcription signalling mechanisms, and that the PI3K/PKB/AKT/GSK3β pathway may be as important as the Mitogen Activated Protein Kinase (MAPK) signalling pathway in some types of hypertrophy and can play a more dominant role in β-adrenergic cardiac hypertrophy (Haq, Choukroun et al. 2000; Badorff, Ruetten et al. 2002; Hardt and Sadoshima 2002).

Moreover, GSK3β may also have a role in protein synthesis during cardiac hypertrophy, working together with other molecules downstream from PI3K/AKT (Hardt and Sadoshima 2002). GSK3β may also be involved in cell survival and regulation of apoptosis, whereby the degree of influence may depend on the cell type and extent of GSK3β inactivation. Activation of PI3K/Akt has shown to be cardioprotective (Matsui, Li et al. 1999) and may be associated with GSK3β inactivation. Tong *et al* have shown that phosphorylation of GSK3β through the PI3K/Akt pathway during pre-conditioning, has proven to have cardioprotective effects during ischaemia (Tong, Imahashi et al. 2002). Despite these initial preliminary studies, there is no evidence that elucidates the distinct role of GSK3β in the human myocardium, in particular the role of GSK3β on growth and death of cardiac myocytes (Hardt and Sadoshima 2002). However a review by Juhaszova and colleagues has elaborated on the distinct cardioprotective role of GSK3β (Juhaszova, Zorov et al. 2009). It is thought that GSK3β acts as an integration point of many cardioprotective signalling pathways and signals downstream to GSK3β act at or near to the mitochondrial permeability transition pore (mPTP), a protein complex that has been highlighted as a key element in reperfusion injury.

Insulin has been associated in playing a role in reperfusion injury salvage kinases such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathways (Bertrand, Horman et al. 2008) and AMP-activated protein kinase (AMPK), may have a role in cardioprotection (Hausenloy and Yellon 2007). As part of a clinical trial, Howell *et al*. evaluated the impact of GIK therapy in patients with left ventricular hypertrophy on AKT and AMPK phosphorylation due to their well-described effects of insulin signalling (Howell, Ashrafian et al. 2011). They showed an increase in phosphorylated AKT and AMPK, postulating that this up-regulation was associated with clinically correlative cardioprotective effects of GIK.

This study aims to establish whether the activation state of AMPK and AKT in the myocardium before cardiac surgery can be correlated with incidence of low cardiac output episode following surgery. The phosphorylation of ACC was used as a surrogate marker for AMPK activity, while the phosphorylation state of GSK3β was used to determine PI3K/AKT pathway activity.

7.2 Methods

7.2.1 Patient sample

Patients recruited into the HYPER trial were the subjects of this study. These patients underwent aortic valve replacement ± CABG for aortic stenosis ± coronary artery disease. Left ventricular hypertrophy was a co-existing pathophysiological response in these patients to long-standing aortic stenosis.

In the initial six hours post reperfusion (removal of the aortic cross clamp), haemodynamic parameters of these patients were optimised and a potential low cardiac output episode treated as outlined in section 2.11.4

A blinded end-points committee adjudicated on patients that met the criteria for a low cardiac output episode (LCOE). A low cardiac output episode was defined as hypotension (MAP <60) with a cardiac index < 2.2 L/min/m^2, in the presence of adequate filling pressures (CVP 8-12 mmHg, PCWP 12–16 mmHg) and heart rate of 70-110bpm; refractory to appropriate intra-vascular volume expansion and following correction or attempted correction of any dysrhythmias.

Five patients who sustained a LCOE and five that did not within the placebo arm of the HYPER trial were identified at random, for the following analyses.

7.2.2 Tissue extraction and preparation

Left ventricle (LV) free wall full thickness biopsies were obtained from non-fibrotic areas between the left anterior descending artery and the first diagonal artery. This was performed using a Trucut biopsy needle (Allegiance Healthcare, McGaw Park, IL), immediately after institution of cardiopulmonary bypass before the onset of ischaemia (placement of the aortic cross clamp). The tissue obtained was immediately snap frozen in liquid nitrogen and then stored in an -80°C freezer until analysis.

7.2.3 Laboratory methods

Tissue samples obtained as outlined above were lysed in RIPA buffer using a Cryolys system. Protein content of the samples was assessed using a BCA Protein Assay Kit (Novagen, USA) according to the manufacturer's protocol. Samples were then diluted to a standard protein concentration in Laemmli Buffer before boiling for 10 minutes followed by a brief (5 second) centrifugation. Samples were then analysed using western blotting.

7.2.4 Western blot analysis

For western blotting, 20µl of sample and 10µl of protein weight marker (Thermo Scientific) were resolved using sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis with 8% or 10% gels where appropriate.

Gels were subjected to 100V constant current in SDS Tris-glycine running buffer to resolve proteins, which were then transferred onto nitrocellulose membrane (Amersham Hybond-ECL, GE Healthcare) using the wet transfer system (BioRad) using 100V constant current for 1 hour. Membranes were then blocked with PBST (PBS + 0.1% Tween-20) with non-fat dried skimmed milk powder. Membranes were incubated with anti-phospho-GSK3β or anti-phospho-ACC primary antibody (1:1000 in 5% BSA, Cell Signalling) with gentle shaking on a rocker overnight at 4°C.

Membranes were then rinsed using PBST, before extended washes (3x10 minutes) in the same buffer with gentle rocking. Goat anti-rabbit IgG, horse-radish peroxidase-linked antibody, was then incubated with the membrane for 1 hour at room temperature before washing with PBST as above and final incubation with enhanced chemiluminescence reagent (GE Healthcare).

Antibody binding to the nitrocellulose membrane was visualized by placing light-sensitive film (Amersham HyperfilmTM Blue) onto the membrane for various times before developing the film in a dark room. The exposure time was optimised based on the quality of the initial film exposure.

In order to assess the consistency of sample loading onto the gel, membranes were re-incubated for 1 hour at room temperature with total-ACC or total-GSK3β antibody

before following the protocol described above (washing, incubation with secondary antibody, etc.).

The resulting films were scanned to create a digital image, which was then densitometrically quantified using ImageJ software. The ratio between the density of the 'phospho' and 'total' bands was then calculated for both GSK3β and ACC and plotted using GraphPad software (Prism).

7.2.5 Statistical analysis

Analysis was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL). Continuous data was assessed for normal distribution and presented as mean ± standard deviation of the mean or median and inter-quartile range. Student's t-test was used to analyse normally distributed data. Skewed data was analysed by Mann-Whitney U-test. Categorical data was analysed by Fisher's exact test. Statistical significance was defined as a $p < 0.05$.

7.3 Results

7.3.1 Sample group and demographics

The baseline demographics are outlined in Table 7-1. All samples were obtained from the control arm of the study. Of the 10 patients, 5 underwent isolated AVR for severe aortic stenosis alone, and the remaining patients had concomitant IHD and underwent AVR + CABG surgery. There was no significant difference in any of the pre-operative variables.

Variable (Median, IQR)/(%)	No LCOE (n=5)	LCOE (n=5)	P Value
Age	74.5 (68.6 – 82.9)	74.4 (57.8 – 81.6)	0.69
BSA	1.76 (1.48 – 1.96)	1.85 (1.70 – 2.23)	0.31
Male: Female	3:2 (60:40)	4:1 (80:20)	1.0
Status			
- Elective	5 (100)	5 (100)	
EuroSCORE	7 (5 – 7.5)	6 (3.5 – 8.5)	1.0
Logistic EuroSCORE	7.41 (3.45 – 8.50)	6.37 (2.58 – 11.25)	0.84
CCS			0.44
- 1	5 (100)	3 (60)	
- 2	0	2 (40)	
NYHA			0.26
- 1	1 (20)	1 (20)	
- 2	4 (80)	2 (40)	
- 3	0	1 (20)	
Previous MI	0	0	
Risk factors			
- Hypercholesterolemia	1 (20)	0	1.0
- Hypertension	1 (20)	0	0.05
Medication			
- ACE	1 (20)	1 (20)	1.0
- A2	0	2 (40)	0.44
- Statin	2 (40)	3 (60)	1.0
- β blocker	1 (20)	1 (20)	1.0
IABP pre surgery	0	0	
IHD	3 (60)	2 (40)	

Table 7-1 Pre-operative variables

The echocardiographic and operative variables are outlined in Table 7-2 and Table 7-3 respectively. There is no significant difference in any of the echocardiographic variables or the operative variables between the groups.

Variable (IQR/%)	No LCOE	LCOE	P value
Ejection fraction	62 (59 – 62)	51 (45 – 51)	0.33
LVID	3.2 (3.2)	5 (4.4 – 5)	0.50
IVSd	1.2 (1.2)	1.6 (1.1 – 1.6)	0.70
PWd	1 (0.9 – 1)	1.1 (0.9 – 1.1)	1.0
Valve Area	0.6 (0.36 – 0.6)	0.85 (0.73 – 1.0)	0.14
Peak gradient	105 (94 – 105)	65.5 (49.8 – 76.75)	0.06
Mean gradient	63.0 (46.8 – 71.0)	38.5 (30.25 – 62.5)	0.11

Table 7-2 Echocardiographic variables

Variable (IQR/%)	No LCOE	LCOE	P Value
Procedure			1.0
- AVR	2 (40)	3 (60	
- AVR + CABG	3 (60)	2 (40)	
Valve type			1.0
- Biological	5 (100)	4 (80)	
- Mechanical	0	1 (20)	
Valve size	23 (21 – 24.5)	23 (20.5 – 23.5)	0.62
Cumulative CPB time	119 (106 – 136)	158 (115 – 236)	0.11
Cum cross clamp time	90 (73 – 99)	81 (66 – 160)	0.45
Hot shot administered	4 (80)	4 (80)	
IABP	0	1 (20)	

Table 7-3 Operative variables

7.3.2 Analysis of ACC phosphorylation

The western blot images acquired for ACC phosphorylation are shown below where total-ACC and phospho-ACC are depicted in Figure 7-4 and Figure 7-5 respectively. The western blots depict samples 1 through to 10 (left to right), where samples 1 – 5 are non-LCOE and 6 – 10 are LCOE.

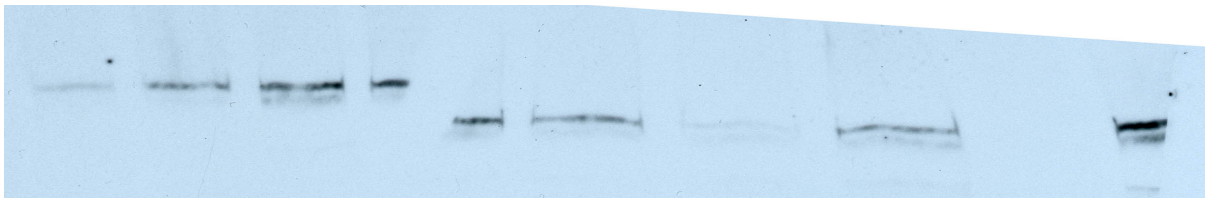


Figure 7-4 Western blot showing total-ACC

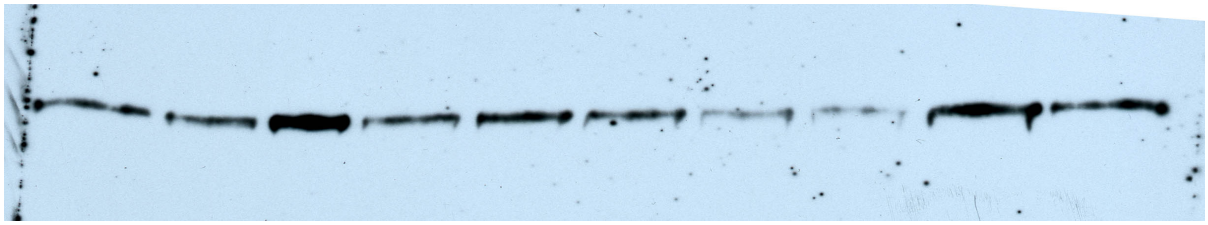


Figure 7-5 Western blot showing phospho-ACC

The ratio of phospho-ACC to total ACC (ACC phosphorylation) was greater in the non-LCOE group compared to the LCOE group (Figure 7-6) although this did not reach significance (p=0.41).

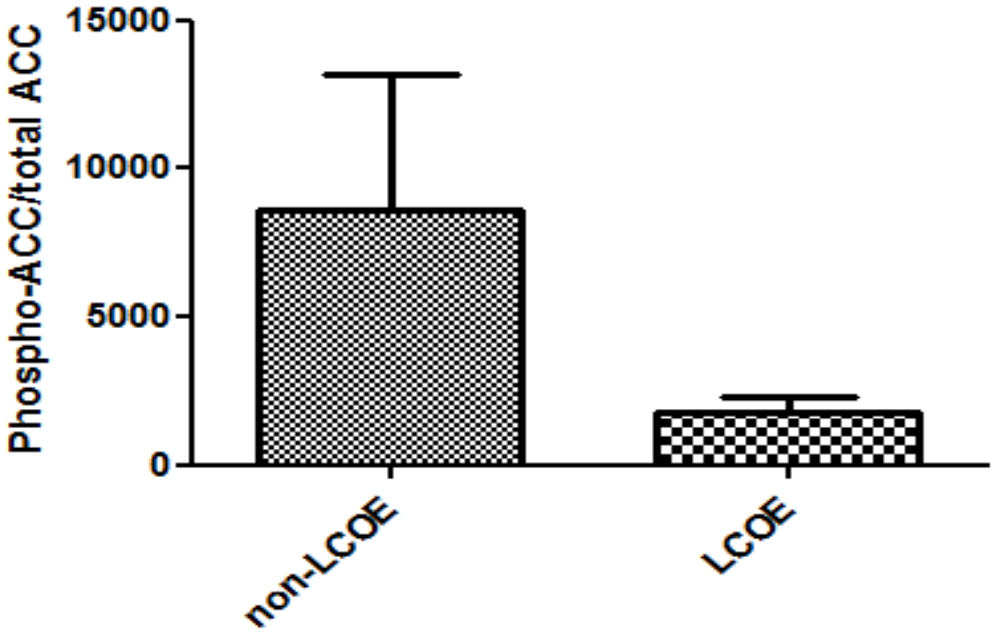


Figure 7-6 Comparison of ACC between LCOE and non-LCOE groups

7.3.3 Analysis of GSK3β

The western blot images acquired for GSK3β phosphorylation are shown below where total-GSK3β and phospho-GSK3β are depicted in Figure 7-7 and Figure 7-8 respectively. The western blots depict samples 1 through to 10 (left to right), where samples 1 – 5 are non-LCOE and 6 – 10 are LCOE.

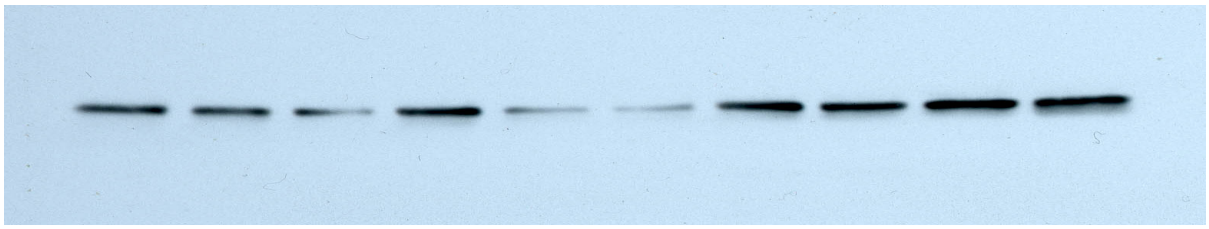


Figure 7-7 Western blot showing total-GSK3β

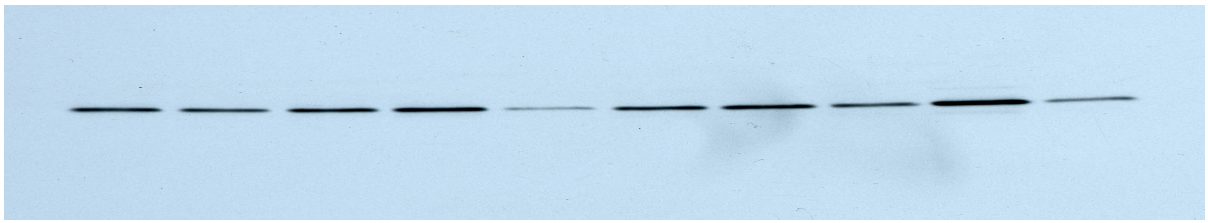


Figure 7-8 Western blot showing phospho-GSK3β

The ratio of phospho-GSK3β to total GSK3β (GSK3β phosphorylation) was greater in the non-LCOE group compared to the LCOE group (Figure 7-9). This difference almost reached statistical significance (p=0.06).

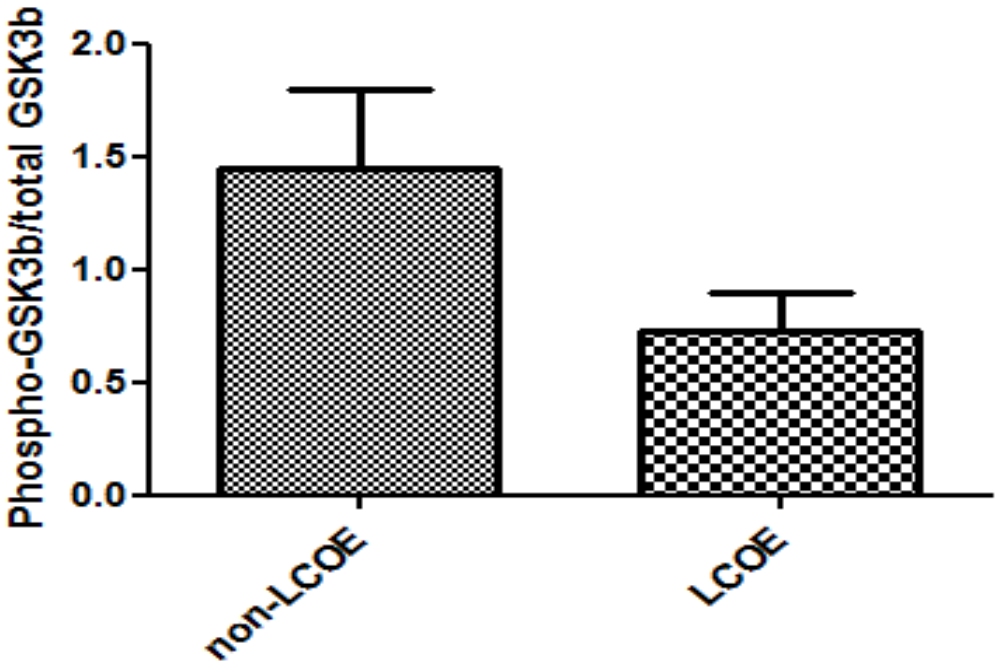


Figure 7-9 Comparison of GSK3-beta between LCOE and non LCOE groups

7.4 Discussion

This study evaluated the activity of ACC and GSK3β on human cardiac tissue and showed an increased phosphorylation of ACC and GSK3β in patients that do not have a low cardiac output episode (LCOE) post cardiac surgery compared to those that sustain a LCOE. Post cardiac surgery, LCOE is reflective of a failing heart and one that requires additional inotropic support to maintain an adequate cardiac function and is associated with increased mortality and morbidity (Maganti, Rao et al. 2005).

The demographic characteristics of the groups were comparable with similar risk stratification and clinical status. The samples analysed were obtained from the placebo cohort and therefore this study projects the true activity of these metabolic regulators in patients that have a LCOE. Increased ACC and GSK3β phosphorylation can be extrapolated to reflect the increased activity of AMPK and AKT respectively.

AMPK acts as the fuel gauge in the heart (Dyck and Lopaschuk 2002) as its activity is controlled by the energy levels; ATP degradation to ADP and eventually to AMP. Therefore AMPK is activated in times of need and this includes the period of myocardial ischaemia. AMPK activity inhibits the formation of malonyl-CoA (phosphorylation of ACC results in the inactive form) and therefore there is a drive towards FFA oxidation. This metabolic process seems to be in paradox to the number of the studies that show inhibition of FFA oxidation is beneficial in the ischaemic period (Wang and Lopaschuk 2007), due to the attenuation of the uncoupling effect of oxidation from phosphorylation, present with increased FFA metabolism. The trend towards increased AMPK activity in patients with no LCOE demonstrated in this study was prior to any ischaemic insult and alludes to the cardioprotective mechanisms of AMPK through its dual faceted role of glucose and fatty acid metabolism; activation of AMPK during ischaemia increases glucose uptake and glycolysis and also increases free fatty acid oxidation during reperfusion (Sambandam and Lopaschuk 2003). Furthermore AMPK activation may increase energy production and inhibit apoptosis during the early ischaemic phase (Dyck and Lopaschuk 2006).

Reperfusion Injuries Salvage Kinases (RISK) pathway (Hausenloy and Yellon 2004) refers to a series of pro-survival protein kinases. The RISK pathway mediates a form of programmed cell survival and there is growing evidence that stimulation of the RISK pathway is associated with cardioprotective benefits (Hausenloy and Yellon 2007). It is believed that activation of the RISK pathway ultimately leads to inhibition of the mitochondrial Permeability Transition Pore (PTP) opening, which offers cardioprotection (Davidson, Hausenloy et al. 2006; Juhaszova, Zorov et al. 2009). Other mechanisms include an improved calcium uptake in the sarcoplasmic reticulum and the

recruitment of antiapoptotic pathways (Yellon and Hausenloy 2007). Originally AKT constituted one of the pro-survival kinases and subsequently several other kinases have been identified including GSK3β (Hausenloy and Yellon 2007), one of the downstream targets of AKT. There is now surmounting evidence that AKT activation and subsequent GSK3β inhibition through its phosphorylation is cardioprotective (Juhaszova, Zorov et al. 2009). Furthermore GSK3β activity has been implicated as a critical element in ischaemic preconditioning (Tong, Imahashi et al. 2002), thought to also have a cardioprotective benefit.

Our group has recently conducted a study on the role of GIK therapy and its impact on AKT and AMPK signalling in patients with LVH undergoing cardiac surgery (Howell, Ashrafian et al. 2011). Howell *et al* demonstrated a 2.5 fold increase in phospho-AKT compared to total-AKT (p=0.03*)* and a 1.7 fold increase in phospho-AMPK to pan-AMPK (p=0.0004*)* in patients treated with GIK therapy, postulating the cardioprotective benefit of GIK therapy, by upregulation of AKT and AMPK activity. This study corroborates these findings to an extent. However this study hypothesises further, in that these cardioprotective properties are innately present in individuals that do not go on to have a LCOE without metabolic modulation (to up-regulate the cardioprotective signalling pathways).

This is the first study to evaluate the activity of these metabolic regulators on pre-ischaemic human myocardium and correlate this to LCOE post cardiac surgery. It appears that in patients that do not sustain a LCOE, there is an inherent cardioprotective benefit of increased AMPK and AKT activity prior to the induction of ischaemia. This correlation is at present purely hypothesis generating and is demonstrated on the hypertrophic myocardium, known to have an altered

pathophysiology and deranged energy metabolism (Sambandam, Lopaschuk et al. 2002).

Although this study demonstrated an increased phosphorylation of ACC and GSK3β in patients that had no LCOE, this did not reach significance, albeit almost reaching significance in the GSK3β analysis. This may be as a result of low numbers in each group and hence is a limitation of this study. The effect of perhexiline therapy on ACC and GSK3β activity was not studied and given the clinical outcomes, perhexiline therapy may not have had a clinically significant impact; however this remains speculative. Although there was no significant difference in the pre-operative echocardiographic findings, the LCOE group may have had marginally more hypertrophy; this assumption is limited by the incomplete echocardiographic data available in this sample group.

This study is preliminary work looking at key master regulators involved in myocardial metabolism in relation to cardiac surgery and its associated ischaemic and reperfusion insults. Future work aims to evaluate the activity of these regulators during ischaemia and in the early reperfusion phase. Furthermore comparison between LVH and non-LVH patients undergoing cardiac surgery and their associated clinical outcomes will help identify if these regulators play a key role within the more susceptible LVH population. Further biochemical studies on human myocardium performed in parallel to their clinical outcomes would help elucidate the fundamental roles of AMPK and AKT activation and their downstream regulators, thereby delineating their cardioprotective mechanisms.

In conclusion this preliminary study showed an inherent increase in AMPK and AKT activity prior to an ischaemic insult in patients who did not sustain a LCOE following

cardiac surgery. The increased activity of these master regulators may provide a cardioprotective benefit. Hence metabolic modulation to activate these pathways may prove beneficial in patients undergoing cardiac surgery and this warrants further examination.

8 DISCUSSION, CONCLUSIONS AND FUTURE WORK

8.1 Clinical trial - HYPER

8.1.1 Perhexiline and myocardial protection during cardiac surgery

The clinical trial was designed to evaluate the hypothesis that metabolic modulation with perhexiline, administered orally prior to cardiac surgery would improve myocardial protection; in patients with left ventricular hypertrophy secondary to aortic stenosis. These patients required an aortic valve replacement for symptomatic and/or prognostic benefit with or without concomitant coronary artery bypass graft surgery for ischemic heart disease. Furthermore the HYPER trial was designed to assess the hypothesis that perhexiline therapy, through its mechanism of action would improve the myocardial energetic state in patients with LVH secondary to AS and hence improve the overall haemodynamic state and cardiac performance post AVR ± CABG. Both these hypothesis have been refuted by the HYPER trial.

The HYPER trial showed no overall benefit from perhexiline therapy as an adjunct to standard myocardial protection in patients undergoing AVR ± CABG. The primary end point, which was the incidence of inotrope use to treat a low cardiac output state appropriately, was not different between the groups. Furthermore there was no benefit of perhexiline therapy in reducing post-operative myocardial injury.

The basis for myocardial protection with perhexiline was to enhance the myocardial metabolic state, prior to cardiac surgery and hence through metabolic modulation, achieve better myocardial protection. Previous studies addressing an improvement in myocardial protection conducted in Birmingham followed the same principles of metabolic modulation (Quinn, Pagano et al. 2006; Ranasinghe, Quinn et al. 2006;

Howell, Ashrafian et al. 2011; Drury NE, Howell NJ et al. 2014). The majority of these studies evaluated the role of GIK associated metabolic modulation and showed an improvement in myocardial protection.

Low cardiac output episode post cardiac surgery has been used as a surrogate marker of myocardial protection in most of these earlier trials. It is well established that during cardiac surgery the heart undergoes a period of ischaemia and following this initial insult, there is the added insult of reperfusion classified as ischaemia-reperfusion injury. Therefore following this, if the myocardium is not adequately protected, the heart is liable to go through a period of low cardiac output with sub-optimal haemodynamic function. This can be detrimental to patient recovery (Hoffman, Wernovsky et al. 2003; Maganti, Rao et al. 2005). Therefore once a low cardiac output episode is identified, inotropic support can be provided to improve the cardiac function thereby improving overall haemodynamic performance. Hence the incidence of inotrope too can be used as a surrogate marker of myocardial protection, as used in the HYPER trial. Perhexiline therapy was thought to improve the pre-ischaemic myocardial metabolism by inhibiting detrimental fatty acid metabolism and hence driving glucose metabolism; this metabolic state was presumed to be beneficial during the ischaemia-reperfusion insult. However, the HYPER trial has disproved the hypothesis that promoting carbohydrate metabolism prior to cardiac surgery, would prime the cardiomyocyte to deal with the stressors of ischaemia and reperfusion. Moreover, HYPER has demonstrated that oral perhexiline therapy does not augment standard myocardial protection in patients with LVH undergoing cardiac surgery.

Although there was no difference in the incidence of appropriate use of inotropic support, the overall incidence of inotrope use was higher in the perhexiline group

during the first 12-hours following reperfusion and was statistically significant in the six to 12 hour period post-reperfusion. This finding was in the context of unchanged parameters in other haemodynamic measurements i.e. filling pressures, heart rate and mean arterial pressures during the same time points. Increased inotrope requirements during the six to 12 hour period of reperfusion, is consist with a significantly reduced cardiac performance measured by CI at 12 hours after reperfusion. These findings are concurrent to a significant reduction in cardiac index at 6 hours albeit insignificant when corrected for baseline cardiac index (reduced in the perhexiline group) in a preceding trial; myocardial protection with perhexiline in coronary artery surgery (CASPER) (Drury NE, Howell NJ et al. 2014). The reason for reduced cardiac function at baseline associated with perhexiline is unclear and remains unanswered (Drury 2012) and given the nominal nature of the statistical test for this analysis, the play of chance remains a plausible explanation of the result not withstanding the apparently low p value.

Perhexiline as a metabolic agent has been extensively studied and it's role as a potent metabolic modulator has been previously advocated. Abozguia *et al* showed perhexiline improved high-energy phosphate ratios, oxygen consumption and NYHA symptoms in patients with hypertrophic cardiomyopathy (Abozguia, Elliott et al. 2010). In an earlier study, Lee and colleagues showed an improvement in oxygen consumption, quality of life and left ventricular ejection fraction in patients with chronic heart failure (Lee, Campbell et al. 2005). In a review of perhexiline, clinical applications in ischaemic heart disease, aortic stenosis and heart failure have been explored and supported (Ashrafian, Horowitz et al. 2007). However the clinical application of perhexiline is limited. In one such study, perhexiline was used in the medical management of symptomatic aortic

stenosis; 13 of 15 elderly patients showed symptomatic improvement when followed up for 30 months (Unger, Robinson et al. 1997). The small number of participants and its non-randomised nature inherently limits this trial. In addition, none of the patients were suitable for surgical intervention and did not undergo cardiac surgery. The application of perhexiline in surgical practice is scarce and this scarcity in the data led to the development of the CASPER trial, which preceded the HYPER trial. These two trials aimed to examine perhexiline therapy in two distinct patient populations; in those with ischaemic heart disease and those with pressure overload hypertrophic ventricles.

A common theme throughout all the studies that support perhexiline, as a metabolic agent is that therapy is prolonged, monitored and optimised over months. This was not logistically possible in the real-world practice of cardiac surgery, reflected by both HYPER and CASPER trial. HYPER was the first to evaluate the role of perhexiline in patients with LVH secondary to AS undergoing cardiac surgery. Further examination would be restricted based on the impotent nature of perhexiline therapy in this surgical population.

8.1.2 Unexpected findings from HYPER

In this study the percentage of patients needing on-going vasopressor infusion in the first six hours of reperfusion was high in both groups (91.4% vs. 88.9%, placebo vs. perhexiline, p=0.76), and within the perhexiline group, was higher during the six to 12 hour reperfusion period (63.7% vs. 79.6%, placebo vs. perhexiline, p=0.09). In previous trials utilizing GIK therapy, GIK in itself may have proved to cause a vasodilatory response, however perhexiline per se is not known to have such properties. This trial used phenylephrine as a vasoconstrictor; although a pure vasoconstrictor with only α-

agonist properties phenylephrine is considered to be weaker than other vasoconstrictors such as noradrenaline, which was used in the previous GIK trials. This may have created an immeasurable bias resulting in a lower threshold to commence vasoconstrictor support. It appears that the increase in vasoconstrictor support during the six to 12 hours of reperfusion coincides with an increase in overall inotropic support use during the same time. Phenylephrine use would supplement overall haemodynamics during a period of low cardiac output, by improving perfusion pressure. It would be speculative to imply that perhexiline has a negative inotropic effect. This correlation has not been extensively studied in patients undergoing anaesthesia or surgery; however similar findings were evident in the CASPER trial and explanation for this remains elusive (Drury 2012).

HYPER has shown that perhexiline therapy has been associated with increased renal impairment (creatinine > 200) in six (11.1%) patients, two (3.4%) requiring haemofiltration and one needing permanent dialysis. Baseline renal function was similar between the groups, with a marginally higher median serum creatinine level in the perhexiline group (not significant). Perhexiline is metabolised in the liver and its metabolites are excreted in the urine (Ashrafian, Horowitz et al. 2007). Renal impairment with creatinine > 200 was an exclusion criteria; the variability of perhexiline metabolism amongst individuals and the associated risk of hepatotoxicity and neurological complications strongly contraindicate the use of perhexiline in patients with renal impairment. To our knowledge there are no other reports implicating renal impairment with perhexiline therapy.

It appears that perhexiline, initially hypothesised as beneficial towards myocardial protection, could now in fact be harmful. However, the unexpected secondary outcomes

remain secondary outcomes and should be interpreted cautiously. Given the neutral primary outcome, the secondary outcomes have to be interpreted as exploratory (Freemantle 2001). Further studies to evaluate these secondary outcomes in greater detail will be restrained due to the neutral clinical implications of perhexiline in this setting.

8.1.3 Limited potency of perhexiline for myocardial protection

Perhexiline is known for its inhibitory properties on CPT action, in particular CPT-1. Fatty acid metabolism is dependent on both CPT-1 and CPT-2 action and as yet there is no certainty that perhexiline inhibits CPT-2 adequately. Furthermore, perhexiline remains a competitive inhibitor of CPT (Ashrafian, Horowitz et al. 2007), competing with the highly potent, endogenous enzyme malonyl-CoA. In addition to the potential inhibitory properties of perhexiline on CPT-1, this action is tested further by the likely overwhelming flood of free fatty acids in circulation, in a patient that has been starved overnight in preparation for theatre. Therefore perhexiline inhibition of CPT-1 may not be effective particularly in the ischaemic and reperfusion phases during cardiac surgery.

To the contrary, one of the benefits of GIK therapy as shown in the previous trials was that GIK therapy continues throughout ischaemia and into reperfusion and hence its effects were readily employed at the end of ischaemia and during reperfusion. This is independent of other cardioprotective properties of GIK such as substrate availability with glucose and the multifactorial cardioprotective benefits of insulin including up-regulation of pro-survival pathways (Howell, Ashrafian et al. 2011).

Drury et al have recently provided further insight into the correlation between tissue and plasma concentrations of perhexiline and its impact (Drury, Licari et al. 2014). This

study has shown good correlation between atrial and ventricular concentrations of perhexiline to plasma, with an atrial to plasma ratio of 21.5, ventricular to plasma ratio of 34.9 and ventricular to atrial ratio of 1.67 and concluded that perhexiline concentration in plasma was predictive of myocardial drug concentrations. However despite a high concentration of perhexiline being found in the human atria and ventricular myocardium, it may not be adequate at steady-state to exert the maximum effects of CPT inhibition required to promote carbohydrate metabolism (Drury, Licari et al. 2014). The lack of metabolic change is further confirmed by a novel metabolomic study assessing the metabolites of myocardial ventricular tissue, which showed that there was no change in metabolism towards a carbohydrate system and no effect on the myocardial metabolome, with exposure to perhexiline (Drury 2012).

Perhexiline has a single known utility of CPT inhibition and therefore this may not be potent enough to provide effective clinical outcomes in the context of cardiac surgery. Despite the clinical outcomes demonstrated in HYPER, experimental models to evaluate the mechanism of perhexiline continue. A recent novel study employing a combined approach of proteomics, metabolomics and computational modelling approach, in a small rat heart model has shown activation of the pyruvate dehydrogenase complex with perhexiline therapy and suggests that perhexiline may have yet unknown complex systemic effects (Yin, Dwyer et al. 2013). Such complex experimental studies may help elucidate some specific actions of metabolic therapies, yet clinical application may not replicate laboratory findings.

8.1.4 Timing of the HYPER trial

The HYPER trial was conceived on the back of a series of myocardial protection studies in Birmingham, and a trend to move away from the labour intensive GIK therapies. Other metabolic therapies apart from GIK were gaining increasing popularity as a potential adjunct to standard myocardial protection strategies, due to their properties of metabolic modulation; perhexiline was one such metabolic therapy and seemed to be a feasible IMP. Our group in Birmingham were the first to study the role of perhexiline in the context of cardiac surgery and myocardial protection. The first of these studies evaluating perhexiline in cardiac surgery was on patients with ischaemic heart disease undergoing CABG (CASPER) and recruitment into this trial had not completed by the time recruitment into HYPER had begun.

Earlier in this thesis I outlined that LVH in itself is a marker of poor outcome post cardiac surgery. Furthermore, LVH is considered to be metabolically deranged, and hence has a greater risk of injury from ischaemia and reperfusion. This principle that LVH is different to the myocardium affected by IHD posed a different research conundrum; effect of perhexiline therapy on LVH should be different to that on IHD. This therefore led to the overlap and simultaneous running of CASPER and HYPER trials. In hindsight, it would have been prudent to await the results of the CASPER trial before embarking on another large randomised control trial using the same IMP.

The HYPER trial was halted early due to futility. A complete futility analysis was conducted in light of the results from the CASPER trial. The CASPER trial failed to improve any of the clinical markers of myocardial protection (Drury NE, Howell NJ et al. 2014). The futility analysis, which consisted of a O'Brien Fleming Alpha spending plan

analysis for the primary outcome demonstrated that it was futile to try and achieve complete recruitment to the trial, as it was likely to reach a neutral outcome on the analysis of the primary outcome. Therefore the DSMB recommended to the trial steering committee that the trial should be halted based on futility, affirming the limited clinical benefit of perhexiline in cardiac surgery. Consequently this had knock-on effects on a sub-study recruiting patients to the IMP, as discussed later in this chapter.

With CASPER being a neutral trial, one would have been more hesitant to instigate another trial using perhexiline as an IMP in cardiac surgery. Yet having embarked down this journey, HYPER has provided ample evidence to add to the literature that perhexiline has it's limited uses and in cardiac surgery perhexiline therapy has no role as an adjunct to standard myocardial protection.

8.1.5 Limitation of HYPER

One of the strongest limitations of HYPER was that 39% of patients were below the therapeutic range of serum perhexiline concentration. A reason for this was the low minimum threshold of four days therapy prior to surgery. This threshold was adapted to optimise recruitment, as it would capture some patients who were listed and then operated on earlier due to waiting list scheduling or more pertinently due to clinical urgency. However it is uncertain what impact this would have had on the clinical outcome if any, as a propensity matched analysis of patients in the therapeutic range from CASPER, showed no difference in the myocardial benefits with perhexiline (Drury 2012).

The power for this trial was calculated on end-points derived from previous GIK trials in the department. With HYPER being the first trial using perhexiline during cardiac

surgery in patients with LVH and with CASPER recruitment incomplete, there were no comparable end-points to utilise, in order to power a phase III trial using the same IMP. Similarly the end-points examined in this trial were based on historical end-points used in previous trials. A validated power calculation or change to any of the end-points may not have changed the overall outcome of this trial, but these concepts were not explored when the trial was designed.

In this trial, very few urgent patients were included; due to the minimum duration of trial therapy required before surgery. This latter limitation disallowed assessment of perhexiline therapy on urgent cases, however given there is no overall clinical benefit this evaluation is less pertinent.

8.1.6 Running a clinical trial

When the HYPER trial was conceived, the department was performing approximately 170 AVRs per year, which included 40 concomitant CABG surgery cases eligible for recruitment. At a recruitment rate of 80%, to recruit the targeted 220 patients would take approximately 30 months. A few months into recruitment, it was evident that this target was not feasible, and the recruitment rate was below 80%. One of the main reasons was due to the resistance of taking an IMP leading up to cardiac surgery (additional oral tablets) by 20% of the eligible cohort, as apposed to an infusion of GIK administered intravenously after anaesthetic induction. Recruitment remained a concern throughout the period of the HYPER trial.

To improve recruitment collaborations with other centres were established. One such centre was Brighton who had recruited into the CASPER trial and was familiar with some of the trial protocols. New collaborations were made with Coventry to increase

recruitment further and at this point HYPER grew into a multicentre trial. Expansion into other centres once trial recruitment had begun in the parent centre proved difficult and challenging. Ethical approval with amendments took their natural time course, which added further delays. Meanwhile, trust approval had to be sought, local consultants had to be convinced of the merits of the trial and trial protocols and local departmental education had to take place. This was a personally demanding time, and to achieve these objectives, required travelling to these institutions on multiple occasions in addition to visiting each centre for every case conducted within the trial.

In addition to this, the throughput within the department in Birmingham fell annually and was lower than the predicted 170 cases per year for each consecutive year of trial recruitment. Furthermore, during trial design it was initially thought that 35% of these patients would be excluded, however in HYPER up to 48% met the exclusion criteria with 22% of patients undergoing AVR ± CABG being diabetic. These constraints were not immediately discernable until over a year into recruitment.

The same limitations and concerns pertaining to recruitment in HYPER were mirrored in the HYPER sub-study, which evaluated the role of perhexiline on cardiac energetics by magnetic resonance spectroscopy.

During the HYPER trial there was a MHRA inspection at University Hospitals Birmingham and the HYPER trial was one of the trials that used an IMP and hence was at random, selected to be investigated. This was a thorough investigation of the entire trial and the sub-study and included a review of the all the trial processes, recruitment, randomisation, standard operating procedures, management of trial patients, data collection and data analysis. As part of this investigation the HYPER trial was criticised

for the manner in which the IMP was handled within the pharmacy and the MHRA recommended that the pharmacy improve their data recording and standard operating procedures on the handling of the IMP. Subsequent to this, in collaboration with pharmacy I wrote new standard operating procedures for the handling of the IMP and this was internally audited and approved.

8.2 MRS and the role of perhexiline on myocardial energetics

Magnetic Resonance Spectroscopy (MRS) is an established research tool to evaluate cellular energetics and in cardiac research it has become increasingly more reliable in the measurement of cardiac energetics (Hudsmith and Neubauer 2009; Beadle and Frenneaux 2010; Holloway, Suttie et al. 2011). Despite some experience with using MRS for the measurement of cardiac energetics (Abozguia, Elliott et al. 2010; Shivu, Abozguia et al. 2010; Shivu, Phan et al. 2010), our group had not undertaken a comprehensive validation study to assess the reliability or reproducibility of the acquisition protocols and analysis in cardiac MRS at 3-Tesla using Image-selected *in vivo* spectroscopy (ISIS). Such a validation study was prudent prior to embarking on the sub-study of HYPER; evaluating the role of perhexiline on myocardial energetics measured by MRS. Hence a validation study was undertaken recruiting healthy volunteers. Once the reliability and reproducibility of our MRS protocols were established, the HYPER sub-study began recruitment.

The HYPER sub-study enrolled patients under the same inclusion and exclusion criterion as the main HYPER trial. In addition it excluded those patients with IHD, in order to recruit a homogenous patient cohort, which allowed the study of myocardial energetics purely on pressure overload hypertrophy and evaluated the impact of

metabolic modulation of perhexiline in these patients. As highlighted earlier, the same recruitment concerns as with the HYPER trial persisted in this study.

When the DSMB recommended the HYPER trial to be halted on the basis of futility, this also referred to the futility in pursuing the use of perhexiline in any form, in patients undergoing cardiac surgery. Therefore the sub-study looking at myocardial energetics also had to cease, reflected by the small number of patients recruited. The small numbers recruited into this sub-study ultimately limits the interpretation of the data and influences the conclusions that can be extrapolated from this study.

In hindsight studies that have the same IMP being evaluated should not be so intrinsically inter-linked and this would avoid one impacting on the other. Evaluation of myocardial energetics and impact of perhexiline in left ventricular hypertrophy should have been a stand-alone study. Recruiting patients that were awaiting cardiac surgery disrupted recruitment and trial progression, due to the clinical urgency for an operation.

The Magnetic Resonance Spectroscopy analysis of cardiac energetics has refuted the hypothesis that perhexiline therapy via metabolic modulation improves the cardiac energetic status in patients with left ventricular hypertrophy secondary to pressure overload, due to aortic stenosis. Furthermore, speckle-tracking echocardiography showed no difference between the groups. This is unsurprising given the neutral findings of the clinical trial and preliminary hypothesis being disproved. It was evident at this stage that any further laboratory studies evaluating the role and impact of perhexiline on cardiac metabolism would be fruitless.

8.3 Metabolomic assessment of the hypertrophic myocardium

The metabolomic study of cardiac tissue is novel in that, to our knowledge the metabolomic assessment of myocardial halves has not been studied before. Furthermore, despite the expected difference between the epicardial and endocardial halves due to known pathophysiological changes, their metabolome was not different. This study has been discussed in detail in Section 6.4. This metabolomic study has disproved the hypothesis that, the pathophysiological changes that take place during pressure overload hypertrophy changes the metabolome between the epicardial and endocardial halves. However, better statistically powered metabolomic studies are warranted to examine this further.

Cardiac tissue is difficult to obtain and most metabolomic studies in the literature are performed on plasma and serum (Mayr 2011). The process of mass spectroscopy based metabolomics destroys the tissue being examined, which limits the number of analyses that can be performed on a specific tissue sample. These constraints impact on the feasibility of metabolomic assessment in cardiac research using cardiac tissue. Our group have performed a number of preliminary studies to optimise the methodology and analysis described in this thesis, initially by using animal tissue and then subsequently running a series of studies related to identifying the metabolomic changes to metabolic therapy in cardiac tissue; first with GIK therapy (Howell 2010) and then with perhexiline therapy (Drury NE, Howell NJ et al. 2014). Due to the lack of clinical benefit with perhexiline in LVH and previous metabolomic studies showing no change in the metabolome with perhexiline, that line of enquiry in LVH was groundless.

8.4 Identifying key regulators involved in low cardiac output

Through the results of the clinical trials and assessment of cardiac energetics, it was evident perhexiline had little if at all no impact on myocardial protection, let alone any clinical outcomes associated with an improved myocardial protection. Therefore this final study was developed to identify key master regulators that may have an inherent mechanistic role in the development of low cardiac output episode (LCOE) post cardiac surgery. Our group had previously identified that GIK therapy may improve myocardial protection by some of the insulin properties, that increase the phosphorylation of AMPK and AKT (Howell, Ashrafian et al. 2011). However it was unclear if some of these regulators were individually responsible towards predisposing a patient to sustain a low cardiac output episode.

This study supports the hypothesis that the key master regulators AMPK and the RI3K/AKT pathway are involved in cardioprotection and there is upregulation of their activity in those patients that do not develop a LCOE in LVH. This study was preliminary work looking into these regulators and therefore is more hypothesis generating, as the study had no statistical significance (almost reaching significance for AKT activity). This study works from a bottom-up approach in trying to understand the difference in metabolic pathways between those that have LCOE compared to those that do not in LVH prior to any metabolic manipulation.

8.5 Conclusions

Perhexiline had promise. Through initial work conducted by our group in patients with cardiomyopathy there was evidence to suggest that perhexiline modulated the myocardial metabolism reflected by an overall improvement in cardiac energetics,

which translated to improved clinical outcomes. The potential to improve myocardial metabolism and make it more efficient by driving the glucose metabolic pathway using an oral agent such as perhexiline, seemed preferable to the labour intensive GIK therapy that had proved to improve myocardial protection in patients undergoing cardiac surgery. Current myocardial protection strategies although adequate in most cases, remain imperfect with some individuals succumbing to a low cardiac output episode post cardiac surgery. Metabolic modulation as an adjunct to standard myocardial protection therefore is still favourable particularly with patients who have metabolic derangement, such as in pressure overload hypertrophy.

Work presented in this thesis, shows that oral perhexiline has no additional benefit in augmenting standard myocardial protection strategies in patients with left ventricular hypertrophy secondary to aortic stenosis. There were no apparent improvements in any of the clinical outcomes. These clinical findings are supported by the cardiac energetic studies in patients with left ventricular hypertrophy.

The validation study on magnetic resonance spectroscopy shows that the protocol for MRS used in the work presented in this thesis, is both reliable and reproducible. Cardiac energetic studies evaluating the role of perhexiline on left ventricular hypertrophy showed no improvement in cardiac energetics with a longer duration of therapy. In addition, perhexiline therapy failed to demonstrate an improvement in overall systolic and diastolic ventricular function in the hypertrophic heart.

The studies using perhexiline as a metabolic modulator in the context of cardiac surgery and in patients with left ventricular hypertrophy, shows no overall metabolic or clinical benefit. This seals the fate of perhexiline and limits its use as a metabolic modulator to

patients who are not cardiac surgical candidates and are refractory to maximal medical therapy. This adds to the body of evidence on perhexiline and further hypothesis generation should be carefully considered against this overwhelming weight of evidence showing no myocardial metabolic benefit in cardiac surgical candidates.

Evaluation of the metabolome of the hypertrophic myocardium although expected to show a difference due to the pathophysiological stressors of hypertrophy, showed no difference in the metabolome between the epicardial and endocardial halves. This suggests that any metabolomic differences that exist are subtle and further evaluation is warranted to corroborate these initial findings.

This thesis finally presents preliminary work in identifying key regulators that may innately be responsible or influence a low cardiac output episode. It is evident that although no statistical significance was reached, there is an increase in AMPK and AKT activity in patients with LVH that do not sustain a low cardiac output episode, highlighting the intrinsic cardioprotective properties. This study was the first step towards identifying key regulators that influence low cardiac output episodes. Subsequent studies can then target metabolic therapies that up-regulate these specific processes and this may improve myocardial protection further.

8.6 Future work

8.6.1 Metabolic manipulation to improve myocardial protection

The neutral findings from the HYPER trial should not detract from the potential therapeutic role of augmenting myocardial protection by metabolic modulation. Through the trials of GIK in cardiac surgery, it is evident that some metabolic therapies are associated with improved myocardial protection. Future research should aim to

identify and evaluate a metabolic modulating agent that is potent, easily administered and monitored and one that is applicable in real-world cardiac surgery. Despite very promising laboratory outcomes for some metabolic therapies such as perhexiline, translating these into clinical practice may not bring to fruition the same expected outcomes. Therefore when using an IMP that has not been used in the same field of study, it is prudent to perform a phase II trial in the first instance and this would also help towards calculating an adequately powered study if the IMP is thought to have adequate efficacy in the cohort studied.

The need to find an appropriate metabolic agent that will augment myocardial protection continues, particularly in the most vulnerable groups of patients, such as in hypertrophic or dilated heart disease. Metabolic therapies should aim both to reduce the uncoupling effect between glycolysis and glucose oxidation and to reduce the burden of fatty acid oxidation during the ischaemic and reperfusion phases of cardiac surgery.

As opposed to modulating the substrate utilised and driving a more efficient metabolic pathway, an alternate mode of metabolic modulation would be to target the Krebs Cycle intermediates. Fumarate is an intermediate within the Krebs Cycle and is reduced to succinate during hypoxia (Laplante, Vincent et al. 1997). Our group have conducted some preliminary work evaluating the cardioprotective properties of fumarate. In a mouse model, inactivation of the gene that breaks down fumarate (fumarate hydratase, Fh1) showed that the Fh1-knock out mice were more tolerate of ischaemia with reduced myocyte necrosis, improved recovery of coronary flow reserve and reduction in troponin release (Ashrafian, Czibik et al. 2012). These findings were consistent with oral supplementation of fumarate. This increased tolerance to ischaemia was found to

be associated with an increased expression of redox sensitive transcription factor Nrf2, a key element in the antioxidant pathway. These findings support the proposal to perform a double blind placebo controlled randomised control trial evaluating the role of oral fumarate as a cardioprotective agent in patients undergoing cardiac surgery, using similar markers of myocardial protection. Tissue obtained from these participants will help understand the mechanism and cardioprotective properties of Nrf2 activation.

8.6.2 Metabolomics and cardiac surgery

The collaboration with the School of Biosciences at the University of Birmingham, where the metabolomic studies were conducted is now well established. Metabolomic studies on cardiac tissue, can be taken further to evaluate the metabolome of tissue from different chambers of the heart; the assessment between right and left ventricular myocardium may help understand the metabolomics of right ventricular dysfunction in some individuals post cardiac surgery. Furthermore a study of the metabolome between pressure overload and volume overload hypertrophy can help map a metabolomic skeleton for the spectrum of hypertrophic disease. Further metabolomic profiling of patients known to have a deranged metabolism such as dilated cardiomyopathy may help identify particular metabolic pathways that can be up-regulated.

8.6.3 Identification of key metabolic regulators

The final study in this thesis examined and identified an increased activity of key regulators AMPK and AKT within the group that did not have a low cardiac output episode. These regulators are known to have cardioprotective properties. Further studies are warranted to evaluate the activity of their down stream targets and potential mechanism of action. This bottom-up approach in identifying regulators that

are inherently more active in patients that do not sustain a low cardiac output episode helps identify the specific targets for manipulation. The activation of AKT is intrinsic to the RISK pathway; therefore pharmacological agents that target this pathway can enhance their cardioprotective properties and improve myocardial protection.

8.6.4 Other potential cardioprotective pharmacological agents

Our group has conducted a retrospective analysis of prospectively collected data on diabetic patients who have undergone cardiac surgery over a 6 year period, and found that there is an increased benefit with reduction in overall mortality and incidence of low cardiac output episodes in patients that have been on metformin compared to those not on metformin (unpublished). These clinical results are insightful. There has been speculation for some time that metformin may have some cardioprotective benefits (Sasali and Leahy 2003), however this needs further thorough evaluation. This is currently underway by way of laboratory based testing on cardiomyocytes subjected to hypoxemic testing, to understand the mechanistic cardioprotective properties if any, with metformin. Retrospective identification of new cardioprotective pharmacological agents should be conducted in parallel with laboratory studies that help elucidate and identify the likely mechanism of cardioprotective action. Further studies to analyse human tissue from patients on metformin undergoing cardiac surgery, are planned.

9 APPENDIX

9.1 ACC/AHA 2008 Valvular heart disease indications for AVR

Class I

1. AVR is indicated for symptomatic patients with severe AS. *(Level of Evidence B)*
2. AVR is indicated for patients with severe AS undergoing coronary artery bypass graft surgery (CABG). *(Level of Evidence C)*
3. AVR is indicated for patients with severe AS undergoing surgery on the aorta or other heart valves. *(Level of Evidence C)*
4. AVR is recommended for patients with severe AS and LV systolic dysfunction (ejection fraction less than 0.5). *(Level of Evidence C)*

Class IIa

1. AVR is reasonable for patients with moderate AS undergoing CABG or surgery on the aorta or other heart valves *(Level of Evidence: B)*

Class IIb

1. AVR may be considered for asymptomatic patients with severe AS and abnormal response to exercise (e.g., development of symptoms or asymptomatic hypotension). (Level of Evidence: C)
2. AVR may be considered for adults with severe asymptomatic AS if there is a high likelihood of rapid progression (age, calcification, and CAD) or if surgery might be delayed at the time of symptom onset. (Level of Evidence: C)
3. AVR may be considered in patients undergoing CABG who have mild AS when

there is evidence, such as moderate to severe valve calcification, that progression may be rapid. (Level of Evidence: C)

4. AVR may be considered for asymptomatic patients with extremely severe AS (aortic valve area less than 0.6 cm2, mean gradient greater than 60 mm Hg, and jet velocity greater than 5.0 m per second) when the patient's expected operative mortality is 1.0% or less. (Level of Evidence: C)

Class III

1. AVR is not useful for the prevention of sudden death in asymptomatic patients with AS who have none of the findings listed under the CLASS IIa/IIb recommendations. *(Level of Evidence: B)*

9.2 Principles of an Aortic Valve Replacement

Conventional aortic valve replacements are performed through a median sternotomy, although minimal access approaches are now practiced. Cardiopulmonary bypass is established with arterial return into the ascending aorta and venous drainage from the right atrium. Myocardial protection is delivered through a cardioplegia cannula placed in the ascending aorta. Once full flow cardio-pulmonary bypass is achieved, an aortic cross clamp is placed on the ascending aorta proximal to the arterial return cannulation and distal to the cardioplegia cannulation site. The heart is then arrested using the potassium rich cardioplegia solution.

Once the heart is adequately arrested a transverse incision is made in the proximal aorta to expose the aortic valve. The valve is excised and the annulus of the aorta is debrided. The valve is sized using the appropriate sizing instruments. The tissue or mechanical valve is implanted using either a semi-continuous suture technique or an interrupted suture technique. The valve is then tied into position and the aortotomy closed.

De-airing maneuverers are performed and temporary pacing wires are placed on the right atrium and ventricle. Once the patient has achieved optimum temperature and biochemical parameters, the patient is gradually weaned off the cardiopulmonary bypass machine.

9.3 HYPER statistical analysis plan

EFFICACY

Primary Outcome

The primary end point was the incidence of inotrope use ± comparison of cardiac index to show an increase > 0.3L/min/m^2 within the first 6 hours from reperfusion

Secondary Outcomes

Cardiac index
Low cardiac output episode
Inotrope usage
Peak cardiac troponin
ECG evidence of new myocardial injury
Composite endpoint of either peak cardiac troponin or ECG evidence of new myocardial injury
Length of stay (Intensive Care, Hospital)

Safety Outcome Measures

Postoperative death
Stroke with residual deficit
Requirement for renal replacement therapy
Reoperation
Chest drainage at 12 hours
Treated infection episodes
Respiratory index (PaO_2/FiO_2) on arrival in Intensive Care and 12 hours postoperatively

STATISTICAL METHODS AND DATA CONSIDERATIONS

Analysis will be carried out using SAS© version 9.2 All statistical tests will be two-sided, and deemed to be statistically significant if $p<0.05$. No adjustments for multiplicity will be made. All analyses will be undertaken stratified for LV function at baseline and elective/urgent surgery. Consultant surgeon will be accounted for as a random effect.

The only data, which will be formally analysed, are those which could potentially be affected by which treatment group they are assigned to. Demographic and other baseline data will not be formally analysed, other than descriptively.

The two treatment groups of patients in this study will be identified in tables and listings as:

Perhexiline
Control

Handling of missing and incomplete data

Primary efficacy variable:
In the event of death before assessment of primary endpoint, LCOE will be assigned.

Secondary efficacy variables:
Missing secondary endpoints will be excluded from the analysis.

ANALYSIS

Primary efficacy variable
The primary objective is to evaluate the effects of perhexiline on the incidence of inotrope use ± comparison of cardiac index to show an increase > 0.3L/min/m^2 within the first 6 hours from reperfusion as defined by the Endpoints Committee. The primary analysis will be a non-linear mixed model accounting for baseline LV function and elective/urgent surgery with surgeon as a random effect. Odds ratio 95% CI and p value will be presented.

Efficacy evaluable population
The efficacy evaluable population will consist of the all-patients-randomised population who received therapy (perhexiline or placebo) and underwent surgery on cardiopulmonary bypass and had a Swan Ganz catheter over the 6hrs post cross clamp removal (reperfusion period), which is required to collect the primary end point data.

Secondary outcome measures

Cardiac index: to detected an increase of 0.3 L/min/m^2 in cardiac index in the first 6 hours from release of aortic cross clamp in the treatment group.

1. Cardiac index at 6 hours will be analysed using generalised linear modelling with baseline cardiac index, elective/urgent surgery, baseline LV function and treatment (Perhexiline/control) as covariates and surgeon as a random effect.

2. Cardiac index up to 12 hours will be analysed using generalised linear modelling with cardiac index (baseline, pre, post, 2, 4, 6, 9 & 12 hours) as repeated measures, adjusted to baseline, and accounting for elective/urgent surgery, LV function and perhexiline as covariates and surgeon as a random effect.

Inotrope usage: assessed by incidence of use based on predetermined protocols and total dosage/kg in the first 6 and 12 hours postoperatively.

Incidence and dose will be assessed:

1. In the first 6 hours after removal of aortic X-clamp (also includes on cardiopulmonary bypass).

2. In the first 12 hours after removal of aortic X-clamp.

The incidence of inotrope use at each time period will be assessed using a non-linear mixed model accounting for elective/urgent surgery and baseline LV function with surgeon as a random effect.

Peak cTnT: in the first 24 hours following release of the aortic cross clamp

1. Linear model with baseline cTnT as covariate accounting for baseline LV function, elective/urgent surgery and with surgeon as a random effect (use peak cTnT at any timepoint in model).

2. Repeated measures analysis – baseline, 6 hours, 12 hours, 24 hours.

ECG evidence of new myocardial injury: assessed by a blinded Cardiologist according to standard criteria using the presence of new Q waves, new bundle branch block or loss of R wave progression.

The presence of ECG evidence of new myocardial injury will be assessed using a non-linear mixed model (logistic) accounting for baseline LV function and elective/urgent surgery with surgeon as a random effect.

Length of stay (LOS): this will include both intensive care and hospital stay within the department of Cardiothoracic Surgery.

The effect of perhexiline on mean length of stay (days) will be assessed using a non-linear mixed model (poisson distribution) accounting for baseline LV function and elective/urgent surgery with surgeon as a random effect.

Safety outcome measures

Postoperative death
Stroke with residual deficit
Requirement for renal replacement therapy
Reoperation
Chest drainage at 12 hours
Treated infection episodes
Respiratory index (PaO_2)/FiO_2 on arrival in Intensive Care and 12 hours postoperatively

Should any patient report the same event more than once then this event will be counted only once in the summary tables and summarised at the highest observed severity and relationship to study drug. Specific adverse events that occur in at least 5% of patients will be formally tested using Fisher's exact test.

9.4 HYPER Patient invitation letter

To be printed on trust letter head

DATE

Dear ……..,

The HYPER trial: Support of the heart with Perhexiline in left ventricular hypertrophy

I have been informed by Prof Pagano that you are due to be admitted to the Queen Elizabeth Hospital, Birmingham in the next few months for your heart operation.

I would therefore like to invite you to participate in a study that we are conducting to help improve protection of the heart during the operation. I enclose a few pages of information regarding the study and would be grateful if you could read through them and consider whether you would like to participate.

Prior to admission for their heart operation, most patients are asked to attend the Queen Elizabeth Hospital for a Pre-Assessment Clinic. I routinely see patients in this clinic and we could discuss the study further then. Otherwise, I will telephone you in the next few weeks.

In the interim, if you have any questions, please feel free to contact myself via the Queen Elizabeth Hospital, Birmingham switchboard on: 0121 472 1311 or my mobile: **07810 251454**.

Yours sincerely,

Mr. Eshan Senanayake

Clinical Research Fellow,
Department of Cardiothoracic Surgery

9.5 HYPER Patient Information Sheet

To be printed on trust letter head

PATIENT INFORMATION

Support of the Heart with Perhexiline in Left Ventricular Hypertrophy

Principal Investigator: Mr D Pagano, Consultant Cardiothoracic Surgeon

AN INVITATION TO PARTICIPATE IN RESEARCH

The heart surgery team at the Queen Elizabeth Medical Centre is inviting patients to participate in research aimed at making aortic valve surgery safer. We would like to recruit patients undergoing this kind of surgery into a clinical trial where potentially advantageous methods of altering the way we treat patients are used to see what differences they actually make to patients' progress. We include a simple and non-technical summary of the reasons for the study and what it will involve for you over and above your routine treatment if you take part.

If you are being approached in the outpatient clinic, we would like you to take this patient information sheet home with you to read and consider participating in this study. At the Pre-admission clinic, the doctor will ask you whether you wish to participate and to sign a consent form. You can only be included in this clinical trial if you give your express permission in the form of signed consent. On admission for your operation, a heart surgical research doctor will be available to discuss the study further and answer any questions.

If you are already an inpatient on the ward, a member of the heart surgical team will be happy to discuss the study with you once you have had at least 24 hours to read through this information sheet.

Questions? Please contact Mr. Eshan Senanayake, Clinical Research Fellow in Cardiac Surgery at University Hospital Birmingham on his mobile: 07810 251454.

WHAT IS THE STUDY ABOUT?

This study is about trying to improve the treatment of patients undergoing aortic valve surgery. To perform this heart operation requires the help of a heart-lung machine, which supports your body's circulation whilst the surgeon removes the diseased valve and inserts a new valve, either biological or mechanical. During your surgery, the heart has to be protected so that it is not injured by the strain of the operation. Despite using standard techniques to protect the heart, some temporary injury still occurs from which the heart gradually recovers during the first few hours and days following the operation. We are seeking ways to improve on these established 'protective techniques' by preparing the heart before surgery with a drug that switches the fuel of the heart from less efficient fatty acids to more efficient sugars.

In summary, taking the drug Perhexiline before your operation has the potential to benefit the way your heart works during periods of strain during and immediately after your surgery.

HOW HAS THIS CLINICAL TRIAL BEEN DESIGNED?

This study is a two-centre double-blind randomised placebo-controlled trial. This means that if you agree to take part, you would be allocated by chance to receive *either* the drug Perhexiline *or* a placebo – a tablet that looks the same but has no medicinal properties. The tablets are coded so that neither you nor the surgical team will know which one you are receiving and therefore the study is called 'double-blind'. The code is revealed once all the patients in the trial have been completed. By giving the tablets in this way, we can determine the benefits of using them in patients undergoing aortic valve surgery.

HOW WILL WE MEASURE THE EFFECTS OF THIS TABLET?

To detect changes in heart function, we need to take measurements of performance before, during and after surgery to show whether the treatment has made a difference. To do this we will make use of small monitoring catheters (plastic tubes) inserted into blood vessels in the neck. These tubes are routinely used in two out of three patients who are not in the trial. These catheters are placed in position in blood vessels whilst you are under anaesthetic (asleep) and are usually removed on the first or second day following surgery. We will record measurements from these catheters during this period at specific time points. We will insert an additional sampling catheter into your heart during your operation to monitor blood in the veins of your heart. This catheter is normally used as an additional way to deliver protection to the heart in patients at high-risk for surgery; in your case, it will be used only to withdraw a sample of blood from your heart veins. It will be removed at the end of the use of the heart-lung machine.

MEASURING THE POSSIBLE BENEFICIAL EFFECTS OF THE TABLET ON THE HEART'S METABOLISM

The sampling of additional blood tests: The blood tests performed on these samples can tell us how well the heart is tolerating the surgery. These blood samples are removed through the same

monitoring lines already mentioned. The total amount of additional blood taken for this study is about a cupful (approximately 60 mls / 2 floz).

Health questionnaire: You may be asked to complete a simple health questionnaire twice during the study: once before starting the trial tablets and once in the routine follow-up clinic at approximately 6 weeks after your operation.

Imaging the heart using a transoesophageal echocardiogram: This is a test that uses ultrasound (sound waves) to produce pictures of the heart. The ultrasound probe is placed in the gullet whilst you are asleep and removed before you are woken up. It allows us to measure the function of the heart during the operation as the previous test cannot be used at this stage. This is performed routinely in all patients undergoing heart valve surgery but extra pictures will be taken to analyse the heart function.

The removal of tiny samples of the heart muscle known as biopsies: This will help tell us how the Perhexiline tablet improves the way the heart works. We would aim to obtain 3 heart muscle biopsies, all whilst you are asleep under the anaesthetic, one each at the beginning, middle and end of being on the heart-lung machine. They will be performed by the operating surgeon and are very small, about so long and thin (▬▬). A suture is placed in the tiny defect left behind and their removal incurs no increased risk during or after the surgery nor has any effect on the strength of the heartbeat. Although there is a hypothetical risk of bleeding in taking this biopsy, we have performed more than 2000 biopsies in patients at the time of writing this information leaflet with no complications.

The removal of samples of superficial muscle and fat: The Perhexiline tablet has an effect on many other parts of the body as well as the heart. We would take small biopsies, weighing approximately one gram, of muscle from the lower end of the normal surgical incision and of fat from around the heart. This will allow us to look at how Perhexiline works in more detail. This will be performed by the operating surgeon and occur while you are asleep under the anaesthetic. It would not affect the strength, appearance or discomfort of the scar after your surgery.

WHAT WILL I HAVE TO DO?

If you decide to participate, there are a number of stages to the study. You will be guided through these stages by us (the research doctors looking after you). You have been given this written information sheet and asked to read it at your leisure at home or on the ward, if you are already an inpatient. Before your surgery, in either the pre-operative assessment clinic or on the ward, a member of the research team will visit so you can ask any questions. If you give signed consent, we will then randomise you to receive either the tablet or the placebo and we will give you the medication with a date to start taking it, usually 1-2 weeks before your admission.

In the anaesthetic room of the operating theatre, the Anaesthetist will give you a general anaesthetic. Once you are asleep, the Anaesthetist will insert the pressure-measuring catheters normally inserted

at this stage. The operation will be conducted in a normal way under the care of the Consultant Cardiac Surgeon who is in charge of your case. The blood samples and biopsies will be performed while you are asleep and you will be unaware of them. The sampling of blood from the veins of the heart will also be performed whilst you are asleep during the use of the heart-lung machine only. When you awake, you will be on the intensive care unit as routine. Only some further blood samples and monitoring tests will remain. The last blood test will be performed at 24 hours after the operation.

In summary, we will be taking measurements from pressure-measuring catheters that are routinely used in heart surgery. The entire sampling of blood for the research will total 60 ml and will not affect your recovery and, because we can use intravenous pressure-measuring lines, there will be no additional need for needles. The biopsies that we take are removed painlessly during the operation and have no effect on the heartbeat.

WHAT ARE THE BENEFITS?

We do not know whether giving patients the tablet Perhexiline will benefit them as an individual – this study is investigating that question. It is hoped that this study will provide information that will benefit future patients undergoing heart valve surgery. In particular it will help us design more suitable treatments for patients who have a very weak heartbeat before surgery and are at high risk of complications after surgery. In the future and after more research, the use of this tablet could significantly improve their recovery from heart surgery.

WHAT ARE THE RISKS?

Previous studies with Perhexiline have shown that it is safe. Some patients (less than 10%) may feel a bit sick after the first few tablets but this usually passes very soon. If these tablets continue to make you feel sick, you can simply stop taking them without harm and your surgery will proceed as planned. If you stop taking these tablets, we would be grateful if you could contact the Research team on 07810 251454.

In some patients who had taken the tablet for long periods of time (many months or years), a very small number developed some numbness or muscle weakness and/or mild liver changes including going yellow (jaundice) and loss of appetite. It has since been shown that this can be avoided by monitoring the level of the drug in the blood. These side effects have never been seen when people take the tablet for just a few weeks and so are very unlikely to affect you. However, if you have any concerns, we would ask you to contact one of the Heart Surgery Research Team at University Hospital Birmingham. You can also contact your GP, who will be given more information about the tablet and the trial if you agree to participate.

All heart operations carry some risk and these will have already been discussed with you. For this study we insert routinely-used monitoring lines to measure heart function. The risks of this are minimal and the possibility of a severe complication is in the region of 1 in 15,000. The only additional

invasive procedure performed on you is the biopsy of the heart muscle. Theoretically, there is a very small risk of bleeding following this procedure. However, we have performed approximately 2000 biopsies and this complication has never happened.

WHAT ARE THE ALTERNATIVES?

If you do not wish to take part in the study, your surgery will be undertaken in the standard manner without any additional measurements, treatments or tests. Your surgeon and anaesthetist may still use the monitoring lines that we have described if they feel that their use is in your best interest.

WHAT HAPPENS TO THE INFORMATION?

All the study data will be collected by Mr Pagano's Research team. It will be stored as paper files and on a hospital computer which are kept in a locked office. The information from the study will be analysed. The information will be presented at scientific meetings and published in scientific journals to inform other doctors and health professionals of the research findings. All data is coded and confidential, ensuring that your identity will not be revealed at any time. All necessary measures will be taken to keep your data safe and confidential and to comply with the Data Protection Act. Only the Heart Surgery Research team and the Research & Development office will have access to this data. Following completion of this trial, the data will be kept for 15 years and then destroyed.

WHAT HAPPENS TO THE SAMPLES?

All of the biopsies will be stored in a coded form in a locked freezer and only the Heart Surgery Research team will have access to these samples. The samples will be analysed to look at how much Perhexiline is taken up by the heart. We will also look at what effect this has on the heart in terms of how it works and how much energy it can store. The biopsies are very small and the simple act of analysing them will destroy the sample. At the end of the study, any remaining samples will be destroyed within 6 months.

WHO ELSE IS TAKING PART?

We will recruit 220 patients to this study.

WHAT IF SOMETHING GOES WRONG?

The standard care of patients undergoing heart surgery involves intensive monitoring. This monitoring allows us to detect any problems early in their development. We do not expect the study itself to cause any problems, however as for all heart surgery, we are in an ideal position to deal with any untoward events during your operation and these will be treated in the normal manner, regardless of the research study. At the time of the measurements, you will be in either the theatre or the ITU where trained staff are at hand at all times. Your safety during and after surgery is paramount, and takes precedence over any research.

In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed due to someone's negligence, you may have grounds for a legal action for compensation against University Hospital Birmingham but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will be available to you. Further information can be obtained from the Patient Advice & Liaison Service (PALS) which is available Monday to Friday 9am to 5pm on 0121 627 8820.

WHAT HAPPENS AT THE END OF THE STUDY?

At the end of the study, your treatment will continue as would that of a patient who had not been involved with the study. You will only need to take the study tablets before your operation.

WHAT IF I HAVE MORE QUESTIONS OR DO NOT UNDERSTAND SOMETHING?

Please ask one of the investigators about any questions or worries that you may have so that any points can be clarified. You should feel free to ask questions at any time by contacting Mr. Eshan Senanayake (Research Fellow) on: 07810 251454. If you are on the ward, ask one of the nurses to contact Mr. Senanayake via switchboard. If he is unavailable, ask for either Mr. Drury (Clinical Lecturer) or Mr. Pagano (Consultant Surgeon).

WHAT HAPPENS NOW IF I DECIDE TO TAKE PART?

We will take some details and ask you to sign a consent form that documents your willingness to participate. We will give you the tablets, which will be either Perhexiline or the placebo, and instructions on when to take them. You will need to take one or more tablets twice per day prior to your operation – in the morning and in the evening. We will also write to your GP to let them know that you have agreed to join this study and giving them more details. You will be listed for surgery as normal. You are free to withdraw from the study after initially consenting without giving reason and without prejudice to your continuing care or the standard care of any future treatment.

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

This research has been organised and developed by the Heart Surgery Research teams at University Hospital Birmingham and the Royal Sussex County Hospital, Brighton. It is being funded by the **British Heart Foundation**.

WHO HAS REVIEWED THIS STUDY?

This study was given a favourable ethical opinion for conduct in the NHS by the Cambridgeshire 1 Research Ethics Committee, United Kingdom.

THANK YOU

Finally the Heart Surgery research team would like to thank you for taking the time to consider this research proposal.

PROPOSED STUDY INVOLVEMENT

This summary flow chart demonstrates what will happen if you consent to join this study:

When you were seen by your Surgeon for the first time to discuss your operation, you will have been invited to join this trial and given this information leaflet.

↓

In your free time, we would like you to read this leaflet and consider joining the study.

↓

In a few weeks, in the pre-operative assessment clinic (or on the ward if you are already an inpatient), you will be seen by a member of Mr Pagano's Research team.

↓

If you are happy to join this trial, we will ask you to give written consent to join the trial.
You will then be randomised to receive either the Perhexiline tablets or the placebo (the dummy tablets). Neither you nor the research team will know which one you are given.
You will then be given a date for your operation and a date to start taking the tablets.
An information leaflet and instructions will be included with your tablets.

↓

On your admission to the hospital for your surgery, you will be seen by a member of the Research team and asked a few questions regarding your wellbeing.

↓

You will then be taken to theatre for your operation as normal.
When you are sleep (under a general anaesthetic), we will do some blood tests, measure how well your heart is working and take the heart biopsies.

↓

After your operation you will be taken to the Intensive Care unit as normal.
We will then make further tests of how well your heart is working.

↓

After the operation, you will be have a several extra blood tests, not using a needle but from the routine monitoring lines that will be in place. After you recover sufficiently, you will be transferred from Intensive Care to the ward and then discharged home.

9.6 HYPER Consent form

Printed on hospital letter head

CONSENT FORM

Support of the Heart with Perhexiline in Left Ventricular Hypertrophy

Principal Investigator: Mr D Pagano, Consultant Cardiothoracic Surgeon

Please initial boxes

I confirm that I have read and understand the information sheet (Version 4a, dated 28/02/10) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
I understand that blood and tissue samples will be kept for the purpose of research and I give permission for these samples to be taken and stored. I understand that the process of analysing the samples will destroy them and that any remaining samples will be destroyed within 6 months of the end of the study.	☐
I confirm that I have read and understand the supplemental information sheet and agree to be included in the sub-study involving additional MRI scans [delete if not applicable].	☐
I agree to my GP being informed of my participation in the study.	☐

I agree to take part in the above study.

Name of Patient	Signature	Date
Name of Person taking consent	Signature	Date

9.7 HYPER Patient information on dosing schedule

PATIENT INFORMATION ON TAKING STUDY TABLETS

Support of the Heart with Perhexiline in Left Ventricular Hypertrophy

Principal Investigator: Mr D Pagano, Consultant Cardiothoracic Surgeon

Thank you for participating in our study. This information sheet provides details of how and when to take the study tablets, so that you don't take too little or too much of the medication.

Your surgery is scheduled for:

Start taking the study tablets on:

For the first 3 days of treatment, take **TWO** tablets in the morning (breakfast-time) and **TWO** tablets in the evening (tea-time). You may take the tablets before or after a meal.

From the 4th day of taking the study tablets ( ) onwards, take **ONE** tablet in the morning and **ONE** tablet in the evening.

Continue taking **one tablet, twice a day** until you are admitted to hospital for your surgery. If you are admitted to hospital for any other reason, let the doctors looking after you know that you are participating in this trial and ask them to contact Mr Senanayake, Clinical Research Fellow in Cardiac Surgery via the Queen Elizabeth Hospital switchboard (including at weekends).

If your surgery is postponed for any reason, we will contact you.

Questions? Please contact either the secretary to Mr D Pagano, Consultant Cardiothoracic Surgeon or **Mr Eshan Senanayake**, Clinical Research Fellow in Cardiac Surgery via the Queen Elizabeth Hospital, Birmingham switchboard on 0121 472 1311 or mobile: **07810251454**.

9.8 HYPER Trial protocols

9.8.1 Anaesthesia and pre-sternotomy

All patients received standardised pre-medication approximately 90 minutes prior to anaesthetic induction. This included temazapam (maximum 30mg), ranitidine 150mg and metoclopramide 10mg.

Anaesthetic induction was achieved with fentanyl 10-15mcg/kg, propofol 0.5-2mg/kg and pancuronium 0.1mg/kg or rocuronium 0.5-1mg/kg. Anaesthesia was maintained with propofol (1-2% neat strength at the discretion of the anaesthetist), infused at 4-8mg/kg/hr and alfentanil 25mg in 50mls (neat) infused at 50mcg/kg/hr. Other volatile anaesthetic agents were not permitted.

All patients required invasive monitoring, which included an arterial line, central venous pressure (CVP) monitoring line and Swann-Ganz, Pulmonary Artery Flotation Catheter (PAFC), which was floated prior to the sternotomy. In addition to arterial and intravenous monitoring, all patients had a trans-oesophageal echocardiogram probe placed.

Prior to cardiopulmonary bypass (CPB), mean arterial pressure (MAP) was maintained between 60-70mmHg using aliquots of phenylephrine (5mg in 20ml of 5% dextrose) or an infusion of phenylephrine (10mg in 50ml of 5% Dextrose). Phenylephrine was used as the first line vasopressor due to its pure α-agonist properties.

In line with unit practice mannitol (20%) was administered 0.5mls/kg as an osmotic diuretic and antibiotic prophylaxis was administered intravenously prior to

sternotomy. An anti-fibrinolytic such as aprotinin or tranaexamic acid was used at the discretion of the surgeon.

Heparin was administered prior to institution of CPB to achieve full anti-coagulation (400units/kg) and this was reversed with protamine (1mg protamine per 100 units of heparin) after termination of CPB.

9.8.2 Operative procedure

After induction with anaesthesia, the patients were transferred into the operating room and appropriately prepped and draped according to standard departmental procedures. If CABG was being performed, conduit was sourced. This usually included the left internal mammary artery and long saphenous vein, which were harvested simultaneously. Approach to the heart was via a median sternotomy.

After adequate conduit was harvested, the patient was instituted on CPB and cooled to 32°C, with venous drainage via a two-stage cannula place in the right atrium and arterial return established by placement of a single arterial cannula in the ascending aorta. Before administration of cardioplegia an aortic cross clamp was placed across the ascending aorta, proximal to the aortic cannula and distal to the coronary arteries.

In patients that received concomitant re-vascularisation with CABG, the distal anastomoses of all free grafts were performed first. Then the aorta was opened through a transverse or hockey shaped incision and the aortic valve explanted and annulus debrided. The aortic annulus was sized and the new valve (biological/mechanical) was implanted as per the individual surgical technique. Throughout the process of valve explantation and implantation, cardioplegia was administered intermittently

(approximately every 20 mins) directly into the coronary ostia, at the surgeons' discretion. The heart was vented either through a direct left ventricular vent placed at the apex or right superior pulmonary vein vent. The heart was then de-aired and the aortotomy closed. If a pedicled coronary artery graft was to be placed, the distal anastomoses of this was performed next. These steps were undertaken during a single cross clamp period. Having completed the above steps, the aortic cross clamp was removed and all proximal coronary artery bypass graft anastomosis were performed using a partial occlusion technique using a side-biting clamp. However if the use of a side biting clamp was difficult due to severe aortic calcification, these proximal anastomosis were performed during a single complete aortic cross clamp period.

The patient was re-warmed on CPB using a heat exchanger to a nasopharyngeal temperature of 36-37°C. Two right atrial and ventricular pacing wires were placed to allow sequential pacing. Patients were gradually weaned and discontinued from CPB with a heart rate of 70 – 110bpm (native or paced), without inotropic support if possible. Drains were placed to the mediastinal and open pleura. The patient was closed with sternal wires and a layered closure to soft tissues, following appropriate haemostasis and a period of haemodynamic stability. The patient was then transferred to the cardiac intensive care unit.

Inta-operative arrhythmias were treated with internal defibrillation at the discretion of the surgeon. Furthermore, intravenous glycopyrollate or atropine was used to improve and/or achieve the above target native cardiac rhythm.

9.8.3 Cardiopulmonary bypass and cardioplegia

Cardiopulmonary bypass was utilised in all patients to perform AVR±CABG and was established in all cases using a roller pump. The lines were primed with 2L Hartman's solution and 10,000 units of heparin. For oxygenation during CPB, a Capiox SX oxygenator was used. CPB was maintained with a flow rate of 2.4 L/min/m^2 and mean radial artery blood pressure was maintained between 50-60 mmHg. During CPB, phenylephrine aliquots or infusion (described above) was used to maintain these pressures.

All patients were actively cooled to 32°C. Blood/water temperature gradient was maintained ≤7°C on the heat exchanger during cooling and re-warming phases. Once the patient reached a nasopharyngeal temperature of 35°C, the heat exchanger temperature was set to 37°C.

Cardioplegia was used as the main, predominate myocardia protection strategy. To achieve and maintain diastolic cardiac arrest, antegrade intermittent cold blood cardioplegia was used. This was initially administered via the aortic root following aortic cross clamp placement. Once the aorta was opened, cardioplegia was administered directly down the coronary arteries. For induction, St Thomas A stock solution (800ml blood + 200ml stock A) was used at 12ml/kg, and for maintenance St Thomas B stock solution (400ml blood + 100ml stock B) was used at 6ml/kg. The composition of St Thomas solution (Martindale Pharma, Brentwood, UK) is outlined in Table 9-1.

The cardioplegia was maintained between 4-7 °C and delivered at a pressure of 120-150 mmHg. Maintenance doses were administered at intervals of 20mins or earlier, or

at the discretion of the surgeon. Supplemental retrograde cardioplegia was not allowed. Supplemental cardioplegia 'hot shots', were allowed at the discretion of the surgeon.

St Thomas A	St Thomas B
898ml Ringers compound sodium chloride	966 Ringers compound sodium chloride
102ml cardioplegia solution	34ml cardioplegia solution
- 16.6g magnesium chloride	- 5.2g magnesium chloride
- 6.1g potassium chloride	- 1.9g potassium chloride
- 1.4g procaine hydrochloride	- 0.4g procaine hydrochloride

Table 9-1 Composition of cardioplegia solution

9.8.4 Post operative care

All patients were managed according to predetermined protocols with regard to their peri-operative and post-operative intensive care management. These are outline below:-

9.8.4.1 Haemodynamic parameters

All patients were maintained within the optimal haemodynamic targets. Filling pressures (pre-load), as advised by the clinical team based on the patient's existing condition and haemodynamic behaviour prior to, during and post CPB were maintained. If no specific targets were set by the surgical team the following targets were maintained: -

- Heart rate 70-110bpm (native or paced rhythm)
- CVP 8-12 mmHg

- MAP 65-85 mmHg
- CI ≥ 2.2 L/min/m^2
- Haemoglobin > 7

To meet these parameters and achieve an ideal preload state intravenous colloid (gelofusin, volulyte or human albumin solution) was used. If blood or blood products were necessary based on drain losses, these were administered at the discretion of the clinical team.

9.8.4.2 Assessment and management of post-operative hypotension

Hypotension was defined as a MAP < 60 mmHg. Prior to any intervention the arterial line calibration, cardiac rhythm, CVP/PAWP and drain losses were assessed and optimised. If the preload was not adequate measured by CVP of < 8 mmHg the patient was resuscitated with volume, guided by the trend in filling pressures and haemodynamic measurements. Depending on serial haemodynamic measurements trends and the above-mentioned parameters, the following actions were taken: -

1. If CI < 2.2 L/min/m^2 with a normal SVR (800-1200 dyne/cm/sec) – the patient was managed as per LCOE.
2. If CI > 3 L/min/m^2 with a low SVR (<800 dyne/cm/sec) – vasoconstrictor support was instituted with an infusion of phenylephrine (10mg in 50 ml 5% Dextrose) and titrated to maintain MAP > 60mmHg.
3. If further vasoconstrictor support was required after reaching the ceiling levels of phenylephrine, vasopressin (20U in 40ml of 5% dextrose) was instituted.

Phenylephrine (10mg in 50ml 5% Dextrose) was the 1st line vasoconstrictor used in this trial. Vasopressin (20U in 40 ml 5% dextrose) was the 2nd line vasoconstrictor. If

further vasoconstriction was required, Noradrenaline (2mg in 50ml 5% dextrose) was then used.

9.8.4.3 Other parameters

Urine output was maintained at ≥ 0.5 ml/kg/hr. Potassium was maintained at 4.5-5.0 mml/L and boluses of 10 and 20mmol of potassium chloride diluted in 30ml 5% dextrose were administered to maintain this. Blood glucose was maintained 4-10mml/L and intravenous insulin therapy as per the unit's policy was administered to maintain these blood sugar levels. Arterial blood gas measurements were performed regularly in addition to the trial requirements and aided the management of the above targets.

Extubation was achieved through an existing nurse-led unit's protocol and the same was followed for the trial patients.

All patients received a continuous infusion of maintenance fluid of 5% dextrose at 1ml/kg/hr. Maintenance of sedation was achieved with propofol 10mg/ml and alfentanil (25mg/50ml 5% dextrose).

Initial post-operative hypertension (BP > 160/90) was treated with glyceryl trinitrate solution (GTN) (50mg/50ml) and/or sodium nitroprusside (SNP) (50mg/50ml 5% dextrose) and/or doxazosin 1-2mg orally.

For patients on the ITU and ward, the unit's policy for the treatment and management of the following standard post-operative conditions were followed: -

- Anti-emetic therapy
- Antibiotic prophylaxis

- Thrombo-embolic prophylaxis
- Analgesia
- Anti-platelet therapy
- Diuresis
- Gastric protection
- Sustained hypertension
- Re-starting cardiovascular drugs and other pre-existing medication

The decision to re-open a patient due to excessive blood loss, cardiac arrest or cardiac tamponade is purely a clinical decision and was at the discretion of the clinical team. In this instance, the findings were recorded and discussed at the end-points meeting.

ITU and ward discharge was at the discretion of the clinical team. The patients' involvement in the trial ceased when they were discharged from the hospital or upon in-hospital death.

9.9 HYPER Trial measurements

9.9.1 Baseline blood samples

Before induction of anaesthesia, fasting arterial blood samples were taken from each patient. These were collected separately into Z Serum Sep Clot Activator, K3E K3EDTA and Z Serum clot Activator (plain) vacuette bottles and immediately centrifuged at 3000g for 5 mins. The supernatant was pipetted out into NUNC cryotubes and frozen at -80 °C. A further sample of blood was collected into a Z Serum Sep Clot Activator vacuette bottle and sent to the clinical chemistry department at the Queen Elizabeth Hospital, to assess the baseline cardiac Troponin.

9.9.2 Haemodynamic studies

The PAFC or Sawn-Ganz has a balloon at the end of the catheter. This was introduced into the Swan-Ganz sheath; at approximately 20cm it sits in the internal jugular vein. The balloon was then inflated and floated through the right atrium, right ventricle and into the pulmonary artery, where it usually wedges itself in one of the pulmonary artery branches. The balloon is then deflated and the tip of this catheter continues to sit in this position. This allows PAFCs to be used to perform haemodynamic flow studies based on the Fick principle of thermodilution. Haemodynamic measurements were thus conducted at specific time points as follows: -

1. Baseline after induction of anaesthesia and ideally before sternotomy.
2. Immediately before administration of protamine after complete discontinuation from CPB, having optimised the rhythm, pre-load, optimum haemodynamic parameters and afterload.

3. Approximately 10 mins after administration of protamine and whilst maintaining optimum haemodynamic parameters.
4. 2, 4, 6, 9 and 12 hrs post AXC removal (these measurements were usually done on the ICU).

The Fick principle of haemodynamic measurements allows the assessment of cardiac output and this in turn is used to calculate the cardiac index based on body surface area (BSA).

The measurement at each time point was an average of 3 successive injections of 10ml of cold (6-10°C) 5% dextrose solution through the proximal port of the PAFC at end expiration. The PAFC allows the measurement of the temperature of the injectate and the temperature at the distal port (sitting in the pulmonary artery). The difference in temperature between these points, against time allows the calculation of cardiac output.

Data on the heart rate, systemic and pulmonary artery pressures, pulmonary artery wedge pressures and central venous pressures were also recorded at the above time points.

9.9.3 Serial blood samples

Arterial blood gas analysis was done intra-operatively and in the peri-operative period on the ITU at the above mentioned time points coinciding with the haemodynamic studies. Further arterial blood samples were collected at 6, 12 and 24 hrs post aortic XC removal into Z Serum Sep Clot Activator, K3E K3EDTA vacuette bottles and centrifuged at 3000g for 5 mins. The supernatant was pipetted out into NUNC cryotubes and frozen at -80 °C. Another set of arterial blood samples were collected at 6, 12 and 24 hrs post

AXC removal into a Z Serum Sep Clot Activator vacuette bottle and sent to the clinical chemistry laboratories at the Queen Elizabeth hospital, for cardiac Troponin analysis. Samples taken overnight were stored in a 4°C blood fridge overnight and processed as above the following morning.

9.9.4 Trans-oesophageal echocardiography

Transoesophageal echocardiography (TOE) was performed in all patients after the induction of anaesthesia and prior to the institution of CPB. This was to quantify and assess the degree of hypertrophy intraoperatively. Furthermore TOE was used to assess biventricular diastolic and systolic function and the aortic valve gradient. Aortic valve area was calculated using the continuity equation; calculating the left ventricular out flow tract diameter, and the continuous and pulse waveform Doppler assessments of velocities at the aortic valve (Shanewise, Cheung et al. 1999; Lang, Bierig et al. 2005). After discontinuation from CPB, the above TOE measurements were repeated to assess ventricular function, valve area and flow velocities.

9.9.5 Electrocardiographic measurements

A baseline 12-lead electrocardiogram (ECG) was performed in all patients pre-operatively. This was compared against a 12-lead ECG that was performed prior to hospital discharge (usually on day 4 post-operatively), to assess for new myocardial injury.

New myocardial injury identified on an ECG was defined as: -

- The presence of new Q wave of ≥ 2mm in ≥ 2 contiguous leads
- The presence of new buddle branch block

OR

- Loss of R wave progression

9.9.6 Myocardial tissue biopsies

Myocardial tissue biopsies were obtained from all possible patients. An intra-operative surgical assessment of tissue quality was undertaken before obtaining the biopsies. Right atrial biopsies were obtained just before the institution of CPB. This biopsy site was incorporated into the surgical technique of placing the right atrial venous drainage cannula. Left ventricle (LV) free wall full thickness biopsies were obtained from non-fibrotic areas between the left anterior descending artery and the first diagonal artery. This was performed using a Trucut biopsy needle (Allegiance Healthcare, McGaw Park, IL). The LV biopsy sites were oversewn and repaired. LV biopsies were obtained at the following time points: -

1. After the institution of CPB but before the application of the aortic XC (onset of ischaemia).
2. Just prior to removal of the aortic XC (end of ischaemia).
3. Approximately 10 mins after the removal of the aortic XC (reperfusion).

When possible, further biopsies were obtained of skeletal muscle (rectus or intercostal), epicardial fat and leg fat (during saphenous vein harvestation) before the institution of CPB.

All biopsies were immediately snap frozen in liquid nitrogen and then stored at -80°C.

9.10 EQ-5D Quality of life questionnaire

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

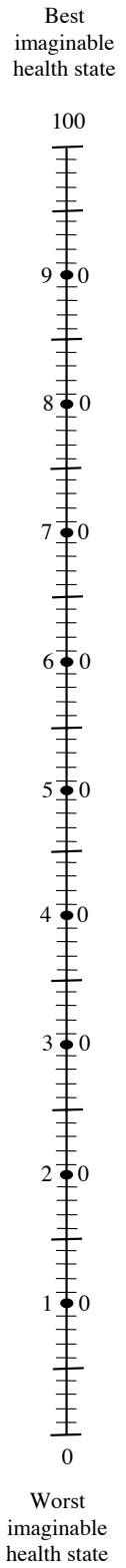
I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



9.11 HYPER Trial safety and standard operating procedures

Report of Serious Adverse event for HYPER trial

Patient ID Number –
Patient Study number –

The research nurse/clinical fellow should report any SAE related to the research procedures and those that are unexpected, by email to eshan.senanayake@uhb.nhs.uk as soon as the SAE is known.

The clinical research fellow (Mr. Eshan Senanayake) will then inform the sponsor through the sponsor's online reporting system and also inform the necessary authorities i.e. MHRA. REC etc

Details of the person reporting
Name:
Designation:
Telephone:
Email:

Type of Event
Death Life threatening Unexpected drug reaction
Persistent or significant disability or incapacity
Congenital anomaly or birth defect Other

Description of the event
Date of the event:
Time of the event:
Location:
Description of the circumstances of the event:

Assessment of Causality
Did the trial drug cause this serious adverse event?
No-unrelated Possibly Probably Definitely

Assessment of Expectedness
Was the serious event expected or unexpected? Expected Unexpected

Declaration
Signature of person reporting:
Print Name:
Date of report:

Code breaking procedure

Responsibilities
Clinical research nurse and clinical research fellow

SOP

- If an event such as a SUSAR (refer to safety reporting procedure) occurs that requires the code to be broken, the following procedure must be followed:
    - Contact pharmacy, who will confirm the bottle number allocated to the subject participant
    - Clinical research fellow, Mr. Eshan Senanayake is informed regarding the need to break the code for a particular participant (contact number 07810251454)
    - Mr Eshan Senanayake will inform the following:
        - Local principal investigator, Mr. Mike Lewis
        - Main PI Mr. Pagano
        - Sponsor

        and contact Mr. Neil Howell (unblinded member of the research team), who has access to the code and group allocation
    - In the event that Mr. Eshan Senanayake is not contactable, Mr. Neil Howell can be contacted directly on 07977454540
    - The local collaborator/PI; Mr. Mike Lewis (consultant cardiothoracic surgeon), must be informed regarding the above events
    - In the event that Mr. Neil Howell is not contactable, Mr. Domenico Pagano (PI for the HYPER trial) must be contacted
    - Once the code is broken and treatment arm is identified standard operating procedures for reporting adverse incidents will be followed as per R&D guidelines
    - All reporting will be done by Mr. Eshan Senanayake and overseen by Mr Domenico Pagano

Requirements
The following persons are required to read the above SOP and follow the above instructions:-
Clinical Research nurse
Clinical research fellow
Local PI

Incidents that do not require reporting as adverse events (SAE, SUSAR)

Responsibilities

Clinical research nurse and clinical research fellow

SOP

- Cardiac surgery alone, by itself is a significant intervention
- There are well known, expected, well recognised complications that would require either an extended ITU stay, ward stay or overall extended stay in hospital
- There is also an expected risk of death and morbidity, which is incorporated to the consent process for cardiac surgery
- Therefore the following expected complications in addition to the side effects outlined in the investigational brochure should not be reported as an adverse event. However, these events are recorded as per the data collection forms.
    - Re-operation
        - Low cardiac output syndrome
        - Tamponade
        - Bleeding
        - Cardiac arrest
    - Respiratory complication
        - Pneumonia
        - CPAP support
        - Re-intubation
        - Tracheostomy
    - Renal complications
        - Renal impairment (Cr>200)
        - CVVH
    - Neurological complications
        - Patient not waking
        - Focal CVA/TIA
        - Type II neurological complication
    - Abdominal complications
        - Ischaemic gut
        - GI bleed
        - Pancreatitis

- Sternal wound dehiscence and infection
- Led wound dehiscence and infection
- Atrial Fibrillation
- Requirement for Permanent pacemaker
- Extended stay due to mobility and social issues

- However all deaths occurring before or after surgery once the patient is recruited into the study should be reported

Requirements

The following persons are required to read the above SOP and follow the above instructions:-

Clinical Research nurse

Clinical research fellow

Local PI

9.12 HYPER DSMB recommendation

DSMB Hyper – Minutes 22nd June 2012

Paul Jordan,

Nick Freemantle

Thank you for the excellent preparations in support of our meeting.

Recommendation

We note that recruitment has proven difficult and have no suggestions on approaches which may improve the rate in this somewhat complex trial.

We are concerned that one patient took a week of trial medication and then withdrew, and thus may have been exposed to the investigational product. We are not convinced that all appropriate steps have been taken by the study team to ensure that the patient's clinical follow up is complete as per protocol.

We believe that, if the trial continues at the current rate, the recruitment objective is unlikely to be achieved in less than 20 months.

We note that we have been asked to assess the trial for futility, in addition to safety and efficacy, and are content to do this, using an O'Brien Flemming alpha Spending approach for futility in addition to our existing schedule.

We assess that the trial is unlikely to achieve its scientific objectives and are content for the trial to be halted at this point, ensuring patients already in follow up receive appropriate care and assessment under the protocol, and for their clinical need.

Further, we are concerned that there are some signs of reduced cardiac output associated with exposure to perhexiline in this trial and in the completed CASPER trial although are unsure of its clinical importance. Given the apparent futility on the scientific objective of the trial we believe that the appropriate course of action is for the Trials Steering Committee to halt recruitment to the trial at this point. We are happy to discuss this recommendation further.

Closed Meeting Minutes

We assessed analyses of the primary and secondary outcomes, and noted differences in cardiac output and cardiac index (see report). In addition we noted the apparent futility of the trial according to the predefined criteria.

We had concerns about the difference in serum creatinine post procedure.

We saw no evidence of clinical benefit associated with the investigational treatment, and note that a similar trial was also neutral on this outcome.

We recognised the likely logistic challenges particularly in staffing associated with completion of the study as originally planned, but regard this as a study management issue rather than one relating to the question of patient safety.

TABLE 2. THE BORG SCALE

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (maximal)

9.14 Dosing regime for extended trial therapy

Dose planning based on Perhexiline assays result on day 7

Perhexiline concentration(mg/L)	Recommended new daily dose (mgs)
0.00-0.05	300
0.05-0.15	250
0.15-1.00	200
1.00-1.50	100
1.50-2.00	50
>2.00	Cease for 1 week then 50 mg on alternative days

Dose planning based on Perhexiline assays result at ≥ 4 weeks on therapy

Perhexiline concentration(mg/L)	Recommended new daily dose (mgs)
<0.15	Double the daily dose
0.15-0.60	No change
0.60-0.90	Reduced dose by 25%
0.90-1.20	Halve the daily dose
>1.20	Cease for 1 week then reduce the daily dose to 25% of the previous dose

9.15 Protocol for tissue preparation and MS analysis

Tissue preparation and extraction for metabolomic analysis

This process used a methanol:chloroform tissue extraction process for very small samples (≤ 10mg)

Equipment required:

Precellys 24 homogeniser

1.8ml glass vials with aluminium lined caps (Fisher TUL 520 006 J)

2ml Eppendorfs

Glass Pasteur Pippettes

Hamilton Syringes (Fisher 500µl)

Solvents requires:

100% MeOH (FPLC Grade)

100% $CHCl_3$ (Pesticide Grade)

100% HPLC grade H_20

Protocol:

- Frozen myocardial biopsy tissue is stored in Precellys tubes with beads in -80°C freezer
- All solvents at 4°C and kept on ice
- Add 32µl/mg of tissue of MeOH and 1.2µl/mg of H_20 keeping precellys tubes on ice
- Place tubes in Precellys 24 homogeniser for 2×10s burst at 6400rpm
- Remove homogenised mixture into a clean 1.8ml glass vial using Pasteur pipette
- Place glass vials back on ice and then add 32µl/mg $CHCl_2$ and 16µl/mg H_2O to each vial
- Vortex vials on full power for 30s each
- Leave on ice for 10 mins
- Centrifuge glass vials at 4000rpm at 4°C for 10 mins
- Set samples on bench at room temperature for 5 mins

At this stage the samples should be biphasic, with protein debris separating the upper (polar) and lower (non-polar) layers.

For the following 2 steps care must be taken not to remove any of the interface regions (1-2mm on either side of the protein layer)

- Using a Hamilton syringe, remove the polar phase into 2 clean eppendorfs (1 postive, 1 negative)
- Using a Hamilton synringe remove the non-polar phase into 2 clean 1.8ml glass viasl (1 positive, 1 negative)
- These are then dried using a Speed Vac Concentrator (approximately 1h in NO heat)
- The samples can then be stored in the -80°C freezer until needed for further analysis

Preparation for analysis of dried polar extracts

The dried polar extracts are re-suspended in 80:20 methanol:water, containing 0.25% formic acid or 20mM ammonium acetate for positive and negative ion analysis respectively. Following this Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) based metabolomic analysis was conducted using a hybrid 7-T LTQ FT (Thermo scientific, Berlin, Germany) equipped with a chip-based direct infusion nanoelectrospray ion source (Triversa, Avion Biosciences, Ithaca, NY).

The nanoelectrospray conditions compromised of a 200ml/min flow rate, 0.3psi backing pressure and +1.7 or -1.7 kV electrospray voltage for positive and negative ion analysis retrospectively, controlled by ChipSoft software (version 8.1.0 Advion Biosciences, UK).

Each sample was analysed in triplicate with FT-ICR MS, collecting multiple adjacent selected ion monitoring (SIM) windows, which are stitched together from m/z 70 to 500. Each window overlaps by m/z 10 (3) with an automatic gain control (AGC) target of 1×10^5 charges and a mass resolution of 100,000. Data was recorded for 5.5 minutes per replicate analysis using Xcalibur software version 2.0 (Thermo Scientific, USA).

Transient data from the FT-ICR detector were processed using the averaging of transients, Hanning apodisation and application of a fast Fourier transformation; using custom written codes in MATLAB version 7.0 (The MathWorks, MA, USA).

Following this the SIM-stitching algorithm was applied (Custom written code, MATLAB Version 7.0 The MathWorks) (Southam, Payne et al. 2007), which stitched together multiple SIM windows, rejected all peaks with a signal-to-noise ratio (SNR) <3.5. This algorithm then internally calibrated each mass spectrum using a pre-defined calibrant list of metabolites.

Probabilistic Quotient Normalisation (PQN) was used to normalise the data. This minimised the effects of particularly high and low intensity peaks (Dieterle, Ross et al. 2006). To avoid the highest intensity peaks from dominating the multivariate analysis and to stabilise the variance across the numerous peaks detected, they were subject to a generalised log transformation (Parsons, Ludwig et al. 2007).

Principle component analysis (PCA) was used to assess the metabolic differences between the sample groups in an unbiased manner, using the PLS_Toolbox (version 3.53, Eigenvector, Manson, WA, USA) within MATLAB (Version 7.0 The MathWorks, Ma, USA) and confirmed using students t-tests (MS Excel, Seatle, USA).

10 REFERENCES

Abozguia, K., P. Elliott, et al. (2010). "Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy." CIrculation **122**(16): 1562-1569.

Agency, E. M. (2013). "European Medicines Agency recommends restricting use of trimetazidine-containing medicines." Retrieved August 2013.

Alam, M., J. Wardell, et al. (1999). "Characteristics of mitral and tricuspid annular velocities determined by pulsed wave Doppler tissue imaging in healthy subjects." J Am Soc Echocardiogr **12**(8): 618-628.

Alderman, C. P., J. D. Hundertmark, et al. (1997). "Interaction of serotonin re-uptake inhibitors with perhexiline." Aust N Z J Psychiatry **31**(4): 601-603.

Allard, M. F., J. D. Flint, et al. (1994). "Calcium overload during reperfusion is accelerated in isolated hypertrophied rat hearts." J Mol Cell Cardiol **26**(12): 1551-1563.

Allard, M. F., S. L. Henning, et al. (1997). "Glycogen metabolism in the aerobic hypertrophied rat heart." CIrculation **96**(2): 676-682.

Allard, M. F., B. O. Schonekess, et al. (1994). "Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts." Am J Physiol **267**(2 Pt 2): H742-750.

Amundsen, B. H., T. Helle-Valle, et al. (2006). "Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging." J Am Coll Cardiol **47**(4): 789-793.

Anderson, P. G., M. F. Allard, et al. (1990). "Increased ischemic injury but decreased hypoxic injury in hypertrophied rat hearts." Circ Res **67**(4): 948-959.

Antos, C. L., T. A. McKinsey, et al. (2002). "Activated glycogen synthase-3 beta suppresses cardiac hypertrophy in vivo." Proc Natl Acad Sci U S A **99**(2): 907-912.

Arad, M., D. W. Benson, et al. (2002). "Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy." J Clin Invest **109**(3): 357-362.

Arad, M., I. P. Moskowitz, et al. (2003). "Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy." CIrculation **107**(22): 2850-2856.

Arany, Z., M. Novikov, et al. (2006). "Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1alpha." Proc Natl Acad Sci U S A **103**(26): 10086-10091.

Ashrafian, H., G. Czibik, et al. (2012). "Fumarate is cardioprotective via activation of the Nrf2 antioxidant pathway." Cell Metab **15**(3): 361-371.

Ashrafian, H., J. D. Horowitz, et al. (2007). "Perhexiline." Cardiovasc Drug Rev **25**(1): 76-97.

ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories (2002). "ATS statement: guidelines for the six-minute walk test." Am J Respir Crit Care Med **166**(1): 111-117.

Aurigemma, G. P., K. H. Silver, et al. (1994). "Impact of chamber geometry and gender on left ventricular systolic function in patients > 60 years of age with aortic stenosis." Am J Cardiol **74**(8): 794-798.

Bache, R. J. (1998). "Vasodilator reserve: a functional assessment of coronary health." CIrculation **98**(13): 1257-1260.

Bache, R. J., T. R. Vrobel, et al. (1981). "Effect of maximal coronary vasodilation on transmural myocardial perfusion during tachycardia in dogs with left ventricular hypertrophy." Circ Res **49**(3): 742-750.

Bache, R. J., T. R. Vrobel, et al. (1981). "Regional myocardial blood flow during exercise in dogs with chronic left ventricular hypertrophy." Circ Res **48**(1): 76-87.

Badorff, C., H. Ruetten, et al. (2002). "Fas receptor signaling inhibits glycogen synthase kinase 3 beta and induces cardiac hypertrophy following pressure overload." J Clin Invest **109**(3): 373-381.

Barclay, M. L., S. M. Sawyers, et al. (2003). "Correlation of CYP2D6 genotype with perhexiline phenotypic metabolizer status." Pharmacogenetics **13**(10): 627-632.

Barger, P. M., J. M. Brandt, et al. (2000). "Deactivation of peroxisome proliferator-activated receptor-alpha during cardiac hypertrophic growth." J Clin Invest **105**(12): 1723-1730.

Barry, W. H., J. D. Horowitz, et al. (1985). "Comparison of negative inotropic potency, reversibility, and effects on calcium influx of six calcium channel antagonists in cultured myocardial cells." Br J Pharmacol **85**(1): 51-59.

Beadle, R. (2013). Metabolic Manipulation in Chronic Heart Failure PhD, University of Aberdeen.

Beadle, R. and M. Frenneaux (2010). "Magnetic resonance spectroscopy in myocardial disease." Expert Rev Cardiovasc Ther **8**(2): 269-277.

Beer, M., T. Seyfarth, et al. (2002). "Absolute concentrations of high-energy phosphate metabolites in normal, hypertrophied, and failing human myocardium measured noninvasively with (31)P-SLOOP magnetic resonance spectroscopy." J Am Coll Cardiol **40**(7): 1267-1274.

Belardinelli, R. and A. Purcaro (2001). "Effects of trimetazidine on the contractile response of chronically dysfunctional myocardium to low-dose dobutamine in ischaemic cardiomyopathy." Eur Heart J **22**(23): 2164-2170.

Belzer, F. O. and J. H. Southard (1988). "Principles of solid-organ preservation by cold storage." Transplantation **45**(4): 673-676.

Benjamini, Y. and Y. Hochberg (1995). "Controlling the false discovery rate - a practical and powerful approach to multiple testing." J R Stat Soc Ser B-Methodol **57**(1): 289-300.

Berg, J. M., J. Tymoczko, et al. (2012). Fatty Acid Metabolism. Biochemistry.

Bers, D. M. (1987). "Ryanodine and the calcium content of cardiac SR assessed by caffeine and rapid cooling contractures." Am J Physiol **253**(3 Pt 1): C408-415.

Bersin, R. M. and P. W. Stacpoole (1997). "Dichloroacetate as metabolic therapy for myocardial ischemia and failure." Am Heart J **134**(5 Pt 1): 841-855.

Bersin, R. M., C. Wolfe, et al. (1994). "Improved hemodynamic function and mechanical efficiency in congestive heart failure with sodium dichloroacetate." J Am Coll Cardiol **23**(7): 1617-1624.

Bertini, M., G. Nucifora, et al. (2009). "Left ventricular rotational mechanics in acute myocardial infarction and in chronic (ischemic and nonischemic) heart failure patients." Am J Cardiol **103**(11): 1506-1512.

Bertrand, L., S. Horman, et al. (2008). "Insulin signalling in the heart." Cardiovasc Res **79**(2): 238-248.

Beyerbacht, H. P., H. J. Lamb, et al. (2001). "Aortic valve replacement in patients with aortic valve stenosis improves myocardial metabolism and diastolic function." Radiology **219**(3): 637-643.

Billman, G. E., B. McIlroy, et al. (1991). "Elevated myocardial calcium and its role in sudden cardiac death." Faseb J **5**(11): 2586-2592.

Birkmeyer, J. D., A. E. Siewers, et al. (2002). "Hospital volume and surgical mortality in the United States." N Engl J Med **346**(15): 1128-1137.

Black, S. C., M. R. Gralinski, et al. (1994). "Effect of ranolazine on infarct size in a canine model of regional myocardial ischemia/reperfusion." J Cardiovasc Pharmacol **24**(6): 921-928.

Bluemke, D. A., R. A. Kronmal, et al. (2008). "The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study." J Am Coll Cardiol **52**(25): 2148-2155.

Bonow, R. O., B. A. Carabello, et al. (2008). "2008 focused update incorporated into the ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to revise the 1998 guidelines for the management of patients with valvular heart disease). Endorsed by the Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons." J Am Coll Cardiol **52**(13): e1-142.

Borg, G. A. (1982). "Psychophysical bases of perceived exertion." Med Sci Sports Exerc **14**(5): 377-381.

Bothe, W., M. Olschewski, et al. (2004). "Glucose-insulin-potassium in cardiac surgery: a meta-analysis." Ann Thorac Surg **78**(5): 1650-1657.

Bottomley, P. A. (1985). "Noninvasive study of high-energy phosphate metabolism in human heart by depth-resolved 31P NMR spectroscopy." Science **229**(4715): 769-772.

Bottomley, P. A. (1994). "MR spectroscopy of the human heart: the status and the challenges." Radiology **191**(3): 593-612.

Bottomley, P. A., E. Atalar, et al. (1996). "Human cardiac high-energy phosphate metabolite concentrations by 1D-resolved NMR spectroscopy." Magn Reson Med **35**(5): 664-670.

Boucher, F. R., D. J. Hearse, et al. (1994). "Effects of trimetazidine on ischemic contracture in isolated perfused rat hearts." J Cardiovasc Pharmacol **24**(1): 45-49.

Braimbridge, M. V., J. Chayen, et al. (1977). "Cold cardioplegia or continuous coronary perfusion? Report on preliminary clinical experience as assessed cytochemically." J Thorac Cardiovasc Surg **74**(6): 900-906.

Braimbridge, M. V., A. J. Clement, et al. (1969). "Triple Starr valve replacement." Br Med J **3**(5672): 683-688.

Braunwald, E. and R. A. Kloner (1982). "The stunned myocardium: prolonged, postischemic ventricular dysfunction." CIrculation **66**(6): 1146-1149.

Bridgewater B, Keogh B, et al. (2008). Sixth National Adult Cardiac surgical Database Report Dendrite clinical systems

Bridgewater, B. and B. Keogh (2009). Sixth National Adult Cardiac Surgical Database Report 2008.

Brooks, W. W., C. H. Conrad, et al. (1995). "Reperfusion induced arrhythmias following ischaemia in intact rat heart: role of intracellular calcium." Cardiovasc Res **29**(4): 536-542.

Buckberg, G. D. (1979). "A proposed "solution" to the cardioplegic controversy." J Thorac Cardiovasc Surg **77**(6): 803-815.

Buckberg, G. D., J. R. Brazier, et al. (1977). "Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass. I. The adequately perfused beating, fibrillating, and arrested heart." J Thorac Cardiovasc Surg **73**(1): 87-94.

Campbell, S. E., J. S. Janicki, et al. (1995). "Temporal differences in fibroblast proliferation and phenotype expression in response to chronic administration of angiotensin II or aldosterone." J Mol Cell Cardiol **27**(8): 1545-1560.

Carabello, B. A. (2002). "Clinical practice. Aortic stenosis." N Engl J Med **346**(9): 677-682.

Carabello, B. A., L. H. Green, et al. (1980). "Hemodynamic determinants of prognosis of aortic valve replacement in critical aortic stenosis and advanced congestive heart failure." CIrculation **62**(1): 42-48.

Cargnoni, A., E. Condorelli, et al. (1992). "Role of timing of administration in the cardioprotective effect of fructose-1,6-bisphosphate." Cardiovasc Drugs Ther **6**(3): 209-217.

Cavassila, S., S. Deval, et al. (2001). "Cramer-Rao bounds: an evaluation tool for quantitation." NMR Biomed **14**(4): 278-283.

Chambers, D. J., G. Astras, et al. (1989). "Free radicals and cardioplegia: organic anti-oxidants as additives to the St Thomas' Hospital cardioplegic solution." Cardiovasc Res **23**(4): 351-358.

Chambers, D. J., M. V. Braimbridge, et al. (1991). "Creatine phosphate (Neoton) as an additive to St. Thomas' Hospital cardioplegic solution (Plegisol). Results of a clinical study." Eur J Cardiothorac Surg **5**(2): 74-81.

Chambers, D. J., K. Haire, et al. (1996). "St. Thomas' Hospital cardioplegia: enhanced protection with exogenous creatine phosphate." Ann Thorac Surg **61**(1): 67-75.

Chambers, D. J. and D. J. Hearse (2001). Cardioplegia and Surgical Ischaemia. Heart Physiology and Pathophysiology. N. Sperelakis, Y. Kurachi, A. Terzic and M. V. Cohen, Academic Press. **4th Edition**.

Chambers, D. J., S. Kosker, et al. (1990). "Comparison of standard (non-oxygenated) vs. oxygenated St. Thomas' Hospital cardioplegic solution No. 2 (Plegisol)." Eur J Cardiothorac Surg **4**(10): 549-555.

Chang, S. A., H. K. Kim, et al. (2010). "Left ventricular twist mechanics in patients with apical hypertrophic cardiomyopathy: assessment with 2D speckle tracking echocardiography." Heart **96**(1): 49-55.

Cheitlin, M. D., W. F. Armstrong, et al. (2003). "ACC/AHA/ASE 2003 guideline update for the clinical application of echocardiography--summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines for the Clinical Application of Echocardiography)." J Am Coll Cardiol **42**(5): 954-970.

Cheitlin, M. D., E. W. Gertz, et al. (1979). "Rate of progression of severity of valvular aortic stenosis in the adult." Am Heart J **98**(6): 689-700.

Cho, Y. W., M. Belej, et al. (1970). "Pharmacology of a new antianginal drug: perhexiline. I. Coronary circulation and myocardial metabolism." Chest **58**(6): 577-581.

Cioffi, G., P. Faggiano, et al. (2011). "Prognostic effect of inappropriately high left ventricular mass in asymptomatic severe aortic stenosis." Heart **97**(4): 301-307.

Coetzee, A., G. Roussouw, et al. (1990). "Preservation of myocardial function and biochemistry after blood and oxygenated crystalloid cardioplegia during cardiac arrest." Ann Thorac Surg **50**(2): 230-237.

Cole, P. L., A. D. Beamer, et al. (1990). "Efficacy and safety of perhexiline maleate in refractory angina. A double-blind placebo-controlled clinical trial of a novel antianginal agent." CIrculation **81**(4): 1260-1270.

Connolly, H. M., J. K. Oh, et al. (1997). "Aortic valve replacement for aortic stenosis with severe left ventricular dysfunction. Prognostic indicators." CIrculation **95**(10): 2395-2400.

Conway, M. A., J. Allis, et al. (1991). "Detection of low phosphocreatine to ATP ratio in failing hypertrophied human myocardium by 31P magnetic resonance spectroscopy." Lancet **338**(8773): 973-976.

Conway, M. A., P. A. Bottomley, et al. (1998). "Mitral regurgitation: impaired systolic function, eccentric hypertrophy, and increased severity are linked to lower phosphocreatine/ATP ratios in humans." Circulation **97**(17): 1716-1723.

Crilley, J. G., E. A. Boehm, et al. (2003). "Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy." J Am Coll Cardiol **41**(10): 1776-1782.

Crompton, M. (1999). "The mitochondrial permeability transition pore and its role in cell death." Biochem J **341 (Pt 2)**: 233-249.

Cunningham, M. J., C. S. Apstein, et al. (1990). "Influence of glucose and insulin on the exaggerated diastolic and systolic dysfunction of hypertrophied rat hearts during hypoxia." Circ Res **66**(2): 406-415.

Cuspidi, C., M. Ciulla, et al. (2006). "Hypertensive myocardial fibrosis." Nephrol Dial Transplant **21**(1): 20-23.

Dalla-Volta, S., G. Maraglino, et al. (1990). "Comparison of trimetazidine with nifedipine in effort angina: a double-blind, crossover study." Cardiovasc Drugs Ther **4 Suppl 4**: 853-859.

Dare, A. J., J. P. Veinot, et al. (1993). "New observations on the etiology of aortic valve disease: a surgical pathologic study of 236 cases from 1990." Hum Pathol **24**(12): 1330-1338.

Davidson, S. M., D. Hausenloy, et al. (2006). "Signalling via the reperfusion injury signalling kinase (RISK) pathway links closure of the mitochondrial permeability transition pore to cardioprotection." Int J Biochem Cell Biol **38**(3): 414-419.

Davies, B. J., J. K. Coller, et al. (2006). "The influence of CYP2D6 genotype on trough plasma perhexiline and cis-OH-perhexiline concentrations following a standard loading regimen in patients with myocardial ischaemia." Br J Clin Pharmacol **61**(3): 321-325.

Davies, S. W., A. H. Gershlick, et al. (1991). "Progression of valvar aortic stenosis: a long-term retrospective study." Eur Heart J **12**(1): 10-14.

de Oliveira, N. C., T. J. Boeve, et al. (1997). "Ischemic intervals during warm blood cardioplegia in the canine heart evaluated by phosphorus 31-magnetic resonance spectroscopy." J Thorac Cardiovasc Surg **114**(6): 1070-1079; discussion 1079-1080.

Depre, C., G. L. Shipley, et al. (1998). "Unloaded heart in vivo replicates fetal gene expression of cardiac hypertrophy." Nat Med **4**(11): 1269-1275.

Deschamps, D., V. DeBeco, et al. (1994). "Inhibition by perhexiline of oxidative phosphorylation and the beta-oxidation of fatty acids: possible role in pseudoalcoholic liver lesions." Hepatology **19**(4): 948-961.

Devereux, R. B., D. R. Alonso, et al. (1986). "Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings." Am J Cardiol **57**(6): 450-458.

Devereux, R. B. and N. Reichek (1977). "Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method." CIrculation **55**(4): 613-618.

Dhalla, N. S., R. M. Temsah, et al. (2001). Calcium overload in Ischemia/Reperfusion injury Heart Physiology and Pathophysiology**:** 949-965.

Dieterle, F., A. Ross, et al. (2006). "Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics." Anal Chem **78**(13): 4281-4290.

Digerness, S. B., V. Vanini, et al. (1981). "In vitro comparison of oxygen availability from asanguinous and sanguinous cardioplegic media." CIrculation **64**(2 Pt 2): II80-83.

Doenst, T., W. Bothe, et al. (2003). "Therapy with insulin in cardiac surgery: controversies and possible solutions." Ann Thorac Surg **75**(2): S721-728.

Doenst, T., R. T. Richwine, et al. (1999). "Insulin improves functional and metabolic recovery of reperfused working rat heart." Ann Thorac Surg **67**(6): 1682-1688.

Doenst, T., D. Wijeysundera, et al. (2005). "Hyperglycemia during cardiopulmonary bypass is an independent risk factor for mortality in patients undergoing cardiac surgery." J Thorac Cardiovasc Surg **130**(4): 1144.

Donal, E., C. Thebault, et al. (2011). "Impact of aortic stenosis on longitudinal myocardial deformation during exercise." Eur J Echocardiogr **12**(3): 235-241.

Drury, N. E. (2012). On perhexiline and its application to myocardial protection during cardiac surgery. PhD PhD, University of Birmingham.

Drury NE, Howell NJ, et al. (2014). "The effect of Perhexiline on myocardial protection during coronary artery surgery: a two-centre randomised double-blind placebo-controlled trial." Eur J Cardiothorac Surg.

Drury, N. E., N. J. Howell, et al. (2011). "The Effect of Perhexiline on Myocardial Protection during Coronary Artery Surgery: a Two-Centre Randomised Double-Blind Placebo-Controlled Trial." Circulation(124): A15650.

Drury, N. E., G. Licari, et al. (2014). "Relationship between plasma, atrial and ventricular perhexiline concentrations in humans: insights into factors affecting myocardial uptake." Br J Clin Pharmacol **77**(5): 789-795.

Dyck, J. R. and G. D. Lopaschuk (2002). "Malonyl CoA control of fatty acid oxidation in the ischemic heart." J Mol Cell Cardiol **34**(9): 1099-1109.

Dyck, J. R. and G. D. Lopaschuk (2006). "AMPK alterations in cardiac physiology and pathology: enemy or ally?" J Physiol **574**(Pt 1): 95-112.

Embi, N., D. B. Rylatt, et al. (1980). "Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase." Eur J Biochem **107**(2): 519-527.

Emery, R. W., A. M. Emery, et al. (2012). Aortic Valve Replacement with a mechanical cardiac valve porsthesis Cardiac surgery in the adult H. L. Cohn. **4th**

Emery, R. W., C. A. Erickson, et al. (2003). "Replacement of the aortic valve in patients under 50 years of age: long-term follow-up of the St. Jude Medical prosthesis." Ann Thorac Surg **75**(6): 1815-1819.

Engler, R. L., M. D. Dahlgren, et al. (1986). "Role of leukocytes in response to acute myocardial ischemia and reflow in dogs." Am J Physiol **251**(2 Pt 2): H314-323.

Engler, R. L., M. D. Dahlgren, et al. (1986). "Accumulation of polymorphonuclear leukocytes during 3-h experimental myocardial ischemia." Am J Physiol **251**(1 Pt 2): H93-100.

Evans, W. E., M. V. Relling, et al. (1993). "Genetic basis for a lower prevalence of deficient CYP2D6 oxidative drug metabolism phenotypes in black Americans." J Clin Invest **91**(5): 2150-2154.

Faggiano, P., G. P. Aurigemma, et al. (1996). "Progression of valvular aortic stenosis in adults: literature review and clinical implications." Am Heart J **132**(2 Pt 1): 408-417.

Fan, Y., A. M. Zhang, et al. (2011). "Glucose-insulin-potassium therapy in adult patients undergoing cardiac surgery: a meta-analysis." Eur J Cardiothorac Surg **40**(1): 192-199.

Fantini, E., L. Demaison, et al. (1994). "Some biochemical aspects of the protective effect of trimetazidine on rat cardiomyocytes during hypoxia and reoxygenation." J Mol Cell Cardiol **26**(8): 949-958.

Fath-Ordoubadi, F. and K. J. Beatt (1997). "Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials." CIrculation **96**(4): 1152-1156.

Fedele, F. A., H. Gewirtz, et al. (1988). "Metabolic response to prolonged reduction of myocardial blood flow distal to a severe coronary artery stenosis." CIrculation **78**(3): 729-735.

Follette, D. M., D. G. Mulder, et al. (1978). "Advantages of blood cardioplegia over continuous coronary perfusion or intermittent ischemia. Experimental and clinical study." J Thorac Cardiovasc Surg **76**(5): 604-619.

Freemantle, N. (2001). "Interpreting the results of secondary end points and subgroup analyses in clinical trials: should we lock the crazy aunt in the attic?" BMJ **322**(7292): 989-991.

Fremes, S. E., M. G. Tamariz, et al. (2000). "Late results of the Warm Heart Trial: the influence of nonfatal cardiac events on late survival." Circulation **102**(19 Suppl 3): III339-345.

Friehs, I., H. Cao-Danh, et al. (2005). "Impaired insulin-signaling in hypertrophied hearts contributes to ischemic injury." Biochem Biophys Res Commun **331**(1): 15-22.

Friehs, I. and P. J. del Nido (2003). "Increased susceptibility of hypertrophied hearts to ischemic injury." Ann Thorac Surg **75**(2): S678-684.

Fulop, N., Z. Zhang, et al. (2007). "Glucosamine cardioprotection in perfused rat hearts associated with increased O-linked N-acetylglucosamine protein modification and altered p38 activation." Am J Physiol Heart Circ Physiol **292**(5): H2227-2236.

Gaasch, W. H. (1979). "Left ventricular radius to wall thickness ratio." Am J Cardiol **43**(6): 1189-1194.

Gaasch, W. H. (1994). "Diagnosis and treatment of heart failure based on left ventricular systolic or diastolic dysfunction." JAMA **271**(16): 1276-1280.

Gaasch, W. H., H. J. Levine, et al. (1976). "Left ventricular compliance: mechanisms and clinical implications." Am J Cardiol **38**(5): 645-653.

Gaasch, W. H., M. R. Zile, et al. (1990). "Tolerance of the hypertrophic heart to ischemia. Studies in compensated and failing dog hearts with pressure overload hypertrophy." Circulation **81**(5): 1644-1653.

Galinanes, M., R. Ferrari, et al. (1992). "PEG-SOD and myocardial antioxidant status during ischaemia and reperfusion: dose-response studies in the isolated blood perfused rabbit heart." J Mol Cell Cardiol **24**(9): 1021-1030.

Galinanes, M., T. Murashita, et al. (1992). "Long-term hypothermic storage of the mammalian heart for transplantation: a comparison of three cardioplegic solutions." J Heart Lung Transplant **11**(4 Pt 1): 624-635.

Gamble, J. and G. D. Lopaschuk (1997). "Insulin inhibition of 5' adenosine monophosphate-activated protein kinase in the heart results in activation of acetyl coenzyme A carboxylase and inhibition of fatty acid oxidation." Metabolism **46**(11): 1270-1274.

Gardiner, S. J. and E. J. Begg (2006). "Pharmacogenetics, drug-metabolizing enzymes, and clinical practice." Pharmacol Rev **58**(3): 521-590.

Gay, W. A., Jr. and P. A. Ebert (1973). "Functional, metabolic, and morphologic effects of potassium-induced cardioplegia." Surgery **74**(2): 284-290.

Gerbode, F. and D. Melrose (1958). "The use of potassium arrest in open cardiac surgery." Am J Surg **96**(2): 221-227.

Gibbon, J. H., Jr. (1954). "Application of a mechanical heart and lung apparatus to cardiac surgery." Minn Med **37**(3): 171-185; passim.

Gonzalez, F. J., R. C. Skoda, et al. (1988). "Characterization of the common genetic defect in humans deficient in debrisoquine metabolism." Nature **331**(6155): 442-446.

Goodney, P. P., G. T. O'Connor, et al. (2003). "Do hospitals with low mortality rates in coronary artery bypass also perform well in valve replacement?" Ann Thorac Surg **76**(4): 1131-1136; discussion 1136-1137.

Gosse, P. and J. Clementy (1995). "Coronary reserve in experimental myocardial hypertrophy." Eur Heart J **16 Suppl I**: 22-25.

Gradinac, S., G. M. Coleman, et al. (1989). "Improved cardiac function with glucose-insulin-potassium after aortocoronary bypass grafting." Ann Thorac Surg **48**(4): 484-489.

Greenberg, J. J. and L. H. Edmunds, Jr. (1961). "Effect of myocardial ischemia at varying temperatures on left ventricular function and tissue oxygen tension." J Thorac Cardiovasc Surg **42**: 84-92.

Griepp, R. B., E. B. Stinson, et al. (1973). "Profound local hypothermia for myocardial protection during open-heart surgery." J Thorac Cardiovasc Surg **66**(5): 731-741.

Griffin, J. L., H. Atherton, et al. (2011). "Metabolomics as a tool for cardiac research." Nat Rev Cardiol **8**(11): 630-643.

Grima, M., J. Velly, et al. (1988). "Inhibitory effects of some cyclohexylaralkylamines related to perhexiline on sodium influx, binding of [3H]batrachotoxinin A 20-alpha-benzoate and [3H]nitrendipine and on guinea pig left atria contractions." Eur J Pharmacol **147**(2): 173-185.

Grimes, C. A. and R. S. Jope (2001). "The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling." Prog Neurobiol **65**(4): 391-426.

Grossman, W. (1980). "Cardiac hypertrophy: useful adaptation or pathologic process?" Am J Med **69**(4): 576-584.

Gunther, S. and W. Grossman (1979). "Determinants of ventricular function in pressure-overload hypertrophy in man." Circulation **59**(4): 679-688.

Guyton, A. and J. E. Hall (2000). Medical Physiology.

Haider, W., W. Schutz, et al. (1982). "[Optimizing myocardial energy potentials by preoperative high-dose insulin administration for myocardial protection in open-heart surgery]." Anaesthesist **31**(8): 377-382.

Halestrap, A. P., S. J. Clarke, et al. (2004). "Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection." Cardiovasc Res **61**(3): 372-385.

Hancock, E. W., B. J. Deal, et al. (2009). "AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part V: electrocardiogram changes associated with cardiac chamber hypertrophy: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society. Endorsed by the International Society for Computerized Electrocardiology." J Am Coll Cardiol **53**(11): 992-1002.

Haq, S., G. Choukroun, et al. (2000). "Glycogen synthase kinase-3beta is a negative regulator of cardiomyocyte hypertrophy." J Cell Biol **151**(1): 117-130.

Hardt, S. E. and J. Sadoshima (2002). "Glycogen synthase kinase-3beta: a novel regulator of cardiac hypertrophy and development." Circ Res **90**(10): 1055-1063.

Hausenloy, D. J. and D. M. Yellon (2004). "New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway." Cardiovasc Res **61**(3): 448-460.

Hausenloy, D. J. and D. M. Yellon (2007). "Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection." Heart Fail Rev **12**(3-4): 217-234.

Hearse, D. J., S. M. Humphrey, et al. (1973). "Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release." J Mol Cell Cardiol **5**(4): 395-407.

Hearse, D. J., S. M. Humphrey, et al. (1975). "Ultrastructural damage associated with reoxygenation of the anoxic myocardium." J Mol Cell Cardiol **7**(5): 315-324.

Hearse, D. J., D. A. Stewart, et al. (1976). "Cellular protection during myocardial ischemia: the development and characterization of a procedure for the induction of reversible ischemic arrest." CIrculation **54**(2): 193-202.

Hearse, D. J., D. A. Stewart, et al. (1980). "The additive protective effects of hypothermia and chemical cardioplegia during ischemic cardiac arrest in the rat." J Thorac Cardiovasc Surg **79**(1): 39-43.

Hein, S., E. Arnon, et al. (2003). "Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms." CIrculation **107**(7): 984-991.

Helmsworth, J. A., S. Kaplan, et al. (1959). "Myocardial injury associated with asystole induced with potassium citrate." Ann Surg **149**(2): 200-206.

Higgins, A. J., M. Morville, et al. (1980). "Oxfenicine diverts rat muscle metabolism from fatty acid to carbohydrate oxidation and protects the ischaemic rat heart." Life Sci **27**(11): 963-970.

Hoffman, T. M., G. Wernovsky, et al. (2003). "Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease." Circulation **107**(7): 996-1002.

Holloway, C. J., J. Suttie, et al. (2011). "Clinical cardiac magnetic resonance spectroscopy." Prog Cardiovasc Dis **54**(3): 320-327.

Holscher, B. (1967). "Studies by electron microscopy on the effects of magnesium choloride-prcain amide or ptassium citrate on the myocardium in induced cardiac arrest " J Cardiovasc Surg (Torino)(8): 163-166.

Horowitz, J. D., I. K. Button, et al. (1995). "Is perhexiline essential for the optimal management of angina pectoris?" Aust N Z J Med **25**(2): 111-113.

Horowitz, J. D., S. T. Sia, et al. (1986). "Perhexiline maleate treatment for severe angina pectoris--correlations with pharmacokinetics." Int J Cardiol **13**(2): 219-229.

Horstkotte, D. and F. Loogen (1988). "The natural history of aortic valve stenosis." Eur Heart J **9 Suppl E**: 57-64.

Hosoda, H., M. Sunamori, et al. (1994). "Effect of pinacidil on rat hearts undergoing hypothermic cardioplegia." Ann Thorac Surg **58**(6): 1631-1636.

Howell, N. J. (2010). Metabolic support in left ventricular hypertrophy. PhD, University of Birmingham.

Howell, N. J., H. Ashrafian, et al. (2011). "Glucose-insulin-potassium reduces the incidence of low cardiac output episodes after aortic valve replacement for aortic stenosis in patients with left ventricular hypertrophy: results from the Hypertrophy, Insulin, Glucose, and Electrolytes (HINGE) trial." Circulation **123**(2): 170-177.

Hrydziuszko, O. and M. R. Viant (2011). "Missing values in mass spectrometry based metabolomics: an undervalued step in the data processing pipeline." Metabolomics: 1-14.

Hudak, W. J., R. E. Lewis, et al. (1970). "Cardiovascular pharmacology of perhexiline." J Pharmacol Exp Ther **173**(2): 371-382.

Hudsmith, L. E. and S. Neubauer (2009). "Magnetic resonance spectroscopy in myocardial disease." JACC Cardiovasc Imaging **2**(1): 87-96.

Hurley, E. J., R. R. Lower, et al. (1964). "Clinical Experience with Local Hypothermia in Elective Cardiac Arrest." J Thorac Cardiovasc Surg **47**: 50-65.

ICH. (1996). "Guideline for Good Clinical Practice E6(R1)." Retrieved 1st March 2011.

ICH. (1997). "General considerations for clinical trials E8." Retrieved 1st March, 2011.

Iivanainen, A. M., M. Lindroos, et al. (1996). "Natural history of aortic valve stenosis of varying severity in the elderly." Am J Cardiol **78**(1): 97-101.

Iliceto, S., D. Scrutinio, et al. (1995). "Effects of L-carnitine administration on left ventricular remodeling after acute anterior myocardial infarction: the L-Carnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) Trial." J Am Coll Cardiol **26**(2): 380-387.

Ingwall, J. S. and R. G. Weiss (2004). "Is the failing heart energy starved? On using chemical energy to support cardiac function." Circ Res **95**(2): 135-145.

Ishihara, M., I. Inoue, et al. (2003). "Impact of acute hyperglycemia on left ventricular function after reperfusion therapy in patients with a first anterior wall acute myocardial infarction." Am Heart J **146**(4): 674-678.

Ishihara, M., S. Kojima, et al. (2005). "Acute hyperglycemia is associated with adverse outcome after acute myocardial infarction in the coronary intervention era." Am Heart J **150**(4): 814-820.

Ito, H. (2006). "No-reflow phenomenon and prognosis in patients with acute myocardial infarction." Nat Clin Pract Cardiovasc Med **3**(9): 499-506.

Ivey, C. L., F. M. Williams, et al. (1995). "Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit. Evidence for a role for C5a and interleukin-8." J Clin Invest **95**(6): 2720-2728.

Iyengar, S. R., E. J. Charrette, et al. (1976). "Myocardial glycogen in prevention of perioperative ischemic injury of the heart: a preliminary report." Can J Surg **19**(3): 246-251.

Jansson, E., V. Bomfim, et al. (1987). "Myocardial energy metabolism in the induction phase of cardioplegia in relation to myocardial temperature during open heart surgery." Clin Physiol **7**(1): 43-49.

Jonassen, A. K., M. N. Sack, et al. (2001). "Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling." Circ Res **89**(12): 1191-1198.

Jonasson, R., B. Jonsson, et al. (1983). "Rate of progression of severity of valvular aortic stenosis." Acta Med Scand **213**(1): 51-54.

Juhaszova, M., D. B. Zorov, et al. (2009). "Role of glycogen synthase kinase-3beta in cardioprotection." Circ Res **104**(11): 1240-1252.

Jung, W. I., L. Sieverding, et al. (1998). "31P NMR spectroscopy detects metabolic abnormalities in asymptomatic patients with hypertrophic cardiomyopathy." Circulation **97**(25): 2536-2542.

Jynge, P., D. J. Hearse, et al. (1981). "The St. Thomas' hospital cardioplegic solution: a characterization in two species." Scand J Thorac Cardiovasc Surg Suppl **30**: 1-28.

Kantor PF (2001). Heart physiology and pathophysiology San Diedo, Academic Press.

Kawasuji, M., T. Yasuda, et al. (1997). "Near-infrared monitoring of myocardial oxygenation during intermittent warm blood cardioplegia." Eur J Cardiothorac Surg **12**(2): 236-241.

Kearney, L. G., K. Lu, et al. (2012). "Global longitudinal strain is a strong independent predictor of all-cause mortality in patients with aortic stenosis." Eur Heart J Cardiovasc Imaging **13**(10): 827-833.

Kelly, T. A., R. M. Rothbart, et al. (1988). "Comparison of outcome of asymptomatic to symptomatic patients older than 20 years of age with valvular aortic stenosis." Am J Cardiol **61**(1): 123-130.

Kempsford, R. D. and D. J. Hearse (1989). "Protection of the immature myocardium during global ischemia. A comparison of four clinical cardioplegic solutions in the rabbit heart." J Thorac Cardiovasc Surg **97**(6): 856-863.

Kennedy, J. A., S. A. Unger, et al. (1996). "Inhibition of carnitine palmitoyltransferase-1 in rat heart and liver by perhexiline and amiodarone." Biochem Pharmacol **52**(2): 273-280.

Khan, M., S. Meduru, et al. (2010). "Trimetazidine, administered at the onset of reperfusion, ameliorates myocardial dysfunction and injury by activation of p38 mitogen-activated protein kinase and Akt signaling." J Pharmacol Exp Ther **333**(2): 421-429.

Killalea, S. M. and H. Krum (2001). "Systematic review of the efficacy and safety of perhexiline in the treatment of ischemic heart disease." Am J Cardiovasc Drugs **1**(3): 193-204.

Kirsch, U., G. Rodewald, et al. (1972). "Induced ischemic arrest. Clinical experience with cardioplegia in open-heart surgery." J Thorac Cardiovasc Surg **63**(1): 121-130.

Knerr, S. M. and M. Lieberman (1993). "Ion transport during hypothermia in cultured heart cells: implications for protection of the immature myocardium." J Mol Cell Cardiol **25**(3): 277-288.

Kohya, T., S. Kimura, et al. (1988). "Susceptibility of hypertrophied rat hearts to ventricular fibrillation during acute ischemia." J Mol Cell Cardiol **20**(2): 159-168.

Kolwicz, S. C., Jr. and R. Tian (2011). "Glucose metabolism and cardiac hypertrophy." Cardiovasc Res **90**(2): 194-201.

Kostler, H., W. Landschutz, et al. (2006). "Age and gender dependence of human cardiac phosphorus metabolites determined by SLOOP 31P MR spectroscopy." Magn Reson Med **56**(4): 907-911.

Kouzu, H., S. Yuda, et al. (2011). "Left ventricular hypertrophy causes different changes in longitudinal, radial, and circumferential mechanics in patients with hypertension: a two-dimensional speckle tracking study." J Am Soc Echocardiogr **24**(2): 192-199.

Koyanagi, S., C. L. Eastham, et al. (1982). "Increased size of myocardial infarction in dogs with chronic hypertension and left ventricular hypertrophy." Circ Res **50**(1): 55-62.

Krayenbuehl, H. P., O. M. Hess, et al. (1988). "Left ventricular systolic function in aortic stenosis." Eur Heart J **9 Suppl E**: 19-23.

Kronon, M., K. S. Bolling, et al. (1997). "The relationship between calcium and magnesium in pediatric myocardial protection." J Thorac Cardiovasc Surg **114**(6): 1010-1019.

Kudo, N., A. J. Barr, et al. (1995). "High rates of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA levels due to an increase in 5'-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase." J Biol Chem **270**(29): 17513-17520.

Kudo, N., J. G. Gillespie, et al. (1996). "Characterization of 5'AMP-activated protein kinase activity in the heart and its role in inhibiting acetyl-CoA carboxylase during reperfusion following ischemia." Biochim Biophys Acta **1301**(1-2): 67-75.

Kupari, M., H. Turto, et al. (2005). "Left ventricular hypertrophy in aortic valve stenosis: preventive or promotive of systolic dysfunction and heart failure?" Eur Heart J **26**(17): 1790-1796.

Kurihara, S. and T. Sakai (1985). "Effects of rapid cooling on mechanical and electrical responses in ventricular muscle of guinea-pig." J Physiol **361**: 361-378.

Kvidal, P., R. Bergstrom, et al. (2000). "Observed and relative survival after aortic valve replacement." J Am Coll Cardiol **35**(3): 747-756.

Kvidal, P., R. Bergstrom, et al. (2000). "Long-term follow-up of morbidity and mortality after aortic valve replacement with a mechanical valve prosthesis." Eur Heart J **21**(13): 1099-1111.

Lafitte, S., M. Perlant, et al. (2009). "Impact of impaired myocardial deformations on exercise tolerance and prognosis in patients with asymptomatic aortic stenosis." Eur J Echocardiogr **10**(3): 414-419.

Lageron, A., J. Scotto, et al. (1981). "Effects of Pexid on liver cell cultures. Ultrastructural and histoenzymological studies." Eur J Clin Pharmacol **19**(6): 417-421.

Lamb, H. J., H. P. Beyerbacht, et al. (1999). "Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism." CIrculation **99**(17): 2261-2267.

Lamb, H. J., J. Doornbos, et al. (1996). "Reproducibility of human cardiac 31P-NMR spectroscopy." NMR Biomed **9**(5): 217-227.

Lang, R. M., M. Bierig, et al. (2005). "Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology." J Am Soc Echocardiogr **18**(12): 1440-1463.

Laplante, A., G. Vincent, et al. (1997). "Effects and metabolism of fumarate in the perfused rat heart. A 13C mass isotopomer study." Am J Physiol **272**(1 Pt 1): E74-82.

Lazdunski, M., C. Frelin, et al. (1985). "The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH." J Mol Cell Cardiol **17**(11): 1029-1042.

Ledingham, S. J., M. V. Braimbridge, et al. (1988). "Improved myocardial protection by oxygenation of St. Thomas' Hospital cardioplegic solutions. Studies in the rat." J Thorac Cardiovasc Surg **95**(1): 103-111.

Lee, L., R. Campbell, et al. (2005). "Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment." Circulation **112**(21): 3280-3288.

Lehman, J. J. and D. P. Kelly (2002). "Transcriptional activation of energy metabolic switches in the developing and hypertrophied heart." Clin Exp Pharmacol Physiol **29**(4): 339-345.

Levy D, Salomon M, et al. (1994). "Prognostic implications of baseline electrocardiographic features and their serial changes in subjects with LVH." CIrculation **90**(4): 1786-1793.

Lewandowski, E. D. (2000). "Metabolic mechanisms associated with antianginal therapy." Circ Res **86**(5): 487-489.

Liedtke, A. J., S. Nellis, et al. (1978). "Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine." Circ Res **43**(4): 652-661.

Liedtke, A. J., S. H. Nellis, et al. (1984). "Effects of reducing fatty acid metabolism on mechanical function in regionally ischemic hearts." Am J Physiol **247**(3 Pt 2): H387-394.

Liu, J., R. B. Marchase, et al. (2007). "Glutamine-induced protection of isolated rat heart from ischemia/reperfusion injury is mediated via the hexosamine biosynthesis pathway and increased protein O-GlcNAc levels." J Mol Cell Cardiol **42**(1): 177-185.

Liu, J., R. B. Marchase, et al. (2007). "Increased O-GlcNAc levels during reperfusion lead to improved functional recovery and reduced calpain proteolysis." Am J Physiol Heart Circ Physiol **293**(3): H1391-1399.

Lopaschuk, G. D., R. Barr, et al. (2003). "Beneficial effects of trimetazidine in ex vivo working ischemic hearts are due to a stimulation of glucose oxidation secondary to inhibition of long-chain 3-ketoacyl coenzyme a thiolase." Circ Res **93**(3): e33-37.

Lopaschuk, G. D., I. M. Rebeyka, et al. (2002). "Metabolic modulation: a means to mend a broken heart." CIrculation **105**(2): 140-142.

Lopaschuk, G. D., S. R. Wall, et al. (1988). "Etomoxir, a carnitine palmitoyltransferase I inhibitor, protects hearts from fatty acid-induced ischemic injury independent of changes in long chain acylcarnitine." Circ Res **63**(6): 1036-1043.

Lopez, J. R., R. Jahangir, et al. (1996). "Potassium channel openers prevent potassium-induced calcium loading of cardiac cells: possible implications in cardioplegia." J Thorac Cardiovasc Surg **112**(3): 820-831.

Lorell, B. H. and B. A. Carabello (2000). "Left ventricular hypertrophy: pathogenesis, detection, and prognosis." CIrculation **102**(4): 470-479.

Lucchesi, B. R. (2001). Myocardial reperfusion Injury - role of free radicals and mediators of inflammation. Heart Physiology and Pathophysiology.

Lund, O. (1990). "Preoperative risk evaluation and stratification of long-term survival after valve replacement for aortic stenosis. Reasons for earlier operative intervention." CIrculation **82**(1): 124-139.

Maganti, M. D., V. Rao, et al. (2005). "Predictors of low cardiac output syndrome after isolated aortic valve surgery." CIrculation **112**(9 Suppl): I448-452.

Mahgoub, A., J. R. Idle, et al. (1977). "Polymorphic hydroxylation of Debrisoquine in man." Lancet **2**(8038): 584-586.

Mamas, M. A., L. Neyses, et al. (2010). "A meta-analysis of glucose-insulin-potassium therapy for treatment of acute myocardial infarction." Exp Clin Cardiol **15**(2): e20-24.

Manning, A. S. and D. J. Hearse (1984). "Reperfusion-induced arrhythmias: mechanisms and prevention." J Mol Cell Cardiol **16**(6): 497-518.

Marcus, M. L., D. B. Doty, et al. (1982). "Decreased coronary reserve: a mechanism for angina pectoris in patients with aortic stenosis and normal coronary arteries." N Engl J Med **307**(22): 1362-1366.

Maron, M. S., I. Olivotto, et al. (2009). "The case for myocardial ischemia in hypertrophic cardiomyopathy." J Am Coll Cardiol **54**(9): 866-875.

Marsin, A. S., L. Bertrand, et al. (2000). "Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia." Curr Biol **10**(20): 1247-1255.

Matsui, T., L. Li, et al. (1999). "Adenoviral gene transfer of activated phosphatidylinositol 3'-kinase and Akt inhibits apoptosis of hypoxic cardiomyocytes in vitro." CIrculation **100**(23): 2373-2379.

Mayr, M. (2011). "Recent highlights of metabolomics in cardiovascular research." Circ Cardiovasc Genet **4**(4): 463-464.

McLenachan, J. M., E. Henderson, et al. (1987). "Ventricular arrhythmias in patients with hypertensive left ventricular hypertrophy." N Engl J Med **317**(13): 787-792.

Mehta, R. H., D. Bruckman, et al. (2001). "Implications of increased left ventricular mass index on in-hospital outcomes in patients undergoing aortic valve surgery." J Thorac Cardiovasc Surg **122**(5): 919-928.

Mehta, S. R., S. Yusuf, et al. (2005). "Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial." JAMA **293**(4): 437-446.

Meissner, A. and J. P. Morgan (1995). "Contractile dysfunction and abnormal Ca2+ modulation during postischemic reperfusion in rat heart." Am J Physiol **268**(1 Pt 2): H100-111.

Melrose, D. G., B. Dreyer, et al. (1955). "Elective cardiac arrest." Lancet **269**(6879): 21-22.

Messerli, F. H., H. O. Ventura, et al. (1984). "Hypertension and sudden death. Increased ventricular ectopic activity in left ventricular hypertrophy." Am J Med **77**(1): 18-22.

Mignot, A., E. Donal, et al. (2010). "Global longitudinal strain as a major predictor of cardiac events in patients with depressed left ventricular function: a multicenter study." J Am Soc Echocardiogr **23**(10): 1019-1024.

Minami, Y., H. Gohra, et al. (2000). "Changes in left ventricular function after cardiac arrest and reperfusion in hypertropheral hearts." Ann Thorac Cardiovasc Surg **6**(5): 309-314.

Miyazaki, S., M. Daimon, et al. (2011). "Global longitudinal strain in relation to the severity of aortic stenosis: a two-dimensional speckle-tracking study." Echocardiography **28**(7): 703-708.

Mohler, E. R., 3rd, M. K. Chawla, et al. (1999). "Identification and characterization of calcifying valve cells from human and canine aortic valves." J Heart Valve Dis **8**(3): 254-260.

Mohler, E. R., 3rd, F. Gannon, et al. (2001). "Bone formation and inflammation in cardiac valves." CIrculation **103**(11): 1522-1528.

Morgan, H. E., J. R. Neely, et al. (1965). "Factors affecting glucose transport in heart muscle and erythrocytes." Fed Proc **24**(5): 1040-1045.

Morisco, C., D. Zebrowski, et al. (2000). "The Akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes." J Biol Chem **275**(19): 14466-14475.

Murakami, T., O. M. Hess, et al. (1986). "Diastolic filling dynamics in patients with aortic stenosis." Circulation **73**(6): 1162-1174.

Murphy, E. S., R. M. Lawson, et al. (1981). "Severe aortic stenosis in patients 60 years of age or older: left ventricular function and 10-year survival after valve replacement." CIrculation **64**(2 Pt 2): II184-188.

Nagueh, S. F., C. P. Appleton, et al. (2009). "Recommendations for the evaluation of left ventricular diastolic function by echocardiography." J Am Soc Echocardiogr **22**(2): 107-133.

Neubauer, S. (2007). "The failing heart--an engine out of fuel." N Engl J Med **356**(11): 1140-1151.

Neubauer, S., M. Horn, et al. (1995). "Contributions of 31P-magnetic resonance spectroscopy to the understanding of dilated heart muscle disease." Eur Heart J **16 Suppl O**: 115-118.

Neubauer, S., M. Horn, et al. (1997). "Cardiac high-energy phosphate metabolism in patients with aortic valve disease assessed by 31P-magnetic resonance spectroscopy." J Investig Med **45**(8): 453-462.

Neubauer, S., T. Krahe, et al. (1992). "31P magnetic resonance spectroscopy in dilated cardiomyopathy and coronary artery disease. Altered cardiac high-energy phosphate metabolism in heart failure." Circulation **86**(6): 1810-1818.

Ng, A. C., V. Delgado, et al. (2011). "Alterations in multidirectional myocardial functions in patients with aortic stenosis and preserved ejection fraction: a two-dimensional speckle tracking analysis." Eur Heart J **32**(12): 1542-1550.

Nyamundanda, G., I. C. Gormley, et al. (2013). "MetSizeR: selecting the optimal sample size for metabolomic studies using an analysis based approach." BMC Bioinformatics **14**: 338.

O'Brien, K. D., D. M. Shavelle, et al. (2002). "Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma." CIrculation **106**(17): 2224-2230.

Olsson, M., C. J. Dalsgaard, et al. (1994). "Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves." J Am Coll Cardiol **23**(5): 1162-1170.

Olsson, M., J. Thyberg, et al. (1999). "Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves." Arterioscler Thromb Vasc Biol **19**(5): 1218-1222.

Opie, L. and G. Lopaschuk (2004). Heart physiology: from cell to circulation. O. LH. Philadelphia, USA, Lippincott Williams & Wilkins.

Orsinelli, D. A., G. P. Aurigemma, et al. (1993). "Left ventricular hypertrophy and mortality after aortic valve replacement for aortic stenosis. A high risk subgroup identified by preoperative relative wall thickness." J Am Coll Cardiol **22**(6): 1679-1683.

Osada, M., T. Netticadan, et al. (1998). "Modification of ischemia-reperfusion-induced changes in cardiac sarcoplasmic reticulum by preconditioning." Am J Physiol **274**(6 Pt 2): H2025-2034.

Otterstad, J. E. (1993). "Ischaemia and left ventricular hypertrophy." Eur Heart J **14 Suppl F**: 2-6.

Otterstad, J. E., M. Davies, et al. (1993). "Left ventricular hypertrophy and myocardial ischaemia in hypertension: the THAMES Study." Eur Heart J **14**(12): 1622-1628.

Otto, C. M., I. G. Burwash, et al. (1997). "Prospective study of asymptomatic valvular aortic stenosis. Clinical, echocardiographic, and exercise predictors of outcome." CIrculation **95**(9): 2262-2270.

Otto, C. M., J. Kuusisto, et al. (1994). "Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies." Circulation **90**(2): 844-853.

Otto, C. M., A. S. Pearlman, et al. (1989). "Hemodynamic progression of aortic stenosis in adults assessed by Doppler echocardiography." J Am Coll Cardiol **13**(3): 545-550.

Pantely, G. A., S. A. Malone, et al. (1990). "Regeneration of myocardial phosphocreatine in pigs despite continued moderate ischemia." Circ Res **67**(6): 1481-1493.

Parker, P. J., F. B. Caudwell, et al. (1983). "Glycogen synthase from rabbit skeletal muscle; effect of insulin on the state of phosphorylation of the seven phosphoserine residues in vivo." Eur J Biochem **130**(1): 227-234.

Parsons, H. M., C. Ludwig, et al. (2007). "Improved classification accuracy in 1- and 2-dimensional NMR metabolomics data using the variance stabilising generalised logarithm transformation." BMC Bioinformatics **8**: 234.

Payne, T. G., A. D. Southam, et al. (2009). "A signal filtering method for improved quantification and noise discrimination in fourier transform ion cyclotron resonance mass spectrometry-based metabolomics data." J Am Soc Mass Spectrom **20**(6): 1087-1095.

Pellikka, P. A., M. E. Sarano, et al. (2005). "Outcome of 622 adults with asymptomatic, hemodynamically significant aortic stenosis during prolonged follow-up." CIrculation **111**(24): 3290-3295.

Pepine, C. J., S. J. Schang, et al. (1974). "Effects of perhexiline on coronary hemodynamic and myocardial metabolic responses to tachycardia." CIrculation **49**(5): 887-893.

Peter, M., A. Hoffmann, et al. (1993). "Progression of aortic stenosis. Role of age and concomitant coronary artery disease." Chest **103**(6): 1715-1719.

Peyton, R. B., R. N. Jones, et al. (1982). "Depressed high-energy phosphate content in hypertrophied ventricles of animal and man: the biologic basis for increased sensitivity to ischemic injury." Ann Surg **196**(3): 278-284.

Philpott, A., S. Chandy, et al. (2004). "Development of a regimen for rapid initiation of perhexiline therapy in acute coronary syndromes." Intern Med J **34**(6): 361-363.

Pilcher, J., J. D. Cooper, et al. (1985). "Investigations of long-term treatment with perhexiline maleate using therapeutic monitoring and electromyography." Ther Drug Monit **7**(1): 54-60.

Pillans, P. I. (1996). "Drug associated hepatic reactions in New Zealand: 21 years experience." N Z Med J **109**(1028): 315-319.

Pisarenko, O. I., V. F. Portnoy, et al. (1987). "Glutamate-blood cardioplegia improves ATP preservation in human myocardium." Biomed Biochim Acta **46**(6): 499-504.

Pound, K. M., N. Sorokina, et al. (2009). "Substrate-enzyme competition attenuates upregulated anaplerotic flux through malic enzyme in hypertrophied rat heart and restores triacylglyceride content: attenuating upregulated anaplerosis in hypertrophy." Circ Res **104**(6): 805-812.

Qiu, Y., M. Galinanes, et al. (1995). "Protective effect of nicorandil as an additive to the solution for continuous warm cardioplegia." J Thorac Cardiovasc Surg **110**(4 Pt 1): 1063-1072.

Quinn, D. W., D. Pagano, et al. (2006). "Improved myocardial protection during coronary artery surgery with glucose-insulin-potassium: a randomized controlled trial." J Thorac Cardiovasc Surg **131**(1): 34-42.

Rabinov, M., X. Z. Chen, et al. (1989). "Comparison of the metabolic response of the hypertrophic and the normal heart to hypothermic cardioplegia. The effect of temperature." J Thorac Cardiovasc Surg **97**(1): 43-49.

Rahimtoola, S. H. (1985). "A perspective on the three large multicenter randomized clinical trials of coronary bypass surgery for chronic stable angina." CIrculation **72**(6 Pt 2): V123-135.

Rajamannan, N. M., M. Subramaniam, et al. (2003). "Human aortic valve calcification is associated with an osteoblast phenotype." CIrculation **107**(17): 2181-2184.

Rampe, D., Z. Wang, et al. (1995). "Voltage- and time-dependent block by perhexiline of K+ currents in human atrium and in cells expressing a Kv1.5-type cloned channel." J Pharmacol Exp Ther **274**(1): 444-449.

Ranasinghe, A. M., D. W. Quinn, et al. (2006). "Glucose-insulin-potassium and tri-iodothyronine individually improve hemodynamic performance and are associated with reduced troponin I release after on-pump coronary artery bypass grafting." Circulation **114**(1 Suppl): I245-250.

Rassias, A. J., A. L. Givan, et al. (2002). "Insulin increases neutrophil count and phagocytic capacity after cardiac surgery." Anesth Analg **94**(5): 1113-1119, table of contents.

Razeghi, P., M. E. Young, et al. (2001). "Metabolic gene expression in fetal and failing human heart." CIrculation **104**(24): 2923-2931.

Roberts, W. C. and J. M. Ko (2005). "Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation." CIrculation **111**(7): 920-925.

Robinson, L. A. and D. L. Harwood (1991). "Lowering the calcium concentration in St. Thomas' Hospital cardioplegic solution improves protection during hypothermic ischemia." J Thorac Cardiovasc Surg **101**(2): 314-325.

Robinson, L. A., G. D. Schwarz, et al. (1995). "Myocardial protection for acquired heart disease surgery: results of a national survey." Ann Thorac Surg **59**(2): 361-372.

Roe, B. B., J. C. Hutchinson, et al. (1977). "Myocardial protection with cold, ischemic, potassium-induced cardioplegia." J Thorac Cardiovasc Surg **73**(3): 366-374.

Roger, V. L., A. J. Tajik, et al. (1990). "Progression of aortic stenosis in adults: new appraisal using Doppler echocardiography." Am Heart J **119**(2 Pt 1): 331-338.

Rosano, G. M., C. Vitale, et al. (2003). "Trimetazidine improves left ventricular function in diabetic patients with coronary artery disease: a double-blind placebo-controlled study." Cardiovasc Diabetol **2**: 16.

Rosenfeldt, F. L., D. J. Hearse, et al. (1980). "The additive protective effects of hypothermia and chemical cardioplegia during ischemic cardiac arrest in the dog." J Thorac Cardiovasc Surg **79**(1): 29-38.

Rosenhek, R., T. Binder, et al. (2000). "Predictors of outcome in severe, asymptomatic aortic stenosis." N Engl J Med **343**(9): 611-617.

Rosenkranz, E. R., G. D. Buckberg, et al. (1983). "Warm induction of cardioplegia with glutamate-enriched blood in coronary patients with cardiogenic shock who are dependent on inotropic drugs and intra-aortic balloon support." J Thorac Cardiovasc Surg **86**(4): 507-518.

Rosenkranz, E. R., F. Okamoto, et al. (1986). "Safety of prolonged aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury." J Thorac Cardiovasc Surg **91**(3): 428-435.

Rosenkranz, E. R., F. Okamoto, et al. (1984). "Safety of prolonged aortic clamping with blood cardioplegia. II. Glutamate enrichment in energy-depleted hearts." J Thorac Cardiovasc Surg **88**(3): 402-410.

Rosenkranz, E. R., J. Vinten-Johansen, et al. (1982). "Benefits of normothermic induction of blood cardioplegia in energy-depleted hearts, with maintenance of arrest by multidose cold blood cardioplegic infusions." J Thorac Cardiovasc Surg **84**(5): 667-677.

Ross, J., Jr. and E. Braunwald (1968). "Aortic stenosis." Circulation **38**(1 Suppl): 61-67.

Rousou, J. A., R. M. Engelman, et al. (1995). "Does interruption of normothermic cardioplegia have adverse effects on myocardium? A retrospective and prospective clinical evaluation." Cardiovasc Surg **3**(6): 587-593.

Rowe, G. G., S. Afonso, et al. (1963). "Systemic and coronary hemodynamic effects of hexadylamine." Proc Soc Exp Biol Med **112**: 545-547.

Ruixing, Y., L. Wenwu, et al. (2007). "Trimetazidine inhibits cardiomyocyte apoptosis in a rabbit model of ischemia-reperfusion." Transl Res **149**(3): 152-160.

Sallustio, B. C., I. S. Westley, et al. (2002). "Pharmacokinetics of the antianginal agent perhexiline: relationship between metabolic ratio and steady-state dose." Br J Clin Pharmacol **54**(2): 107-114.

Sambandam, N. and G. D. Lopaschuk (2003). "AMP-activated protein kinase (AMPK) control of fatty acid and glucose metabolism in the ischemic heart." Prog Lipid Res **42**(3): 238-256.

Sambandam, N., G. D. Lopaschuk, et al. (2002). "Energy metabolism in the hypertrophied heart." Heart Fail Rev **7**(2): 161-173.

Sasali, A. and J. L. Leahy (2003). "Is metformin cardioprotective?" Diabetes Care **26**(1): 243-244.

Sasayama, S., J. Ross, Jr., et al. (1976). "Adaptations of the left ventricle to chronic pressure overload." Circ Res **38**(3): 172-178.

Schaefer, S., E. Prussel, et al. (1995). "Requirement of glycolytic substrate for metabolic recovery during moderate low flow ischemia." J Mol Cell Cardiol **27**(10): 2167-2176.

Schiller, N. B., P. M. Shah, et al. (1989). "Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms." J Am Soc Echocardiogr **2**(5): 358-367.

Schonekess, B. O., M. F. Allard, et al. (1995). "Propionyl L-carnitine improvement of hypertrophied heart function is accompanied by an increase in carbohydrate oxidation." Circ Res **77**(4): 726-734.

Schulz, K. F., D. G. Altman, et al. (2010). "CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials." BMJ **340**: c332.

Schwartz, G. G., C. Greyson, et al. (1994). "Inhibition of fatty acid metabolism alters myocardial high-energy phosphates in vivo." Am J Physiol **267**(1 Pt 2): H224-231.

Schwarz, F., P. Baumann, et al. (1982). "The effect of aortic valve replacement on survival." Circulation **66**(5): 1105-1110.

Selvetella, G., E. Hirsch, et al. (2004). "Adaptive and maladaptive hypertrophic pathways: points of convergence and divergence." Cardiovasc Res **63**(3): 373-380.

Selzer, A. (1987). "Changing aspects of the natural history of valvular aortic stenosis." N Engl J Med **317**(2): 91-98.

Semenovsky, M. L., V. I. Shumakov, et al. (1987). "Protection of ischemic myocardium by exogenous phosphocreatine. II. Clinical, ultrastructural, and biochemical evaluations." J Thorac Cardiovasc Surg **94**(5): 762-769.

Shah, R. R. (2006). "Can pharmacogenetics help rescue drugs withdrawn from the market?" Pharmacogenomics **7**(6): 889-908.

Shah, R. R., N. S. Oates, et al. (1982). "Impaired oxidation of debrisoquine in patients with perhexiline neuropathy." Br Med J (Clin Res Ed) **284**(6312): 295-299.

Shandelya, S. M., P. Kuppusamy, et al. (1993). "Evaluation of the role of polymorphonuclear leukocytes on contractile function in myocardial reperfusion injury. Evidence for plasma-mediated leukocyte activation." CIrculation **87**(2): 536-546.

Shanewise, J. S., A. T. Cheung, et al. (1999). "ASE/SCA guidelines for performing a comprehensive intraoperative multiplane transesophageal echocardiography examination: recommendations of the American Society of Echocardiography Council for Intraoperative Echocardiography and the Society of Cardiovascular Anesthesiologists Task Force for Certification in Perioperative Transesophageal Echocardiography." J Am Soc Echocardiogr **12**(10): 884-900.

Sheldon, M. G., M. V. Braimbridge, et al. (1969). "Effect of glucose and insulin on the failing myocardium in an isolated heart preparation." Br Heart J **31**(3): 393.

Shivu, G. N., K. Abozguia, et al. (2010). "(31)P magnetic resonance spectroscopy to measure in vivo cardiac energetics in normal myocardium and hypertrophic cardiomyopathy: Experiences at 3T." Eur J Radiol **73**(2): 255-259.

Shivu, G. N., T. T. Phan, et al. (2010). "Relationship between coronary microvascular dysfunction and cardiac energetics impairment in type 1 diabetes mellitus." CIrculation **121**(10): 1209-1215.

Simonsen, S. and J. K. Kjekshus (1978). "The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man." CIrculation **58**(3 Pt 1): 484-491.

Singlas, E., M. A. Goujet, et al. (1978). "[Perhexilline maleate: relationship between side-effects, plasma concentrations and rate of metabolism (author's transl)]." Nouv Presse Med **7**(19): 1631-1632.

Singlas, E., M. A. Goujet, et al. (1978). "Pharmacokinetics of perhexiline maleate in anginal patients with and without peripheral neuropathy." Eur J Clin Pharmacol **14**(3): 195-201.

Sink, J. D., G. L. Pellom, et al. (1981). "Response of hypertrophied myocardium to ischemia: correlation with biochemical and physiological parameters." J Thorac Cardiovasc Surg **81**(6): 865-872.

Sloan, T. P., A. Mahgoub, et al. (1978). "Polymorphism of carbon oxidation of drugs and clinical implications." Br Med J **2**(6138): 655-657.

Smith, C. S., P. A. Bottomley, et al. (2006). "Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium." Circulation **114**(11): 1151-1158.

Smith, N., J. H. McAnulty, et al. (1978). "Severe aortic stenosis with impaired left ventricular function and clinical heart failure: results of valve replacement." CIrculation **58**(2): 255-264.

Sodi-Pallares, D., M. R. Testelli, et al. (1962). "Effects of an intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report." Am J Cardiol **9**: 166-181.

Sondergaard, T., E. Berg, et al. (1975). "Cardioplegic cardiac arrest in aortic surgery." J Cardiovasc Surg (Torino) **16**(3): 288-290.

Sones, F. M., Jr. (1958). "Results of open heart surgery with elective cardiac arrest by potassium citrate in patients with congenital and acquired heart disease." Dis Chest **34**(3): 299-316.

Sorensen, L. B., R. N. Sorensen, et al. (2003). "Polymorphic hydroxylation of perhexiline in vitro." Br J Clin Pharmacol **55**(6): 635-638.

Sorokina, N., J. M. O'Donnell, et al. (2007). "Recruitment of compensatory pathways to sustain oxidative flux with reduced carnitine palmitoyltransferase I activity characterizes inefficiency in energy metabolism in hypertrophied hearts." CIrculation **115**(15): 2033-2041.

Southam, A. D., T. G. Payne, et al. (2007). "Dynamic range and mass accuracy of wide-scan direct infusion nanoelectrospray fourier transform ion cyclotron resonance mass spectrometry-based metabolomics increased by the spectral stitching method." Anal Chem **79**(12): 4595-4602.

Spann, J. F., A. A. Bove, et al. (1980). "Ventricular performance, pump function and compensatory mechanisms in patients with aortic stenosis." Circulation **62**(3): 576-582.

Stanley, W. C., L. A. Hernandez, et al. (1996). "Pyruvate dehydrogenase activity and malonyl CoA levels in normal and ischemic swine myocardium: effects of dichloroacetate." J Mol Cell Cardiol **28**(5): 905-914.

Stanley, W. C., K. M. Kivilo, et al. (2003). "Post-ischemic treatment with dipyruvyl-acetyl-glycerol decreases myocardial infarct size in the pig." Cardiovasc Drugs Ther **17**(3): 209-216.

Stanley, W. C., G. D. Lopaschuk, et al. (1997). "Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions." Cardiovasc Res **33**(2): 243-257.

Stanley, W. C., F. A. Recchia, et al. (2005). "Myocardial substrate metabolism in the normal and failing heart." Physiol Rev **85**(3): 1093-1129.

Steenbergen, C., E. Murphy, et al. (1990). "Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart." Circ Res **66**(1): 135-146.

Stevens, A. and J. Lowe (2001). Contractile cells. Human histology

Stewart, S., D. W. Voss, et al. (1996). "Relationship between plasma perhexiline concentration and symptomatic status during short-term perhexiline therapy." Ther Drug Monit **18**(6): 635-639.

Stone, D., V. Darley-Usmar, et al. (1989). "Hypoxia-reoxygenation induced increase in cellular Ca2+ in myocytes and perfused hearts: the role of mitochondria." J Mol Cell Cardiol **21**(10): 963-973.

Surgeons, S. o. T. (2012). "Adult Cardiac Surgery Database Executive Summary 10 Years." Retrieved 3rd January 2013, 2013.

Swamy, R. S. and R. M. Lang (2010). "Echocardiographic quantification of left ventricular mass: prognostic implications." Curr Cardiol Rep **12**(3): 277-282.

Szwed, H., Z. Sadowski, et al. (2001). "Combination treatment in stable effort angina using trimetazidine and metoprolol: results of a randomized, double-blind, multicentre study (TRIMPOL II). TRIMetazidine in POLand." Eur Heart J **22**(24): 2267-2274.

Taegtmeyer, H., G. W. Goodwin, et al. (1997). "Substrate metabolism as a determinant for postischemic functional recovery of the heart." Am J Cardiol **80**(3A): 3A-10A.

Takamura, T., K. Dohi, et al. (2010). "Left ventricular contraction-relaxation coupling in normal, hypertrophic, and failing myocardium quantified by speckle-tracking global strain and strain rate imaging." J Am Soc Echocardiogr **23**(7): 747-754.

Takemoto, N., H. Kuroda, et al. (1994). "Effect of magnesium and calcium on myocardial protection by cardioplegic solutions." Ann Thorac Surg **57**(1): 177-182.

Takeuchi, M., W. B. Borden, et al. (2007). "Reduced and delayed untwisting of the left ventricle in patients with hypertension and left ventricular hypertrophy: a study using two-dimensional speckle tracking imaging." Eur Heart J **28**(22): 2756-2762.

Tian, R., N. Musi, et al. (2001). "Increased adenosine monophosphate-activated protein kinase activity in rat hearts with pressure-overload hypertrophy." CIrculation **104**(14): 1664-1669.

Ting, W., A. J. Spotnitz, et al. (1994). "Protection of the acutely injured heart--deleterious effects of hypothermia." J Surg Res **56**(1): 108-111.

Tomanek, R. J., P. J. Palmer, et al. (1986). "Morphometry of canine coronary arteries, arterioles, and capillaries during hypertension and left ventricular hypertrophy." Circ Res **58**(1): 38-46.

Tong, H., K. Imahashi, et al. (2002). "Phosphorylation of glycogen synthase kinase-3beta during preconditioning through a phosphatidylinositol-3-kinase--dependent pathway is cardioprotective." Circ Res **90**(4): 377-379.

Turina, J., O. Hess, et al. (1987). "Spontaneous course of aortic valve disease." Eur Heart J **8**(5): 471-483.

Tyers, G. F., N. J. Manley, et al. (1977). "Preliminary clinical experience with isotonic hypothermic potassium-induced arrest." J Thorac Cardiovasc Surg **74**(5): 674-681.

Tyler, D. J., Y. Emmanuel, et al. (2009). "Reproducibility of 31P cardiac magnetic resonance spectroscopy at 3 T." NMR Biomed **22**(4): 405-413.

Tyler, D. J., L. E. Hudsmith, et al. (2008). "A comparison of cardiac (31)P MRS at 1.5 and 3 T." NMR Biomed **21**(8): 793-798.

Unger, S. A., J. A. Kennedy, et al. (2005). "Dissociation between metabolic and efficiency effects of perhexiline in normoxic rat myocardium." J Cardiovasc Pharmacol **46**(6): 849-855.

Unger, S. A., M. A. Robinson, et al. (1997). "Perhexiline improves symptomatic status in elderly patients with severe aortic stenosis." Aust N Z J Med **27**(1): 24-28.

van der Vusse, G. J., M. van Bilsen, et al. (2000). "Cardiac fatty acid uptake and transport in health and disease." Cardiovasc Res **45**(2): 279-293.

van Echteld, C. J., J. H. Kirkels, et al. (1991). "Intracellular sodium during ischemia and calcium-free perfusion: a 23Na NMR study." J Mol Cell Cardiol **23**(3): 297-307.

Vanhamme, L., T. Sundin, et al. (2001). "MR spectroscopy quantitation: a review of time-domain methods." NMR Biomed **14**(4): 233-246.

Vaturi, M., A. Porter, et al. (1999). "The natural history of aortic valve disease after mitral valve surgery." J Am Coll Cardiol **33**(7): 2003-2008.

Villari, B., S. E. Campbell, et al. (1993). "Influence of collagen network on left ventricular systolic and diastolic function in aortic valve disease." J Am Coll Cardiol **22**(5): 1477-1484.

Vlasselaers, D. (2011). "Glucose-insulin-potassium: much more than enriched myocardial fuel." Circulation **123**(2): 129-130.

Wagner, S. and A. Selzer (1982). "Patterns of progression of aortic stenosis: a longitudinal hemodynamic study." Circulation **65**(4): 709-712.

Walker, B. D., S. M. Valenzuela, et al. (1999). "Inhibition of HERG channels stably expressed in a mammalian cell line by the antianginal agent perhexiline maleate." Br J Pharmacol **127**(1): 243-251.

Wambolt, R. B., S. L. Henning, et al. (1999). "Glucose utilization and glycogen turnover are accelerated in hypertrophied rat hearts during severe low-flow ischemia." J Mol Cell Cardiol **31**(3): 493-502.

Wang, W. and G. D. Lopaschuk (2007). "Metabolic therapy for the treatment of ischemic heart disease: reality and expectations." Expert Rev Cardiovasc Ther **5**(6): 1123-1134.

Weber, K. T. and C. G. Brilla (1991). "Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system." CIrculation **83**(6): 1849-1865.

Weber, K. T., J. S. Janicki, et al. (1990). "Myocardial fibrosis and pathologic hypertrophy in the rat with renovascular hypertension." Am J Cardiol **65**(14): 1G-7G.

Weber, K. T., Y. Sun, et al. (1994). "Collagen network of the myocardium: function, structural remodeling and regulatory mechanisms." J Mol Cell Cardiol **26**(3): 279-292.

Weber, R. J. M., A. D. Southam, et al. (2011). "Characterization of isotopic abundance measurements in high resolution FT-ICR and Orbitrap mass spectra for improved confidence of metabolite identification." Anal Chem **83**(10): 3737-3743.

Weber, R. J. M. and M. R. Viant (2010). "MI-Pack: Increased confidence of metabolite identification in mass spectra by integrating accurate masses and metabolic pathways." Chemometrics and Intelligent Laboratory Systems **104**(1): 75-82.

Whitehouse, S., R. H. Cooper, et al. (1974). "Mechanism of activation of pyruvate dehydrogenase by dichloroacetate and other halogenated carboxylic acids." Biochem J **141**(3): 761-774.

Wicker, P. and R. C. Tarazi (1982). "Coronary blood flow in left ventricular hypertrophy: a review of experimental data." Eur Heart J **3 Suppl A**: 111-118.

Wikman-Coffelt, J., S. Wagner, et al. (1991). "Alcohol and pyruvate cardioplegia. Twenty-four-hour in situ preservation of hamster hearts." J Thorac Cardiovasc Surg **101**(3): 509-516.

Willoughby, S. R., S. Stewart, et al. (2002). "Beneficial clinical effects of perhexiline in patients with stable angina pectoris and acute coronary syndromes are associated with potentiation of platelet responsiveness to nitric oxide." Eur Heart J **23**(24): 1946-1954.

Wright, G. J., G. A. Leeson, et al. (1973). "Proceedings: The absorption, excretion and metabolism of perhexiline maleate by the human." Postgrad Med J **49**: Suppl 3:8-15.

Wu, H., A. D. Southam, et al. (2008). "High-throughput tissue extraction protocol for NMR- and MS-based metabolomics." Anal Biochem **372**(2): 204-212.

Wu, J. and P. B. Corr (1992). "Influence of long-chain acylcarnitines on voltage-dependent calcium current in adult ventricular myocytes." Am J Physiol **263**(2 Pt 2): H410-417.

Ye, Y., G. Gong, et al. (2001). "High-energy phosphate metabolism and creatine kinase in failing hearts: a new porcine model." Circulation **103**(11): 1570-1576.

Yellon, D. M. and D. J. Hausenloy (2007). "Myocardial reperfusion injury." N Engl J Med **357**(11): 1121-1135.

Yin, X., J. Dwyer, et al. (2013). "Effects of perhexiline-induced fuel switch on the cardiac proteome and metabolome." J Mol Cell Cardiol **55**: 27-30.

Yotti, R. and J. Bermejo (2011). "Left ventricular hypertrophy in aortic valve stenosis: friend or foe?" Heart **97**(4): 269-271.

Zhang, Y. and S. Xu (1995). "Increased vulnerability of hypertrophied myocardium to ischemia and reperfusion injury. Relation to cardiac renin-angiotensin system." Chin Med J (Engl) **108**(1): 28-32.

Zorov, D. B., C. R. Filburn, et al. (2000). "Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes." J Exp Med **192**(7): 1001-1014.

Zucchi, R., R. Poddighe, et al. (1989). "Protection of isolated rat heart from oxidative stress by exogenous creatine phosphate." J Mol Cell Cardiol **21**(1): 67-73.