

# **APPLICATION OF REMOTE ISCHAEMIC PRECONDITIONING TO HUMAN CORONARY ARTERY BYPASS SURGERY**

by

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## ABSTRACT

This thesis reports a clinical study designed to assess myocardial, renal and lung outcomes following cardiac surgery. In a single centre, prospective randomized, placebo intervention-controlled trial the effects of intermittent upper limb ischaemia (remote ischaemic preconditioning (RIPC)) were compared in non-diabetic adult patients undergoing on-pump multi-vessel coronary artery surgery. Patients, investigators, anaesthetists, surgeons and critical care teams were all blind to group allocation. Subjects were randomized(1:1) to RIPC(or placebo) stimuli (3x upper limb (or dummy arm) 5 minute cycles of 200mmHg cuff inflation/deflation) during sternotomy and conduit procurement. Anaesthesia, perfusion, cardioplegia and surgical techniques were standardized.

Groups were well matched on demographic and operative variables. In contrast to prior smaller studies, RIPC did not reduce troponin T (48 hour area under the curve (AUC); 6hour and peak) release, improve post-operative haemodynamics (cardiac indices; low cardiac output episodes incidence; IABP usage; inotrope and vasoconstrictor use; M mode, 2D contrast-enhanced echocardiography and tissue Doppler imaging) or offer anti-arrhythmic benefit (*de novo* left bundle branch block or Q waves; ventricular tachyarrhythmia incidence). RIPC did not afford renal (peak creatinine, AUC urinary albumin-creatinine ratios, dialysis requirement) or lung protection (intubation times, 6hour and 12 hour pO<sub>2</sub>/FiO<sub>2</sub> ratios). Case urgency did not influence RIPC effect.

## **DEDICATION**

I would like to dedicate this work to my mother, Shakeela, and my father, Altaf, for providing me with so many opportunities; to my wife, Hina, for helping me to maximise those opportunities; and to my daughter, Tamara, for being my inspiration.

## **ACKNOWLEDGMENTS**

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I would also like to thank my co-supervisor Professor Michael Frenneaux, Regius Professor of Medicine and mentor Dr Rick Steeds, Consultant Cardiologist. Dr Howard Marshall, Consultant Cardiologist, facilitated my investigations of arrhythmia analysis. The specific help of Peter Nightingale, Statistician, was crucial to this work. Echocardiography was performed by Ms Anne Marie Marsh, Dr Lynne Williams and Mr Ador Alvior. Primary work on myocardial specimens was done by Dr Hussain Contractor.

I would also acknowledge the contributions of the co-authors Mr Jorge Mascaro, Dr Peter Gosling, Dr Peter Townsend, Dr John Townend and Dr David Green on our published manuscripts

## **PERSONAL CONTRIBUTION**

The work of this thesis was undertaken during the tenure of a British Heart Foundation Fellowship (2006-9). I was attached to the Department of Cardiac Surgery, under the guidance of Professor Robert Stuart Bonser, at the University Hospital Birmingham NHS Foundation Trust. The thesis was registered with the University of Birmingham. The Fellowship had clinical responsibility for all 162 trial patients and additional time was dedicated to research. This thesis details the clinical trial that was undertaken.

At the time of this work, in coronary artery bypass grafting (CABG), adverse outcomes predominantly related to cardiomyocyte injury due to myocardial protection failure presented a current problem that was projected to grow as a more aged population with increased co-morbidities presented for surgery. A need to conserve cardiac function and myocyte integrity (myocardial protection) in the peri-operative period and thereafter had been identified. Early experimental and clinical evidence suggested that RIPC could attenuate such injury safely and practically whilst also offering renal and lung protection. Informed consent of patients, management of the trial, data accrual and analysis of data was my own responsibility. Echocardiographic image analysis was performed by myself under the guidance of Dr Rick Steeds. Continuous ECG monitoring data disks was analysed solely by myself. I observed the techniques for analysis of myocardial specimens by Dr Hussain Contractor and was involved in analysis. The writing of this thesis is wholly my own endeavour.

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## ABBREVIATIONS AND ACRONYMS

### A

A1M	alpha-1-microglobulin
A2C	apical 2 chamber view
A4C	apical 4 chamber view
ACE-I	angiotensin converting enzyme inhibitor
ACP	antegrade cardioplegia
ACR	albumin creatinine ratio
AF	atrial fibrillation
AKI	acute kidney injury
AlRed	aldose reductase
ALI	acute lung injury
Angio	angiography
ANOVA	analysis of variance
ARDS	adult respiratory distress syndrome
ARF	acute renal failure
ARF-D	acute renal failure requiring dialytic therapy
ARTS	Arterial Revascularization Therapies Study
ATP	adenosine triphosphate
AUC	area under curve
AV	atrioventricular
AVR	aortic valve replacement
AXC	aortic cross clamp

### B

BARI	Bypass Angioplasty Revascularisation Investigation
Bcl-2	antiapoptotic protein
BHF	British Heart Foundation
BPM	beats per minute
BSA	body surface area

### C

°C	degrees centigrade
Ca <sup>2+</sup>	calcium ions
CABG	coronary artery bypass grafting
CABRI	Coronary Angioplasty vs Bypass Revascularisation Investigation
cAMP	cyclic adenosine monophosphate
CAO	coronary artery occlusion
CASS	Coronary Artery Surgery Study
CCS	Canadian Cardiovascular Society
CGRP	calcitonin gene-related peptide
CHD	coronary heart disease
CI	cardiac index
CKMB	creatine kinase-MB
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
COS	cardiac output study
COX-2	cyclooxygenase 2

CPB	cardio-pulmonary bypass
CPK	creatine phosphokinase
cTnI	cardiac Troponin I
cTnT	cardiac Troponin T
CPD	citrate phosphate dextrose
Cr	creatinine
CrCl	creatinine clearance
Cryo	cryoprecipitate
CVA	cerebrovascular accident
CVP	central venous pressure

## D

DAG	diacylglycerol
DC	direct current
DHES	deferoxamine-conjugated hydroxyethyl-starch
DNA	deoxyribonucleic acid
DoB	date of birth
Dopa	dopamine
DoS	date of surgery
Dr	Doctor
DVT	deep vein thrombosis

## E

e'	early myocardial relaxation velocity
E/e'	transmitral to mitral relaxation velocity ratio
ECG	electrocardiogram
ECSS	European Coronary Surgery Study
EF	ejection fraction
Epi	epinephrine
ERACI	Argentine Estudio Argentino de Angioplastia vs Cirugia
Erk	extracellular signal regulated kinase
EVAR	endovascular repair

## F

FEV <sub>1</sub>	forced expiratory volume in one second
FFP	fresh frozen plasma
FiO <sub>2</sub>	fraction of inspired oxygen
FMD	flow mediated dilatation
FT	Foundation Trust
FVC	forced vital capacity

## G

GIK	glucose insulin potassium
GSK-3 $\beta$	glycogen synthase kinase 3 $\beta$
GTN	glyceryl trinitate

## H

HB	haemoglobin
HD	haemodilution
HDU	High Dependency Unit

HMR-1098	specific sarcolemmal KATP channel blocker
HO	Heme oxygenase
HOE-140	inhibitor of bradykinin B2 receptors
HP	hypoxic preconditioning
HSP	heat shock protein

## I

IABP	intra-aortic balloon pump
ICCF	intermittent crossclamp fibrillation
ICU	Intensive Care Unit
IKKbeta	signalling molecule in the activation of the NF-kappaB pathway
IL	interleukin
iNOS	inducible nitric oxide synthase
INR	international normalised ratio
IP	ischaemic preconditioning
IR	ischaemia reperfusion
ITU	Intensive Therapy Unit
iv	intravenous
IVA	isovolumic acceleration
IVSd	intraventricular septum in diastole

## K

K <sub>ATP</sub> Mito	ATP-dependent potassium channel in mitochondria
K <sub>ATP</sub> SARC	ATP-dependent potassium channel in sarcolemma
KCl	potassium chloride
kD	kilo Daltons
kDA	kilo Daltons
kg	kilogram

## L

L	litre
LA	left atrium
LAD	left anterior descending
LBbB	left bundle branch block
LCOE	low cardiac output episode
LIMA	left internal mammary harvest
LMS	left main stem
LOS	length of stay
LREC	local research ethics committee
LV	left ventricle
LVEDV(i)	left ventricular end-diastolic volume (index)
LVEF	left ventricular ejection fraction
LVESV(i)	left ventricular end-systolic volume (index)
LVIDd	left ventricular internal dimension in diastole
LVPWd	left ventricular posterior wall dimension in diastole
LVSWI	left ventricular stroke work index

## M

MACCE	major adverse cardiac or cerebrovascular event
MABP	mean arterial blood pressure

MAO	mesenteric artery occlusion
MAP	mean arterial pressure
MASS-II	Medicine Angioplasty or Surgery Study
MI	myocardial infarction
min	minute
mitoK <sub>ATP</sub>	mitochondrial membrane ATP-sensitive K <sup>+</sup> channels
mL	millilitres
mmHg	millimetres mercury
MnSOD	manganese superoxide dismutase
MOPS	3-(N-morpholino)propanesulfonic acid
mPT	mitochondrial permeability transition
mPTP	mitochondrial permeability transition pore
Mr	Mister
MRI	magnetic resonance imaging

## N

NF <sub>κ</sub> B	nuclear factor kappa-B
NKH477	potent activator of adenylyl cyclase
NGAL	earliest responding biomarker for acute kidney injury
NHS	National Health Service
NIM811	mitochondrial permeability transition inhibitor
NSTEMI	non-ST elevated myocardial infarction
NO	nitric oxide
NorE	norepinephrine
NSAID	non steroidal anti-inflammatory drug
NYHA	New York Heart Association

## O

ON-CABG	on-pump coronary artery bypass grafting
OP-CABG	off-pump coronary artery bypass grafting
OPCAB	off-pump coronary artery bypass
ODP	operating department practitioner
ORT	operating room technician

## P

PA	preconditioned acceptor
PaO <sub>2</sub>	partial pressure of oxygen in arterial blood
PAWP	pulmonary artery wedge pressure
PCI	percutaneous coronary intervention
PCWP	pulmonary capillary wedge pressure
PD	preconditioned donor
PEEP	positive end expiratory pressure
PKC	protein kinase C
PLC	phospholipase C
PLD	phospholipase D
PMI	perioperative myocardial infarction
PO	'per os' (by mouth)
POD	postoperative day
Postop	postoperative
PPM	permanent pacemaker

PRBC	packed red blood cells
Preop	preoperative
PRN	'pro re nata' (as the situation arise)
PTCA	percutaneous transluminal coronary angioplasty
PVDF	Polyvinylidene fluoride

## **R**

RA	room air
RBC	red blood cells
RCP	retrograde cardioplegia
RCT	randomised controlled trial
RIPC	remote Ischaemic preconditioning
RISK	reperfusion injury salvage kinase
RITA	Randomised Intervention Treatment of Angina trial
RMANOVA	repeated measure analysis of variance
ROS	reactive oxygen species
RV	right ventricle
RVEF	right ventricular ejection fraction
RVIDd	right ventricular internal dimension in diastole

## **S**

s'	systolic myocardial velocity
SBP	systolic blood pressure
SD	standard deviation
SDS	sodium dodecyl sulfate
SES	Sirolimus eluting stent
SNP	sodium nitroprusside
SoS	Stent or Surgery
SR	sinus rhythm
STEMI	ST elevated myocardial infarction
SVR	systemic vascular resistance

## **T**

T-SOD/MDA	superoxide dismutase/malondialdehyde
TAPSE	tricuspid annular systolic annular plane excursion
THAM	tris(hydroxymethyl)aminomethane
TIA	transient ischaemic attack
TLR	target lesion revascularisation
TNF-alpha	tumour necrosis factor alpha
TRIS	Tris(hydroxymethyl)-aminomethane

## **V**

VA	Veterans Administrative Coronary Artery Bypass Surgery Study
VAC	Vacuum assisted closure
VF	ventricular fibrillation
VT	ventricular tachycardia

# **CHAPTER 1: THE DEVELOPMENT AND CURRENT STATUS OF SURGERY FOR ISCHAEMIC HEART DISEASE**

## **Section 1: Development of coronary artery surgery and cardiopulmonary bypass**

### **1.0 Introduction**

Currently surgery on the heart and/or great vessels (cardiac surgery) represents an established form of medical practice. Its applications have extended to include treatment of ischaemic, congenital, valvular heart disease and transplantation.

In the financial year ending 2008, 22846 patients underwent isolated coronary artery bypass surgery, on symptomatic and prognostic grounds, with a mortality rate of 1.5% [1]. Access to the heart is a recent phenomenon and its history is linked with controversy.

### **2.0 Early History of Cardiac Surgery**

In 1801 Francisco Romero, a Catalan physician, performed an open pericardiostomy to treat a pericardial effusion and became credited with the title of 'The First Heart Surgeon' [2]. He went on to present his work at the Society of the School of Medicine in Paris in 1815 but considered too aggressive in his procedure his work was silenced for many years.

The future, at this stage for cardiac surgery, continued to look bleak and in 1881, the founding father of modern abdominal surgery, Theodore Billroth announced:

*‘Anyone who would attempt to operate on the heart should loose the respect of his colleagues’*

By 1896 Stephen Paget, surgeon and son of the distinguished surgeon and pathologist Sir James Paget, wrote:

*‘Surgery of the heart has probably reached the limits set by Nature; no new methods and no new discovery can overcome the natural difficulties that attend a wound of the heart’*

In 1893, Dr. Daniel Hale Williams (Figure 1.1) a surgeon from Chicago, successfully operated on a 24-year-old man who had been stabbed during a fight by suturing an artery and vein inside the chest wall and a tear in the pericardium [3].

**Figure 1.1: Dr Daniel Hale Williams**



A wound in the right ventricle was noted but was not bleeding, so Williams did not place a stitch through the heart wound. The patient recovered and Williams reported this case four years later [3][4]. This operation was the first successful surgery involving a documented stab wound to the heart. At the time, Williams' surgery was considered bold and daring, and although he did not actually place a stitch through the wound in the heart, his treatment seems

to have been appropriate. Under the circumstances, he most likely saved the patient's life.

On the 4<sup>th</sup> of September 1895 the first surgery on the heart itself was performed, albeit unsuccessfully, by the Norwegian surgeon Axel Cappelen at Rikshospitalet in Kristiania, now Oslo.

A 24 year old man presented in deep shock following a stab injury to the left axilla. Cappelen accessed the chest through a left thoracotomy and ligated the bleeding coronary artery. The young man awoke and appeared to respond well in the first 24 hours but then became pyrexial and passed away from mediastinitis on the third postoperative day [5].

A year on, after suturing a stab wound to the right ventricle without any complications, Ludwig Rehn (Figure 1.2) had performed the first successful surgery of the heart. In his own words:

*... Today the patient is cured. He looks very good. His heart action is regular. I have not allowed him to work physically hard. This proves the feasibility of cardiac suture repair without a doubt! I hope this will lead to more investigation regarding surgery of the heart. This may save many lives.*

Ten years after Rehn's initial repair, he had accumulated a series of 124 cases with a mortality of 60% [6]. His successful work was inspirational to other pioneers and reversed the prevailing belief of inviolability of the heart and marked the beginning of cardiac surgery [7].

**Figure 1.2: Dr Ludwig Rehn**



### **3.0 A brief history of Coronary Artery Surgery**

The dawn of coronary artery surgery was heralded by Alexis Carrel in a canine model who published in 1910 [8]:

*I attempted to perform an indirect anastomosis between descending aorta and the left coronary artery. It was for many reasons a difficult operation. On account of the continuous motion of the heart, it was not easy to dissect and to suture the artery. In one case, I implanted one end of a long carotid artery, preserved in a cold storage, on the descending aorta. The other end was passed through the pericardium and anastomosed to the pericardial end of the coronary near the pulmonary artery. Unfortunately, the operation was too slow. Three minutes after the interruption of the circulation fibrillary contractions appeared, but the anastomosis took five minutes. By massage of the heart, the dog was kept alive, but he died less than two hours afterwards. It shows that the anastomosis must be done in less than three minutes.*

Claude Beck, in 1930, tried to revascularise myocardium in experimental models by attaching adjacent tissues (pericardium, pericardial fat, pectoralis muscle, omentum) in the hope of forming collateral blood flow to the ischaemic myocardium [9][10]. Postmortem examination revealed that

anastomotic vessels had developed between these tissues and the myocardium. Beck subsequently performed this operation with modifications on 16 patients.

In 1946 Arthur Vineberg reported successful implantation of the internal mammary artery through a tunnel in the myocardium but not actually anastomosing the left internal mammary artery to the coronary artery [11]. Mason Sones later validated Vineberg's concept by demonstrating communications between the graft in the myocardium and the coronary system by angiography in two patients operated on 5 and 6 years earlier. In the middle 1960s the Vineberg operation with many variations was performed at many institutions in the United States and Canada [12].

Coronary arterial endarterectomies were also attempted for the treatment of ischemic coronary disease but mortality was high, and the procedure was abandoned as an isolated operation [13].

From 1960 to 1967, isolated cases of coronary grafting were reported. None had an impact on the development of coronary surgery. In 1960 Robert Goetz performed the first coronary artery bypass operation in a human, which was successful [14]. The right internal mammary artery was connected to the right coronary artery using a non suture technique. The patient was asymptomatic for a year, then developed recurrent angina and died of a myocardial infarction.

The first clinical case of successful coronary artery bypass surgery using autogenous saphenous vein was performed by Garrett, Dennis and DeBakey on a 42-year-old man in 1964 [15]. The vein was placed from the aorta to the left anterior descending. The internal mammary artery had been anastomosed

to the left coronary artery in 1952 by Vladimir Demikhov, in a canine model [16] and Longmire performed the first internal mammary to coronary artery anastomosis in humans [17].

In 1967 Kolessov reported his experience with mammary artery–coronary artery anastomoses for treatment of angina pectoris in six patients [18] through a left thoracotomy without extracorporeal circulation or preoperative coronary angiography. The following year, Green et al [19] and Bailey and Hirose [20] separately published reports in which the internal mammary artery was used for coronary artery bypass in patients. Bailey and Hirose carried out the anastomosis on the beating heart and then Green et al advocated using cardiopulmonary bypass, fibrillating the vented heart, cross-clamping the aorta, and washing all blood from the coronary system while performing the anastomosis.

Favalaro used saphenous vein for bypassing coronary obstructions [21]. However, the start of modern coronary bypass surgery took place in 1969 when Johnson et al reported a series of 301 patients who had undergone various operations for coronary disease since early 1967 [22].

Johnson presented guidelines for direct surgery:

*One: Do not limit grafts to proximal portions of large arteries....*

*Two: Do not work with diseased arteries. Vein grafts can be made as long as necessary and should be inserted into distal normal arteries.*

*Three: Always do end-to-side anastomosis....*

*Four: Always work on dry, quiet field. Consistently successful fine vessel anastomoses cannot be done on a moving, bloody target....*

*Five: Do not allow the haematocrit to fall below 35.*

The direct anastomosis between the internal mammary artery and the coronary artery was not initially as popular as the vein graft technique; however, due to the persistence of Drs. Green, Loop, Grondin, and others, internal mammary artery grafts eventually became the conduit of choice when their superior long-term patency became known [23].

#### **4.0 The development of cardiopulmonary bypass**

Successful cardiopulmonary bypass development requires understanding of cardiovascular physiology, a mechanism to pump blood, and a technique to ventilate blood whilst preventing it from clotting [24].

Credit for anticoagulation in the heart-lung machine is owed to McLean in 1916 [25][26] who discovered heparin [27][28]. The work of John Gibbon led to the first successful demonstration that life could be maintained by an artificial heart and lung and that the native heart and lungs could resume function [29]. Dennis et al went on to develop a heart-lung machine to support circulation intraoperatively in a young girl during atrial septal defect closure [30][31]. Although the machine was successful in purpose the patient did not survive.

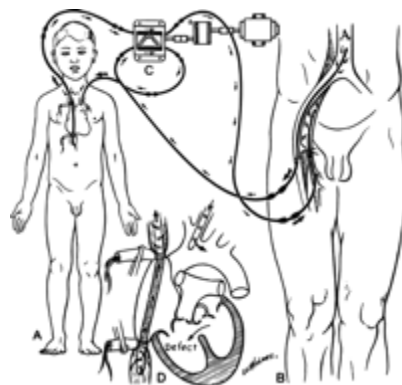
With the help of General Motors in 1952 Dodrill and colleagues developed a mechanical blood pump [32] that successfully bypassed the left-side of the human heart. Dodrill went on to successfully perform right-sided heart bypass [33] and in 1955 transferred use of the oxygenator to human models.

John Gibbon, with the assistance of the International Business Machines (IBM) Corporation, built a machine which contained a rotating vertical cylinder oxygenator and a modified DeBakey rotary pump. The machine was

successful in canine models and Gibbon went on to develop a machine with a larger oxygenator planned for human models [34].

Whilst Gibbons early experience in experimental and human models with the heart lung machine was mixed [35] Lillehei studied 'controlled cross-circulation' [24] in canine models. The circulation of one dog was temporarily used to support that of a second dog while the second dog's heart was temporarily stopped and opened. After a simulated repair in the second dog, the animals were disconnected and allowed to recover. This technique with a blood pump and bubble oxygenator was then extended to human models [24][36][37] (Figure 1.3).

**Figure 1.3: Crosscirculation by Lillehi**



In 1955 Kirklin started an open heart program at the Mayo Clinic [38] using his own modified Gibbon-IBM heart-lung machine [39]. This propelled many international university groups by 1957 to have followed suit and start the era of modern cardiac surgery.

An international drive in the pursuit of excellence continues to reduce complication rates and increase recovery time of patients undergoing cardiac surgery. Today with the refinement of anaesthetic, surgical and perfusion protocols cardiac surgical procedures are routine and commonplace.

## **Section 2:           The need for myocardial protection**

### **1.0   Introduction**

Coronary heart disease (CHD) is the leading cause of death world-wide and can manifest as acute myocardial ischaemia-reperfusion injury with detrimental effects.

Myocardial injury has been found to occur in 10-40% of cases during coronary intervention and can be characterized by a slight increase of markers of myocardial necrosis, without symptoms, electrocardiographic changes or impairment of cardiac function [40]. Coronary artery bypass graft (CABG) surgery, the procedure of choice for coronary artery revascularisation in a large number of patients with severe CHD, can also result in myocardial injury leading to worse short and long-term clinical outcomes. As a trend in progressively increased preoperative patient risk profile has been observed worse outcomes are envisaged in the future.

This projection of poorer outcomes has alerted the medical fraternity to the need for treatment strategies designed to protect the heart in terms of reducing myocardial injury and preserving left ventricular systolic function.

Microvascular protection during the acute event has become the focus of a variety of emerging technologies. The goal of these mechanical and pharmacologic therapies is the restoration of normal metabolic function at the myocyte level [41].

Myocardial protection aims at preventing myocardial tissue loss and current methods include cardioplegia, off-pump coronary artery bypass surgery

(OPCAB), ischaemic preconditioning, on-pump beating heart surgery and intermittent ischaemic arrest.

## **2.0 Brief history of myocardial protection**

Early work on circulatory arrest in 1914 by Alexis Carrel found that:

*....it was possible to clamp the pedicle of the heart (aorta and pulmonary artery) for two and a half or three minutes without any subsequent trouble. As soon as the clamp was removed, the heart resumed its pulsations, and after a very short time, the pulsations were again normal. [42]*

Hooker in 1929 suggested that potassium inhibition induced by an excess of potassium chloride could be used to stop the heart leading Melrose in 1955 [43] to present the first experimental study describing induced arrest by potassium-based cardioplegia. Blood cardioplegia was used:

*"to preserve myocardial energy stores at the onset of cardiac ischemia."*

Melrose goes on to state that

*"... they have succeeded in evolving a reliable method of stopping and restarting the heart at both normal and reduced body temperatures."*

Unfortunately, the Melrose solution proved to be toxic to the myocardium, and as a result, cardioplegia was not used widely for several years.

Gay and Ebert [44] and Tyres [45] demonstrated that cardioplegia with lower potassium concentrations was safe. Studies by Kirsch [46] Bretschneider [47] and Hearse [48] demonstrated the effectiveness of cardioplegia with other constituents and renewed interest in this technique.

Gay and Ebert in 1973 demonstrated a significant reduction in myocardial oxygen consumption during potassium-induced arrest when compared with

that of the fibrillating heart [44]. They also showed that the problems in the use of the Melrose solution in the early days of cardiac surgery probably were due to its hyperosmolar properties and perhaps not to the high potassium concentration.

Follette, in 1978, [49] demonstrated in experimental and clinical studies that hypothermic, intermittent blood cardioplegia provided better myocardial protection than normothermic, continuous coronary perfusion and/or hypothermic, intermittent blood perfusion without cardioplegia solution.

Since the 1980s the use of cardioplegia as a form of myocardial preservation technique has become established and commonplace in surgical practice. Debate remains over the ideal composition of cardioplegia solution. Formulations, methods of delivery, and recommended temperature continue to refine and evolve.

### **3.0 Reperfusion Injury**

An increasing body of evidence suggests a multifactorial mechanism for myocyte injury and microvascular collapse that is associated with a profound impact on long-term outcomes.

During cardiac surgery, the reperfusion of blood following prolonged ischaemia (e.g. aortic crossclamping) provides oxygen and substrate for the return of oxidative metabolism and rewarming increases the metabolic rate toward normal. There is potential, with reperfusion, to add further injury to the ischaemic damage sustained by cells through a localised inflammatory response whose basic components are:

- Free radical injury
- Calcium overload
- Complement activation
- Neutrophil activation
- Cytokine system
- Endothelial response to ischaemia/reperfusion injury

## **4.0 Cardioplegia**

### *4.1 Introduction to cardioplegia*

Over thirty years ago, the development of hyperkalaemic cardioplegic solutions revolutionised cardiac surgery by offering effective chemically-induced cardiac arrest and myocardial protection during global ischaemia.

Despite remaining the most widely-used cardioplegic technique, hyperkalaemia can have detrimental effects due to the Na and Ca loading of the cardiac cell induced by depolarisation of the cell membrane. Efforts over the last two decades to establish better cardioplegic agents have mainly remained limited to animal experiments. The failure of these approaches to progress to clinical trials may be due to a lack of clear criteria that a cardioplegic agent should meet at a cellular level and, more importantly, at a system level [50].

### *4.2 Principles of Cardioplegia*

During the interruption of coronary circulation by the intraoperative cross-clamping of the aorta cardioplegia is used to arrest the heart and reduce myocardial metabolism. In the absence of cardioplegia cross-clamping alone

would induce anaerobic metabolism, depletion of myocardial energy stores and cause severe myocardial dysfunction. Cardioplegic solutions today are able to provide myocardial protection of over 3 hours without adversely affecting myocardial function [51].

The optimal results from cardioplegia are based on the principles of prompt diastolic arrest, buffering, reducing calcium levels, an adequate delivery system, temperature and the addition of substrates designed to optimize myocardial metabolism or prevent cell damage (Table 1.1).

**Table 1.1: Principles and composition of cardioplegia [51]**

PRINCIPLE	COMPOSITION
Prompt diastolic arrest	KCl 20-25 mEq.L <sup>-1</sup>
Buffering	THAM, bicarbonate
Reduction of calcium levels	Citrate-phosphate-dextrose (CPD)
Adequate delivery	Antegrade+/-retrograde administration
Temperature	Cold vs. tepid vs. warm
Substrate additives to optimize myocardial metabolism or prevent cell damage	Aspartate glutamate Na <sup>+</sup> -H <sup>+</sup> exchange inhibitors Insulin Magnesium Procainamide L-arginine Calcium channel blockers
THAM = tris(hydroxymethyl)aminomethane; CPD = citrate-phosphate-dextrose	

#### 4.2.1 Prompt diastolic arrest

Prompt diastolic arrest of the heart is achieved by administering a solution containing approx 20-25 mEq/L of potassium chloride (KCl). Three methods of

delivery currently employed are crystalloid cardioplegia, blood cardioplegia and miniplegia.

#### *4.2.1.1 Crystalloid cardioplegia*

In crystalloid cardioplegia potassium is added to a crystalloid solution that is administered undiluted. The resulting solution provides little substrate and no oxygen to the heart during ischaemic arrest. It functions primarily by arresting the heart at cold temperatures and has the potential to be oxygenated by bubbling oxygen through the solution although this is uncommon [51].

#### *4.2.1.2 Blood cardioplegia*

When potassium is concentrated in a smaller bag of crystalloid solution and is administered in a mixture with blood in varying ratios (most commonly 4:1 blood) blood cardioplegia is produced. This form of cardioplegia provides oxygen, natural buffering agents, antioxidants and free-radical scavengers. Standard additives include buffers such as THAM to achieve an alkaline pH, citrate-phosphate-dextrose (CPD) to lower calcium levels and mannitol to maintain slight hyperosmolarity. The cardioplegia mixture formed then passes through a separate heater/cooler system in the extracorporeal circuit and infusion rate and pressure can be controlled [52].

#### *4.2.1.3 Miniplegia*

When potassium is added directly to blood to minimize haemodilution miniplegia is formed.

#### *4.2.2 Reduction in cardiac oxygen demand*

By arresting the heart at normothermia cardiac oxygen demand is reduced by nearly 90%. Maintenance of arrest during the cross-clamp period is achieved by readministering the solution every 15-20minutes so as to deliver potassium and washout the metabolic byproducts that have been generated. A low potassium solution ( $12-15\text{mEq.L}^{-1}$ ) is used to maintain the arrest while avoiding an excess potassium load. A high potassium solution should be used if the heart resumes any activity [51].

In the case of cold blood cardioplegia alone, this can be given retrograde into the coronary sinus as an alternative to subsequent doses of cardioplegia to optimise tissue oxygenation and metabolism while minimising potassium load [51].

#### *4.2.3 Temperature*

The reduction in myocardial metabolism attributable to hypothermia is quite insignificant compared with that achieved by diastolic arrest. Nonetheless, systemic hypothermia supplemented by use of topical cold saline and topical cooling devices that surround the left ventricle and protect the phrenic nerve from cold injury is routinely used in patients receiving cold cardioplegia [51].

Since enzymatic and cellular reparative processes function better at normothermia, some surgeons use 'warm cardioplegia' for myocardial protection. However because of the tendency for the heart to resume electrical activity at normothermia, this must be given continuously or with only brief interruptions to protect the heart. When given continuously it can obscure the operative field [51].

Warm cardioplegia can be used as an adjunct to cold cardioplegia when given at the beginning and the end of the period of aortic cross-clamping. Administering 500mL of warm cardioplegia immediately after aortic cross-clamping may be beneficial in actively ischaemic hearts with energy depletion by providing a brief period of time during which oxygen can be used to repair cell damage and replace energy stores.

Terminal warm blood cardioplegia ('hot shot') is commonly given just before the removal of the aortic cross clamp as it has been shown to improve myocardial metabolism. The heart tends to remain asystolic for several minutes after removal of the aortic cross clamp, during which time the heart is able to 'repair' cellular processes or replenish energy stores while the oxygen demand is low [53][54].

#### *4.2.4 Route of delivery*

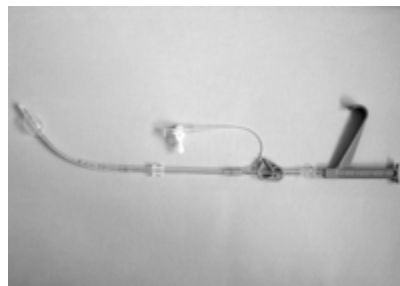
Cardioplegia is initially administered antegrade (Figure 1.4) into the aortic root and then may be given retrograde through a catheter placed in the coronary sinus (Figure 1.5).

**Figure 1.4: Antegrade cardioplegia cannula with a side port for venting**



The efficacy of antegrade cardioplegia (ACP) delivery may be compromised by severe coronary artery stenosis and is often dependent on collateral flow. Sufficient root distension may not be achieved in patients with more than mild aortic insufficiency and it can be cumbersome to readminister ACP during aortic and mitral valve operations.

**Figure 1.5: Retrograde cardioplegia catheter with self-inflating balloon for measuring coronary sinus pressure**



Retrograde cardioplegia (RCP) is easy to administer, either intermittently or continuously, and does not interrupt the flow of an operation. RCP does provide excellent myocardial protection but there is concern about maldistribution of the solution especially to the right ventricle. Careful monitoring of coronary sinus pressure during the administration of RCP is required to avoid coronary sinus rupture.

Routes of delivery of cardioplegia should be complementary and not exclusive. Contrast echocardiography has demonstrated left ventricular perfusion to be better with warm antegrade delivery than retrograde delivery. Delivery to the right ventricle is poor with either approach especially in right coronary artery occlusion [51].

In patients undergoing elective coronary artery bypass grafting, retrograde continuous infusion of cardioplegia by gravitational force combined with antegrade cardioplegia has been shown to provide satisfactory myocardial protection and to eliminate the need for inotropic support when compared with antegrade delivery alone [55].

#### *4.2.5 Cardioplegia additives*

A variety of medications that might potentially be cardioprotective when given in the cardioplegia solution have been studied. Those showing the greatest promise include the  $\text{Na}^+\text{-H}^+$  exchange inhibitors (such as cariporide), adenosine and  $\text{L}$ -arginine. Aspartate and glutamate are Krebs' cycle intermediates that have been used to improve myocardial energy metabolism with variable success. Other drugs include procainamide, magnesium (which has been shown to reduce arrhythmias) and free-radical scavengers. The insulin cardioplegia trial involved use of tepid cardioplegia enriched with glucose and insulin. This demonstrated metabolic and functional benefits to patients undergoing elective but not urgent surgery [51].

#### *4.3 Cardioplegia Strategy*

Variations in cardioplegia strategies and solutions have made it difficult to ascertain which specific element provides true benefit. The extent of coronary disease and coronary flow can significantly impact the efficacy of both antegrade and retrograde flow and confound any analysis. Clinical studies have not demonstrated the superiority of one type of cardioplegia in routine cases but have suggested patients with more advanced left ventricular

dysfunction fare better with blood cardioplegia especially given both antegrade and retrograde.

Studies suggest that in low-risk groups, multidose cold crystalloid, or cold or warm blood cardioplegia, whether given antegrade and/or retrograde, produce relatively comparable clinical results. In general, protection of the right ventricle is suboptimal with all strategies, especially in patients with right coronary artery disease. In high-risk cases a combination of ACP and RCP cold or tepid blood cardioplegia may offer the best results [51].

#### *4.4 Blood vs. crystalloid cardioplegia*

A meta-analysis of 34 RCTs (18/34 undergoing elective CABG surgery) found superior myocardial protection provided by blood cardioplegia, deduced from lower low output syndrome rates and CKMB release after surgery at 24hours, when compared to crystalloid [56]. Blood cardioplegia provides a closer approximation to normal physiology and this most likely translates into the measurable clinical benefits and advantage.

A survey of UK practice has found 56% of surgeons use cold blood cardioplegia, 14% use warm blood cardioplegia, 14% use crystalloid cardioplegia, 21% use retrograde infusion and 16% do not use any cardioplegia [57].

Although blood cardioplegia has been shown to be 'superior' to crystalloid this advantage is marginal and might explain the continuous use of crystalloid cardioplegia by some surgeons [58].

#### *4.5 Directed cardioplegia*

To eliminate the steal of cardioplegia, during nonselective antegrade cardioplegia, from severely stenosed vessels by other less diseased arteries 'direct cardioplegia' is a novel approach that is being developed. To protect poorly perfused myocardial regions the surgeon occludes the other main branches and directs a certain volume of cardioplegic solution into the severely diseased coronary artery. Early results in severe left main coronary artery disease have been promising [59].

### **5.0 Volatile anaesthetics**

#### *5.1 Introduction to volatile anaesthetics*

Volatile anaesthetic agents have been shown to have direct protective properties against ischaemic myocardial injury by a mechanism termed 'anaesthetic preconditioning' [60][61]. The cardioprotective effects occur at therapeutic doses and are independent of anaesthetic and haemodynamic effects [62].

It is suggested that the cardiac depressant effects of volatile anaesthetics decrease myocardial oxygen demand and may thus improve the myocardial oxygen balance during ischemia whilst also directly protecting from ischemic myocardial damage [63].

#### *5.2 Enflurane, Sevoflurane, Desflurane and Isoflurane*

Both Enflurane [64] and Sevoflurane have been shown independently to enhance postischaemic functional recovery in patients undergoing coronary surgery. Sevoflurane decreases postoperative release of brain natriuretic

peptide, the sensitive biochemical marker of myocardial contractile dysfunction [65].

Preischaemic administration of isoflurane also protects against prolonged ischaemia with functional recovery after CPB, increases mean cardiac index and reduces necrosis (cTnl) in patients undergoing coronary artery bypass graft surgery. Experimentally isoflurane has been shown to provide myocardial protection through a signal transduction cascade that is similar to the pathways identified in ischaemic preconditioning [66].

Meta-analysis' have demonstrated that desflurane and sevoflurane reduce postoperative mortality and incidence of myocardial infarction following cardiac surgery with advantages in postoperative cardiac troponin release, need for inotrope support, time on mechanical ventilation, intensive care unit and overall hospital stay [61][62][63]. In support of current evidence The American College of Cardiology/American Heart Association have recommended volatile anaesthetic agents to be used during non-cardiac surgery for the maintenance of general anaesthesia in patients at risk for myocardial infarction [61][62][63].

### *5.3 Volatile vs. non-volatile anaesthesia*

The advantage of volatile anaesthesia, against total intravenous anaesthesia, on cardiac troponin release in off-pump coronary artery bypass grafting (OPCAB) has been found in multicenter randomized controlled studies [67]. This benefit has also been shown to extend to patients undergoing CABG with CPB with reduced cardiac troponin release, reduced need for postoperative inotropic support and trends toward a reduction in number of Q-wave

myocardial infarction, time on mechanical ventilation, intensive care unit and overall hospital stay [68].

A systematic overview and meta-analysis of all randomized trials comparing volatile with non-volatile anaesthesia in CABG surgery identified 27 trials that included 2979 patients. Post-bypass, patients randomized to receive volatile anaesthetics had 20% higher cardiac indices, lower troponin I serum concentrations and lesser requirement for inotropic support. Duration of mechanical ventilation was reduced by 2.7 h and there was a 1 day decrease in hospital length of stay [69].

## **6.0 Ischaemic conditioning**

'Conditioning' of the heart to harness its endogenous cardioprotective capabilities using either brief ischaemia or pharmacological agents provides a potentially novel approach to myocardial protection during cardiac surgery by limiting myocardial injury, preserving left ventricular systolic function and potentially improving morbidity and mortality in patients with CHD [70][71]. The conditioning stimulus can be given locally or distant to the organ and before or after a prolonged period of ischaemia.

Ischemic postconditioning by brief episodes of ischemia performed just at the time of reperfusion has been shown to reduce infarct size in animal models, in the clinical settings of percutaneous coronary intervention and in adult patients undergoing valve replacement [72].

Brief episodes of non-lethal ischaemia and reperfusion to an organ or tissue remote from the heart, thereby obviating the need to 'condition' the heart directly has been termed remote ischaemic conditioning and it is suggested

there is potential for widespread systemic protection to other organs which are susceptible to acute ischaemia-reperfusion injury such as the brain, liver, intestine or kidney [71].

## **7.0 Nicorandil**

This potassium channel activator in intravenous preparation has been shown to have beneficial effects. When administered during CABG surgery with CPB lowers concentrations of cTnT were observed [73] suggesting its potential for myocardial protection.

## **8.0 Statins**

The perioperative use of statin therapy has demonstrated improved short and long-term cardiac outcomes following noncardiac surgery. Its beneficial effects have been attributed to its positive effects on plaque stability (pleiotropic) [74], lipid lowering effects and anti-inflammatory properties [75]. In cardiac surgery, one series found pretreatment with statins to reduce the incidence of death and myocardial infarction following coronary artery bypass grafting surgery [76] supporting its role as a cardioprotective agent.

## **9.0 Aprotinin**

Aprotinin, a serine protease inhibitor, can limit systemic inflammation, and has been associated with myocardial, pulmonary and cerebral protection in addition to its proven haemostatic efficacy [77]. It has antithrombotic, antifibrinolytic, and antiinflammatory effects and is effective in reducing

bleeding and the need for blood transfusions after cardiac surgery with cardiopulmonary bypass [77][78].

Recent controversy has started since a large randomized controlled trial comparing antifibrinolytics in patients undergoing cardiac surgery was stopped after a preliminary analysis suggested a trend toward an increase in all-cause 30-day mortality associated with aprotinin [78]. Further investigation is currently under way.

## **10.0 Conclusions**

The dangers of insufficient myocardial protection during cardiac surgery represents a real worry to surgeons as our population ages and the patient presenting have more severe comorbidities. A number of techniques have been identified to tackle this issue and continue to be tested and refined.

The topic of investigation of this thesis and the consecutive chapters is the applicability of remote ischaemic preconditioning as a novel technique to aid in the armamentarium of the surgical team to reduce myocardial injury and enhance myocardial preservation.

### **Section 3:           The current status of coronary artery bypass graft surgery (CABG)**

#### **1.0    Adult Cardiac Surgical Database Report 2008**

The Society of Cardiothoracic Surgeons of Great Britain and Ireland 6<sup>th</sup> National Adult Cardiac Surgical Database Report (2008) [1] documents the nature of adult cardiac surgical practice in the United Kingdom and Ireland. It provides an invaluable resource for the extraction of data, analysis and comment on the current practice of coronary artery surgery.

##### *1.1    Patient populations treated by CABG*

In the financial year ending 2008 22,846 patients underwent isolated CABG. Analyses of patients' risk profiles show quite marked changes, with surgeons operating on progressively higher-risk patient year-on-year:

A recent review confirms the feelings that a higher-risk population has started to develop. The most current data reports 75% of patients undergoing isolated CABG are overweight & about one-third are obese. The number of elderly patients presenting for surgery is increasing with the over 75s now making up more than 20% of all cardiac surgery and the over 80s making up over 5%. The average age of patients undergoing isolated CABG between 1991 and 2007 has increased from 58 to 66 years.

There are marked changes in the proportion of female patients with increasing age: in patients under the age of 51 years of age 13% of patients are female, in those over the age of 80 it rises to nearly 30%.

The incidence of comorbidities of patients presenting for coronary surgery between 2001 and 2008 has increased: 33% in diabetics and more than 15% in hypertensives. While left main stem (LMS) disease rates appear to have stabilised now, they did increase between 2001 and 2006. Approximately half the patients have had a previous myocardial infarction (MI) and the proportion of patients who have suffered a heart attack within the previous 30 days has gone up from under 19% in 2004 to over 34% in 2008.

An increase to over 8% of patients presenting for CABG have had prior percutaneous coronary intervention (PCI) with the vast majority having undergone PCI during a previous hospital admission. There has also been a small increase in the proportion of patients with preoperative dialysis-dependent renal failure, extra-cardiac arteriopathy and a greater proportion of urgent cases.

There is suggestion that an increase in the longevity of conduit used is being observed. Fewer patients are requiring repeat operations and the time between first- and second-time operations is increasing.

## *1.2 Survival following CABG*

The outcomes for patients undergoing coronary artery bypass surgery as an elective operation are excellent, with low mortality, low morbidity and good medium-term survival. Operative mortality rates for isolated CABG fell from 2.3% in 2001 to 1.5% in 2008. In those patients with no history of previous MI in the year to March 2008 the mortality rate was 0.8% and for those who had 2 or more previous MIs it was 3.6%.

The operative mortality of elective surgical patients under the age of 70 years is less than 1%. Survival appears to be worse for patients who are older, female, undergoing urgent surgery, diabetic, suffering from impaired cardiac function or in renal failure. In terms of improving outcome, surgical procedures have seen a trend towards increased usage of arterial grafts.

Following surgery body habitus plays a role in outcome as the extremes of weight (under and morbidly obese) have prolonged in-hospital stays. Those that are underweight fair poorly with an observed mortality rate of 4.1%. Obese and morbidly obese patients have the same medium-term survival rates as patients of normal weight.

As an indicator of improved care and treatment strategies the operative mortality has continued to fall whilst the proportion of patients over 75 and 80 has continued to increase. The mortality rate for patients over the age of 75 has also fallen from 5.0% in 2004 to 3.4% in 2008. Kaplan-Meier survival rate at 5 years post-operatively for the under 66 year age group is over 90% and in the over 80 group is 69%. Gender has been shown to affect outcome as women have an in-hospital mortality that is nearly twice that of their male counterparts and worse medium-term survival.

The impact of comorbidities on prognosis remains significant. Patients who have had a previous heart attack are twice as likely to die at the time of surgery compared to patients who have not had a heart attack. However, the in-hospital mortality rate of diabetics has decreased in spite of a 50% increase in the diabetic proportion between 2001 and 2008. The mortality for diabetics remains higher than for non-diabetic patients. Diabetics have a longer length-

of-stay and a medium-term survival rate of 85% compared to 90% of non-diabetics.

In the most recent year of study there is no significant increase in operative mortality on inpatients with hypertension. Hypertension does remain associated with longer in-hospital stay and worse medium-term survival. Severe angina remains an important risk factor for operative mortality. Those with LMS disease have significantly higher rates of mortality. LMS disease is associated with a significantly greater length of in-hospital stay and a worse medium-term survival.

Priority is an important predictor of operative mortality with urgent and emergency cases having mortality rates of 2.2% & 8.3% respectively. Positively, in-hospital mortality in this group has fallen in spite of an increase in proportion presenting compared to elective cases. Urgent and emergency patients consistently stay in hospital longer than elective patients.

Patients undergoing isolated CABG within 24 hours of a PCI have an overall mortality rate of 7.9%. Moderate and severe dyspnoea and extra cardiac arteriopathy are associated with an increased operative mortality and the mortality associated with redo surgery is nearly four times that of first-time surgery.

Any degree of renal disease is associated with a marked increase in length-of-stay. Renal disease is a powerful predictor of poor post-operative survival: for patients on dialysis the medium-term survival rate at 5 years after surgery is 50% compared to 90% for those without renal disease.

## **2.0 Revascularisation vs. medical therapy**

Three multicentre randomised controlled trials demonstrated early on the benefit of CABG over medical therapy: Veterans Administration Coronary Artery Bypass Surgery (VA) Study [79], Coronary Artery Surgery Study (CASS) [80] and the European Coronary Surgery Study (ECSS) [81]. Following these studies, there has been impetus to refine understanding and identify the best responders to CABG.

In CASS [80] 780 patients with stable ischaemic heart disease were randomly assigned equally to receive surgical or nonsurgical treatment and quality of life was assessed. At ten year follow up these patients were found to have no difference in cumulative survival, percentage free of death and nonfatal myocardial infarction. Patients with an ejection fraction (EF) <50% exhibited a better survival with initial surgery whilst those with EF ≥50% exhibited a higher proportion free of death and myocardial infarction with initial medical therapy. The 10 year follow up results of CASS confirmed the early reports that patients with left ventricular dysfunction have long-term benefit from surgery [82].

In the European Coronary Surgery Study 768 men under 65 with mild to moderate angina, at least two-vessel disease and good left ventricular function were recruited. CABG was shown to significantly improve survival in patients with three-vessel disease and in those with stenosis in the proximal third of the left anterior descending artery constituting a component of either two or three vessel disease [81][83].

To secure the position of CABG as a beneficial treatment strategy, an overview of seven randomised trials over ten years found those patients with

stable coronary heart disease receiving initial CABG (n=1324) had lower mortality at 5, 7 and 10 years than those receiving initial medical therapy (n=1325) [84]. Risk reduction was greater in those with left main artery disease than in those with three vessel disease or one or two vessels. The absolute benefits of CABG were more pronounced in patients in the highest risk categories.

### **3.0 Revascularisation vs. percutaneous intervention**

#### **3.1 CABG vs. balloon angioplasty**

Coronary-artery bypass grafting (CABG) and percutaneous transluminal coronary angioplasty (PTCA) are alternative methods of revascularization in patients with coronary artery disease. A number of large trials exist comparing the two treatment strategies:

Randomised Intervention Treatment of Angina (RITA) trial compared the long-term effects of PTCA with CABG in patients with one, two, or three diseased coronary arteries and randomised 1011 patients. Although recovery after the more invasive CABG took longer than PTCA it did result in less risk of angina and fewer additional diagnostic and therapeutic interventions in the first 2 years with no significant difference in risk of death or myocardial infarction [85].

The Bypass Angioplasty Revascularisation Investigation (BARI) in patients with multivessel disease found PTCA did not significantly compromise five-year survival in patients with multivessel disease, however once again subsequent revascularization was required more often with this strategy. For treated diabetics five-year survival was significantly better after CABG [86].

The multinational, multicentre Coronary Angioplasty versus Bypass Revascularisation Investigation (CABRI) in 1054 patients with symptomatic multivessel coronary disease found PTCA patients more likely to have reinterventions [87].

In another study the 5-year prognosis of patients with isolated proximal left anterior descending coronary artery stenosis was good and both PTCA and CABG improve clinical status, but revascularization was needed more frequently after PTCA. [88]

To draw together the experiences a meta-analysis of eight randomised trials including 3371 patients comparing CABG (n=1161) with PTCA (n=1710) was performed. Overall there was a substantial similarity in outcome across the trials. The combined evidence showed no difference in prognosis between these two initial revascularisation strategies. However, the treatments differed markedly in the subsequent requirement for additional revascularisation procedures and in the relief of angina [89].

In terms of long-term mortality, pooled patient data (n=7812) from ten randomised trials with PCI achieved with balloon angioplasty in six trials and with bare-metal stents in four trials found it to be similar after CABG and PCI in most patient subgroups with multivessel coronary artery disease [90]. More recently the SYNTAX trial, a noninferiority comparison of 1800 patients with three-vessel or left main coronary artery disease for the primary endpoint of a major adverse cardiac or cerebrovascular event (MACCE) (i.e., death from any cause, stroke, myocardial infarction, or repeat revascularization) during the 12-month period after randomization was performed. It was recommended that CABG remain the standard of care for patients with three-vessel or left

main coronary artery disease, since the use of CABG, as compared with PCI, resulted in lower rates of the combined end point of major adverse cardiac or cerebrovascular events at 1 year [91].

### 3.2 *CABG vs. coronary stenting*

Following the arrival of coronary stenting as a method of coronary revascularisation studies have been performed to evaluate its efficacy as compared to the longer established conventional CABG:

ERACI II found in symptomatic 450 patients with multivessel coronary artery disease randomly assigned to stenting (225) or CABG (225) during the first 30 days, stented patients had lower major adverse events (death, myocardial infarction, repeat revascularization procedures and stroke [92].

ARTS (Arterial Revascularization Therapies Study) trial assessed the relationship between completeness of revascularization and adverse events at one year. In 1,205 randomly assigned patients with multivessel disease, complete revascularization was found to be more frequently accomplished by bypass surgery than by stent implantation. One year after bypass, there was no significant difference in event-free survival between surgically treated patients with complete revascularization and those with incomplete revascularization, but patients randomized to stenting with incomplete revascularization had a greater need for subsequent bypass surgery. [93]

The Stent or Surgery (SoS) trial assessed the effect of stent-assisted percutaneous coronary intervention (PCI) (n=488) versus CABG (n=500) in the management of patients with multivessel disease. The use of coronary stents reduced the need for repeat revascularisation when compared with

previous studies that used balloon angioplasty, though the rate remained significantly higher than in patients managed with CABG [94]. The six-year follow up from the Stent or Surgery Trial (SoS) demonstrated a continued survival advantage for patients managed with CABG [95].

It was felt by some clinicians that the above trials and others individually were underpowered to properly assess safety end points like death, stroke, and myocardial infarction in stented patients. Since pooling data from randomized controlled trials increases the statistical power and allows better assessment of the treatment effect in high-risk subgroups a meta-analysis with 5-year patient level data from the ARTS, ERACI-II, MASS-II and SoS trials was performed [96].

The pooled analysis of 3051 patients in 4 randomized trials evaluating the relative safety and efficacy of PCI with stenting and CABG at 5 years for the treatment of multivessel coronary artery disease found PCI with stenting was associated with a long-term safety profile similar to that of CABG. However, as a result of persistently lower repeat revascularization rates in the CABG patients, overall major adverse cardiac and cerebrovascular event rates were significantly lower in the CABG group at 5 years [97].

Stents themselves have evolved since their initial application with the arrival of drug-eluting stents to add to the armamentarium, along with bare metal stents, of the Cardiologist.

A randomized, double-blind trial comparing a sirolimus-eluting stent with a standard stent in 1058 patients found it to reduce the rates of restenosis and associated clinical events in all subgroups analyzed [97]. A further study has found that Sirolimus eluting stents (SES) are not inferior to CABG or bare

metal stents for the treatment of patients with multivessel coronary disease including involvement of the proximal LAD [98].

Reports continue to be promising with drug-eluting stents. Paclitaxel when compared to bare metal stents in a patient population with complex lesions reduces clinical and angiographic restenosis [99].

A significant body of longer term data comparing revascularisation techniques is awaited. However some data is becoming available. In a series of patients undergoing revascularisation between 2000 and 2007 (n=363) for unprotected left main stem coronary artery stenosis, CABG (n=216), PCI with drug eluting stents (n=94) and PCI with bare-metal stent (n=53) were compared. It was found that CABG patients had higher target lesion revascularisation (TLR)-free survival and revascularisation-free survival than rival PCI groups. Cardiac death did not significantly differ between the 3 groups [100].

More long term data on the safety and outcomes of these techniques is still required and will certainly mark a turning point in the future of coronary revascularisation therapies on offer to specific patient populations.

## **CHAPTER 2: ISCHAEMIC PRECONDITIONING (IP) IN CARDIAC SURGERY: A SYSTEMATIC REVIEW OF EXPERIMENTAL AND CLINICAL LITERATURE**

### **1.0 Introduction**

#### *1.1 Local (or 'Cardiac') Ischaemic Preconditioning*

In 1986 Reimer et al found that ATP levels in dog hearts subjected to serial ischaemia were higher than in those exposed to a single ischaemic event. This finding was the first description of ischaemic preconditioning (IP) [101]. This initial breakthrough by Reimer was confirmed by the finding by Murray et al in the same year that paradoxically intermittent ischaemia may not have an additive effect but rather a protective affect against subsequent ischaemia. [102]

Murray et al described that myocardial ATP concentrations, on canine hearts, fell during the first brief episode of ischaemia. These concentrations were then preserved during further identical coronary artery occlusions and no necrosis occurred. They demonstrated that pre-treatment with repeated periods of ischaemia triggered adaptive changes that protected the myocardium from the effects of subsequent prolonged ischaemic insult "ischaemic preconditioning (IP)" [102].

Murray et al established that 4 cycles of 5 minutes coronary occlusion and 5 minutes of reperfusion were sufficient to precondition the dog heart. They reported that the infarct size produced by 40 minutes of sustained coronary artery occlusion in anaesthetized open-chest dogs averaged approximately 30% of the myocardium at risk. In contrast, in dogs that received 4 cycles of 5

minutes episodes of brief ischaemia immediately before the sustained test occlusion only 7% of the myocardium at risk became necrotic. Thus, despite a longer cumulative duration of coronary occlusion, infarct size in preconditioned dogs averaged only one fourth of that seen in controls. Reduction of infarct size is now considered the “gold standard” of IP.

Yellon et al were the first to report that IP protects the human heart during cardiac surgery by demonstrating conservation of myocardial adenosine triphosphate (ATP) content in biopsy specimens. [103] They showed that 2 cycles of 3 minutes ischaemia with 2 minutes reperfusion was effective during CABG surgery in preconditioning the human myocardium. They found that, as in the experimental canine model, myocardial ATP stores after 10 minutes of sustained ischaemia, in this case induced by aortic cross clamping and fibrillation during coronary artery bypass surgery, were significantly preserved in patients who first received two 3 minute episodes of intermittent crossclamping when compared with controls who received the sustained ischaemia alone. Subsequent studies by these investigators further documented a reduction in the release of cardiac troponin T (an index of cellular injury) in patients initially preconditioned before undergoing bypass. [104]

The majority of surgeons today utilise cardioplegic arrest for cardioprotection. The first study during cardiac surgery with cardioplegia was performed with warm blood cardioplegia and the investigators found that preconditioning increased the early release of creatine kinase MB and the transmyocardial lactate gradient shifted toward production, this would suggest that preconditioning may increase the injury. This study initiated several studies,

some of which found a positive effect of preconditioning whereas others either found no effect or even a worsened postoperative outcome indicated by increased inotropic support. [105]

Perrault et al reported that in the presence of cardioplegic arrest, there was no difference in the release of biochemical markers (CK-MB) between preconditioned and control groups. This lack of additional protection conferred by IP was further confirmed by the absence of difference in the post-arrest myocardial levels of ribonucleic messengers coding for cardioprotective heat shock proteins between the 2 groups. Similar negative results were reported by Kaukoranta, DiSalvo et al, who went on to report that in the presence of hypothermia, no beneficial effect of preconditioning was observed in similar patients undergoing surgery with intermittent fibrillation techniques. [106]

### *1.2 Remote Ischaemic Preconditioning (RIPC)*

More than 20 years on since the breakthrough by Reimer et al the effect of intermittent upper limb ischaemia during coronary artery bypass surgery on the human heart has been investigated [107]. This technique has been coined “Remote Ischaemic Preconditioning (RIPC)”.

RIPC was first suggested by Przyklenk et al after demonstrating that brief circumflex artery occlusion preconditioned the area of myocardium perfused by the left anterior descending artery. [108]

Subsequent animal studies have expanded on the principle of RIPC to show that ischaemia of different organs such as the kidney, mesenterium, intestine and brain can also protect the heart. At the same time all organs tested demonstrate the presence of the preconditioning response. Furthermore,

RIPC of the brain appears not only to protect the heart but also preserves endothelial function of aortic rings in vitro. [105][109]

IP is a powerful protective mechanism that appears to have immediate and delayed protective effects, the importance of which varies between species and organ systems. It is a multifactorial process requiring the interaction of numerous signals, second messengers and effector mechanisms. Stimuli other than ischaemia, such as hypoxic perfusion, tachycardia and pharmacological agents, including isoflurane have preconditioning-like effects. Currently IP is used during minimally invasive cardiac surgery without cardiopulmonary bypass to protect the myocardium against ischaemic injury during the anastomosis. [109]

Other situations in which ischaemia occurs naturally or is induced by therapeutic manipulations include unstable angina before infarction, percutaneous transluminal coronary angioplasty and coronary artery bypass surgery with intermittent cross-clamp fibrillation. [110]

The data accruing thus far for RIPC in both the medical and surgical cardiological arenas is promising and at present it appears to be one of the most important potential myocardial protective adjuncts so far identified.[111]

Progress has been made in elucidating the mechanistic pathways underlying 'conditioning' cardioprotection, and have resulted in the identification of novel targets for pharmacological preconditioning.[70] Future clinical applications of IP and RIPC will depend on the clarification of the underlying biochemical mechanisms, the development of pharmacological methods to induce preconditioning, and controlled trials in humans showing improved outcomes.[109]

## **2.0 Mechanisms Involved in Conditioning**

### *2.1.1 Ischaemic Preconditioning*

The underlying mechanism of IP remains to be unravelled in its entirety. Hypotheses have been postulated and relative mimetics have been identified but the clear underlying pathway is still not known.

Once the molecular mechanisms of IP and the conditions that confer optimal protection in the human heart are defined then pharmacological intervention that mimic IP may be developed and used as an adjunct, rather than alternative to cardioplegia.[109]

The first reported corollary of infarct size reduction with preconditioning is the observation by Murray et al that the rate of depletion of adenosine triphosphate (ATP) during the initial minutes of the sustained test occlusion was markedly attenuated in preconditioned dogs versus controls.[102]

IP appears to provide ischaemia protection by a variety of physiological mechanisms. Energy requirements are reduced, conserving substrates and diminishing metabolism. Acid base and electrolyte homeostasis is therefore better controlled. Preconditioned tissues also demonstrate reduced oxidative stress, neutrophil activation and cytokine production and apoptosis.[112]

Prolonged myocardial ischaemia, in addition to inflicting lethal injury on myocytes, also has a deleterious effect on the coronary vasculature, typically manifesting as a loss in both endothelium-dependent and endothelium-independent coronary vasodilator reserve. However, whether the benefits of IP extend beyond the myocyte and protect the vasculature from sustained ischaemia is a topic of some controversy.[113]

More relevant to cardiothoracic surgical practice, Yellon et al showed that IP

had positive effects on reducing ATP utilisation by cardiomyocytes during cardiopulmonary bypass.[103] Patients with angina within 24 hours of myocardial infarction had smaller infarct size. Improved left ventricular function and enhanced survival after reperfusion therapy.[112]

It is suggested that the cardioprotection achieved with preconditioning is a receptor-mediated phenomenon. In the simplest terms, brief antecedent ischaemia is thought to stimulate one or more receptors on the myocyte membranes, thereby initiating one or more signal transduction pathways that culminate in the phosphorylation of an effector protein and ultimately render the myocytes resistant to subsequent sustained ischaemia. However, definitive identification of the receptor, the end effector, and most notably the intermediate signal transduction pathway evoked by brief antecedent ischaemia has proven to be far from simple.[113]

### *2.1.2 Cellular Events*

IP is a powerful mechanism in reducing post-ischaemic myocardial injury. Brief periods of myocardial ischaemia result in the breakdown of ATP and so releasing adenosine. Adenosine receptor occupancy has been implicated to serve as both a trigger and a mediator of infarct size reduction with preconditioning.[113]

A1 receptors are located on myocardial cells and A2 receptors are located on coronary endothelial and leucocyte/platelet membranes. Pretreatment of dog heart with selective A1 receptor agonists prior to an ischaemic event mimics the protection derived from IP, hence A1 receptors play a role in IP. Adenosine infusion prior to CABG surgery has been shown to improve

haemodynamics after cardiopulmonary bypass and decreases CPK release.[105] Adenosine has demonstrated a role in infarct size reduction with preconditioning in the rabbit, dog and swine models, however the rat is the exception, suggesting adenosine receptor activation is not the sole trigger for infarct size reduction with preconditioning.[113]

The mechanisms of preconditioning probably vary between acute and delayed models and between local versus remote.[105] The initial phase of preconditioning may be similar between classic and delayed models and may start by release of a trigger substance during the brief episodes of ischaemia and reperfusion that may bind to surface receptors coupled to G<sub>1</sub> proteins.

Preconditioning–induced cardioprotection can be initiated by more than one receptor. Adenosine,  $\alpha_1$ adrenergic receptors, muscarinic M<sub>2</sub>, angiotensin II, bradykinin B<sub>2</sub>,  $\sigma$ -opioid receptors have been shown to play a contributory or additive role, or in some cases redundant roles. The common theme is that all receptors implicated are coupled to pertussis toxin-sensitive inhibitory G proteins. It appears that the stimulation of G<sub>1</sub> protein-coupled receptors during brief antecedent ischaemia serves as a trigger for preconditioning-induced cardioprotection.[113] Other trigger substances have been identified as prostanoids, nitric oxide and low doses of reactive oxygen intermediates and natriuretic peptides. Tumour necrosis factor alpha has been indicated as a trigger of the preconditioning response.[105][113]

The intracellular message in IP is generated through coupling of agonists of G-proteins that span the cell membrane. Adenosine, norepinephrine and bradykinin receptors are coupled to G proteins which when stimulated increase activity of phospholipase C and D. These enzymes catalyse

hydrolysis of membrane phospholipids, increasing diacylglycerol and inositol triphosphate levels.

Diacylglycerol, a second messenger, activates protein kinase C (PKC), an enzyme believed to play a key role in the biochemistry of IP through its phosphorylation of effector proteins.[113]

PKC is a hotly debated second messenger implicated to play a role in preconditioning. Brief episodes of myocardial ischaemia also result in transient increase in cytosolic calcium concentration and calcium is known to participate as a second messenger in many signalling pathways.[113]

Tyrosine kinases as well as mitogen activated protein kinases appear involved in the signalling cascade in several species, although which kinase cascade is upstream or downstream is currently an issue of debate.[105]

The next step in the signalling pathway is activation of transcription factors by protein kinases, reactive oxygen species, and nitric oxide, of which particularly nuclear factor  $\kappa$ -B (NF $\kappa$ B) has been investigated in both classic and delayed models. NF $\kappa$ B is a redox sensitive transcription factor that regulates transcription of battery of genes most of which are associated with proinflammatory effects such as cytokines, chemokines, and leucocyte adhesion molecules. Some candidate genes for organ protection in preconditioning are also regulated by NF $\kappa$ B. Pharmacologic blocking of NF $\kappa$ B translocation inhibits preconditioning in both classic and delayed models.[105]

The importance of transcription factors is easy to grasp in models of delayed preconditioning in which the time frame for translation of protective substances is ample. However, the fact that transcription factors appear important for protection in classic models is less easy to grasp. The

cardioprotective effect of NF $\kappa$ B in immediate models may be due to an upregulation of its own inhibitor I $\kappa$ B $\alpha$  thus contributing to reducing a NF $\kappa$ B-dependent inflammatory response during sustained ischaemia. However, abolishing classic preconditioning effects through employing the transcriptional inhibitor actinomycin D indicates that the process is complex. Other endogenous cardioprotective substances that are suggested as upregulated and protective are heat shock proteins of the 70kDA or 27kDA families. These have their own regulatory factors but do influence the activation of NF $\kappa$ B. Antioxidants, inducible nitric oxide synthase and inducible cyclooxygenase, all regulated by NF $\kappa$ B, have furthermore been indicated as organ effectors of protection. A last possible route of NF $\kappa$ B mediated cardioprotection is through the antiapoptotic effect of preconditioning. NF $\kappa$ B regulates several antiapoptotic molecules, such as Bcl-2, surviving, and inhibitor of apoptosis protein-1, as well as X-linked inhibitor of apoptosis protein-1.[105]

In 1992 Gross et al proposed that opening of K<sub>ATP</sub> channel may be involved in the reduction in infarct size seen in preconditioned myocardium. They found the selective K<sub>ATP</sub> channel antagonist glibenclamide in dogs either before, or immediately after, the brief preconditioning stimulus gave infarct sizes greater than those observed in preconditioned dogs. They also found that K<sub>ATP</sub> agonists including bimakalim and aprikalim mimicked, the reduction in infarct size achieved by preconditioning and demonstrated a connection or synergy between adenosine receptor activation, opening of K<sub>ATP</sub> channels, and cardioprotection.[113][114]

Opening of the K<sub>ATP</sub> channel is initiated by low intracellular concentrations of

ATP (i.e. as seen during ischaemia). The resultant efflux of potassium from the myocytes into the interstitium has thus been suggested to protect the cells via local “cardioplegia”. Opening of the  $K_{ATP}$  channel also results in membrane hyperpolarisation and shortening of the action potential duration. These electrical responses have further been associated with a reduction in calcium influx via voltage-sensitive calcium channels, and thus may attenuate myocyte death by attenuating calcium overload.  $K_{ATP}$  channels thus do not mediate infarct size reduction with preconditioning solely via more rapid or pronounced shortening of the action potential.[113] Activation of  $K_{ATP}$  channel causes release of reactive oxygen species and one may speculate that their role in preconditioning is linked to this.[105]

There is only general agreement that G protein-coupled receptors initiate and  $K_{ATP}$  channels mediate infarct size reduction with preconditioning. The sequence of cellular events occurring between receptor activation and eventual cardioprotection are subject of considerable controversy.[113]

In anaesthetized rabbits, transient systemic ischaemia, followed by HD with D-HES, can enhance early cardiac functional recovery following coronary artery occlusion and reperfusion, functioning as a powerful mechanism of IP. Transient systemic ischaemia is associated with increased endogenous nitric oxide (NO) production or release during reperfusion, which may have contributed to the improved early myocardial functional recovery in the rabbits.[115]

In summary, stimulation of G protein-coupled receptors (most notably the adenosine A1 and A3 receptors) trigger or initiate the adaptation to later ischaemia and  $K_{ATP}$  channels appear to play role in “end effector” (Figure

2.1).[113]

### 2.1.3 cAMP

Reduction of cAMP has been implicated in the protection of IP against myocardial necrosis. Evidence for this is even in the absence of ischaemia, elevations in cAMP have been shown to produce myocardial necrosis. High levels of cAMP increase sarcolemmal calcium entry and increase the activation of cardiac lipases, effects that are known to aggravate ischaemic injury.

In a rabbit heart model, 10 to 30 minutes of sustained regional ischaemia is accompanied by a nearly twofold increase in cAMP levels. IP induced in this model with a single cycle of transient ischaemia and reperfusion (IR) prior to sustained ischaemia, caused the increase in cAMP to be reduced. Furthermore three cycles of IR preceding the sustained ischaemia prevented the increase in cAMP.[116]

How IP prevents the ischaemia-induced elevation in cAMP levels remains unclear. It has been suggested that the mechanism of cAMP reduction by IP does not involve activation of protein kinase C (PKC) or reduced responsiveness of the  $\beta$ -adrenergic effector pathway. It is suggested that the lack of elevation in cAMP levels observed during sustained ischaemia in IP hearts is mediated by an attenuation of norepinephrine release.

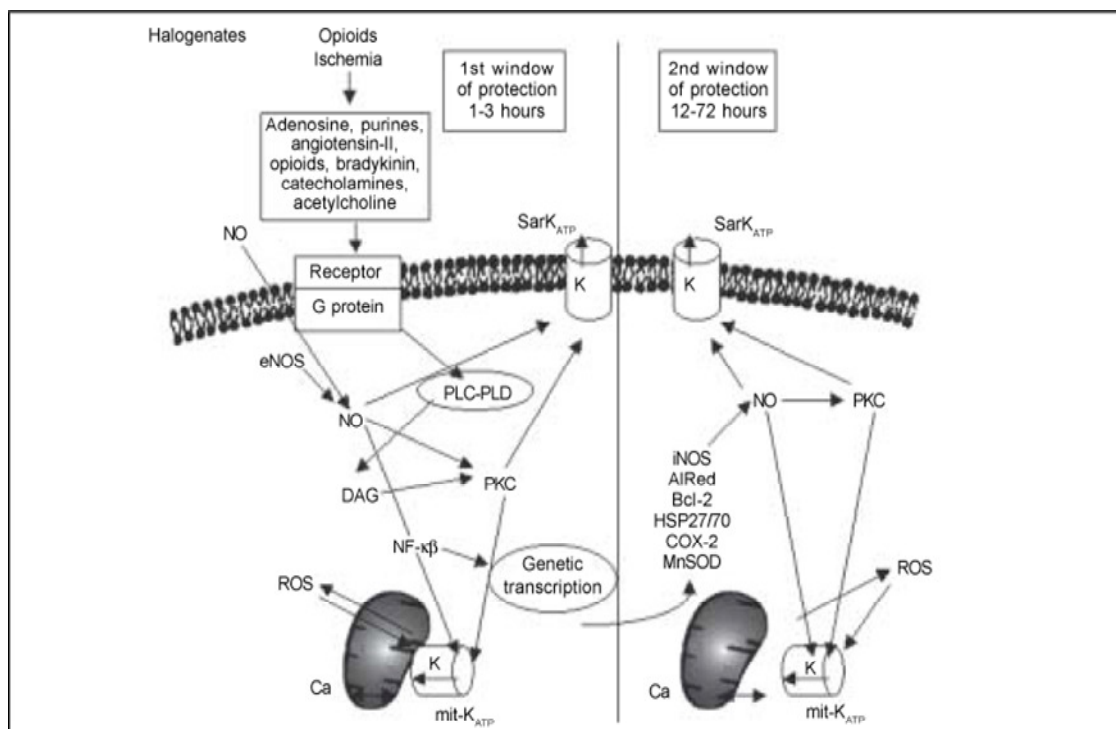
Activation of adenylyl cyclase with NKH477 increases cAMP levels during sustained ischaemia but does not increase necrosis in control hearts or block the protection of IP against necrosis. It has been shown that the administration of either adenosine A<sub>1</sub> receptor agonists or muscarinic M<sub>2</sub>

receptor agonists can mimic the positive effect of IP. In cardiac myocytes, both these receptors are coupled to adenylyl cyclase through  $\beta$ -adrenergic  $G_1$  proteins and stimulation of these receptors inhibit cAMP formation.[116]

Evidence has emerged that activation of PKC is the final effector pathway responsible for the protection of IP against necrosis and postischaemic dysfunction. Activation of PKC is also known to produce several effects on the cAMP-generating and degrading pathways. However, the net effect of PKC activation on myocardial cAMP levels is difficult to predict from available studies, because one of these effects would be expected to decrease cAMP (e.g. uncoupling of adenylyl cyclase-coupled receptors from  $G_s$  protein), whereas others would be expected to increase cAMP (e.g. ablation of  $G_1$  function and direct sensitisation of adenylyl cyclase).[116]

IP has also been shown to protect against arrhythmias in several species, including the conscious rabbit. High levels of cAMP are casually linked to the occurrence of arrhythmias and reducing cAMP levels with drugs such as  $\beta$ -adrenergic receptor blockers have antiarrhythmic effects. Elevating cAMP levels with NKH477 have been shown to significantly increase the occurrence of fibrillation in both control and IP groups, thereby suggesting that high levels of cAMP are proarrhythmic.[116]

**Figure 2.1: Ischaemic preconditioning cascade. The first window of protection is shown on the left and the second window of protection on the right. ARed = aldose reductase; Bcl-2 = antiapoptotic protein; DAG = diacylglycerol; COX-2 = cyclooxygenase 2; endothelial nitric oxide synthase; G-protein = protein that contains cell membrane receptors; HSP27 and HSP70 = heat shock proteins; iNOS = inducible nitric oxide synthase;  $K_{ATP}$  Mito and  $K_{ATP}$  SARC = ATP-dependent potassium channels in the mitochondria and sarcolemma respectively; MnSOD = manganese superoxide dismutase;  $NF_{\kappa\beta}$  = nuclear factor  $\kappa\beta$ ; NO = nitric oxide; PKC = protein kinase C; PLC/PLD = phospholipases C and D; ROS = reactive oxygen species [334].**



## 2.2 *Remote Ischaemic Preconditioning*

RIPC appears to involve adenosine, bradykinin, nitric oxide, protein kinase C, and inducible nitric oxide synthase. The apparent general effect of IP may be mediated by humoral or neurogenic factors or through substances in blood released from the preconditioned organ.[105]

Previous studies in animals have indicated that remote preconditioning seems to involve release of adenosine, bradykinin or norepinephrine and activation of K<sub>ATP</sub> channels and bears mechanistic resemblance to local preconditioning. In addition to humoral factors, remote preconditioning may also involve the autonomic nervous system or modulate the functions of circulating cells such as platelets.[117]

There is evidence that endothelial dysfunction and reduced NO bioavailability are important components of IR injury, possibly limiting the extent of reperfusion after ischaemia and amplifying IR injury. RIPC might therefore reduce tissue injury by limiting endothelial damage during ischaemia reperfusion. Ventricular function during ischaemia has been seen to improve in preconditioned animals, suggesting a direct effect on the myocardium, possibly modifying the metabolic response to ischaemia. Whether endothelial preconditioning contributes directly to the reduction in myocardial IR injury remains to be determined, but results suggest that RIPC prevents tissue injury in animal and human models.[117]

Data from a randomized controlled trial investigating the effects of RIPC on children undergoing cardiac surgery were consistent with an acute modification of inflammatory pathways. No significant difference in the mean level of IL-6, IL-8, IL-10 or TNF-alpha was found but there were significant

differences in variance of levels of IL-10 and TNF-alpha. High levels of TNF-alpha are known to have a deleterious effect e.g. myocardial depression, capillary leak and pulmonary dysfunction.[118]

In a human forearm model, RIPC has been shown to reduce neutrophil activation and endothelial dysfunction. Neutrophil activation leads to release of proteases and oxygen radical species that impair mechanical properties of the lung.[118]

In a wild-type mouse hind limb model, RIPC protected left ventricular function and reduced infarct size during reperfusion. It was concluded that delayed cardioprotection induced by hind limb preconditioning involved signalling through nuclear factor kappa-B (NF- $\kappa$ B) and inducible nitric oxide synthase (iNOS).[119]

Although the mechanism of both IP and RIPC has begun to be unravelled there still remains much that is not clear or well-defined. This represents an area that would benefit from large scale studies.

### *2.3 Mitochondrial Transition Pore Opening*

Mitochondrial permeability transition (mPT) is a key event in cell death after ischaemia-reperfusion. Opening of the non-specific mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane results in the collapse of the membrane potential, uncoupling of the respiratory chain, and efflux of cytochrome c and other proapoptotic factors that may lead to either apoptosis or necrosis. Ca<sup>2+</sup> overload and excessive production of reactive oxygen species (ROS) in the early minutes of reflow trigger opening of the mPTP and are crucial events in reperfusion injury.[120]

A study on anaesthetised open-chest rabbits who underwent ischaemia-reperfusion and control hearts that underwent no additional intervention found that postconditioning, preconditioning, and NIM811 (mPTP inhibitor) significantly limit infarct size.[120]

The  $\text{Ca}^{2+}$  load required to open the mPTP in postconditioning, preconditioning and NIM811 was significantly higher than in controls and the study concluded that postconditioning inhibits opening of the mPTP and provides a powerful anti-ischemic protection. The data strongly suggested that mPT is an important mediator of cardioprotection.[120]

In an adult rat myocyte model, known inhibitors of mPT (cyclosporine A and N-methyl-4-valine-cyclosporin A) demonstrated an increase in time taken to induce the mPT compared with control groups. Hypoxic preconditioning (HP) and pharmacological preconditioning, using diazoxide or nicorandil also increased the time taken to induce mPT compared with control groups. The effects of HP, diazoxide and nicorandil were shown to be abolished in the presence of mitochondrial ATP-sensitive  $\text{K}^+$  ( $\text{K}_{\text{ATP}}$ ) channel blockers glibenclamide and 5-hydroxydecanoate but were maintained in the presence of the sarcolemmal  $\text{K}_{\text{ATP}}$  channel blocker HMR-1098.[121]

This evidence suggested that preconditioning protected the myocardium by reducing the probability of the mPT, which normally occurs during ischaemia-reperfusion in response to oxidative stress.[121]

Further studies on isolated rat hearts have promoted the theory that IP and mitochondrial  $\text{K}_{\text{ATP}}$  channel activation may protect the myocardium by inhibiting mPTP opening at reperfusion.[122]

An isolated rat heart study concluded diazoxide (pharmacological

preconditioning) and mitochondrial uncoupling requires transient mPTP opening also and ROS to mediate protection.[123]

Furthermore, in open-chest beagle dogs, mitochondrial and sarcolemmal  $K_{ATP}$  channels have been shown to independently play an important role in the limitation of infarct size in the heart by IP.[124]

However, in one study on male Wistar rat hearts it was found that mPTP opening in mitochondria occurred more readily than control mitochondria, implying that mPTP inhibition by IP in situ was secondary to factors such as decreased calcium overload and oxidative stress. In addition, the hearts perfused with inhibitors of mPTP, also recovered better from ischaemia than controls. These agents were less effective than IP at preventing mPTP opening. The data suggested that protection from reperfusion injury was better achieved by reducing factors that induce mPTP opening rather than by inhibiting mPTP directly.[125]

The evidence seems to indicate that the inner mitochondrial membrane ATP-sensitive  $K^+$  channels (mito $K_{ATP}$ ) appear to be activated during preconditioning and the mitochondrial and cellular consequences of this activation promote ischaemic protection. The effects seen include decreased loss of tissue ATP through reverse activity of ATP synthase due to increased mitochondrial matrix volumes and lower transport of adenine nucleotides into the matrix.[126]

Mito $K_{ATP}$  also decreases the release of mitochondrial ROS by promoting mild uncoupling in concert with  $K^+/H^+$  exchange. Finally, mito $K_{ATP}$  activity may inhibit mitochondrial  $Ca^{2+}$  uptake during ischaemia, which, together with decreased reactive oxygen release, can prevent mPT, loss of organelle

function, and loss of physical integrity.[126]

## *2.4 Postconditioning*

Brief episodes of myocardial ischemia-reperfusion employed during reperfusion after a prolonged ischemic insult may attenuate the total ischemia-reperfusion injury. This phenomenon has been termed ischemic postconditioning.

In humans, preservation of flow mediated dilatation by postconditioning is consistent with increased nitric oxide availability during reperfusion, either due to decreased nitric oxide inactivation and/or increased nitric oxide synthesis. In one study IR-induced endothelial injury resolved spontaneously within 60 minutes of reperfusion, consistent with 'endothelial stunning' rather than necrosis in response to 20 minutes of ischaemia.[127]

Postconditioning was initially proposed to act by reducing neutrophil-mediated damage in postischaemic myocardium but this cannot be the sole mechanism of action, because it is protective in isolated perfused hearts and cell culture systems that are neutrophil free. There may be effects to reduce reactive oxygen species production and calcium overload. Moreover evidence indicates that protection may be dependent on adenosine receptor stimulation, opening of mitochondrial  $K_{ATP}$  channels, activation of the prosurvival kinases PI3K-Akt and MEK $^{1/2}$ -Erk $^{1/2}$ , and inhibition of mitochondrial permeability transition pore opening, key factors implicated in ischaemic preconditioning-induced protection. These observations suggest that postconditioning and IP have common signalling pathways, which may explain why these protective phenomena are equally effective in protecting against IR injury.[127]

## 2.5 *Cardiopulmonary bypass*

Cardiopulmonary bypass (CPB) is known to induce a systemic inflammatory reaction that is believed to be responsible for increased morbidity. More recent studies have demonstrated that CPB can also trigger preconditioning and be cardioprotective.

CPB has been shown to trigger an activation of the kinase cascade that is mechanistically linked to opening of potassium channels.[128] It has been shown to stimulate alpha adrenergic and adenosine-1 receptors. It is implicated in a general inflammatory response involving generation of reactive oxygen species and increase of cytokines such as tumour necrosis factor alpha that may also be triggers of the preconditioning response.[105]

The generation of free radical species occurs soon after the institution of CPB and so free radicals have been implicated as the primary cause of cardioprotection. The administration of oxygen free radicals scavengers during the first reperfusion period has been shown to block the beneficial effect of preconditioning on infarct size in dogs. The generation of low amounts of free radicals during the short ischaemic episode is not sufficient to cause cell necrosis but enough to modify cellular activity and induce preconditioning.[129]

The opening of mitochondrial  $K_{ATP}$  channels triggers protection through the generation of free radicals that activate protein kinase C, an obligatory step in the signal transduction mechanism of IP.[130]

Interleukin-1 and tumour necrosis factor- $\alpha$ , the production of which is increased by CPB, cause an elevation in tissue manganese-superoxide dismutase, which was demonstrated when brief sublethal ischaemia or anoxic

insults were induced. However, this thesis is unlikely, because the production of cytokines is a late event in response to CPB that requires more than 10minutes.[131][132]

The induction of CPB affects the body haemodynamics that may provoke a number of tissue responses. The loss of atrial and ventricular filling may stimulate a sympathetic-receptor-mediated release of local catecholamines, whereas the interruption of pulsatile systolic and diastolic blood flow to the adrenal glands may stimulate a systemic catecholamine release. Therefore, an altered adrenergic state may also be partially responsible for CPB-associated preconditioning in human myocardium. Several investigators have observed that norepinephrine or phenylephrine triggers preconditioning and that this protection is prevented by adrenergic blockade.[133]

Certainly, more studies are required to elucidate the mechanism of cardioprotection effected by CPB.

## *2.6 Neural Factors*

One of the most intriguing questions regarding RIPC is how the transfer of the protective signal from the site of preconditioning to remote tissue occurs. There is evidence for humoral mediators that may include endogenous opioids. In addition, a neurogenic pathway has also been suggested, with evidence for involvement of the autonomic nervous system in the mechanism of the early phase and sensory C-fibres in the late phase.[134]

One study, infusing the N<sub>N</sub>-cholinergic antagonist trimetaphan camsylate, demonstrated in humans in vivo that RIPC prevents endothelial injury in conduit vessels with two temporally distinct phases of protection. An early

(short) phase is activated immediately and disappears within 4h; a second late (prolonged) phase presents 24h after the application of the RIPC stimulus and is sustained for at least 48h. Both phases of RIPC are dependent on an intact autonomic function.[134]

Time-control studies confirmed that trimetaphan, an autonomic ganglion blocker, had no direct effect on FMD, or the endothelial response to IR injury. However, when administered during RIPC, it blocked early and late protective effects on endothelial IR injury. It is possible that release of local triggers of IP (including bradykinin and adenosine) activate the autonomic nervous system either directly or via sensory nerves, and transfers the signal to the myocardium or other remote tissues. The present study data does not indicate which component of autonomic function (muscarinic or adrenergic) is involved.[134]

One potential limitation of this study was the lack of specificity of trimetaphan which also has alpha-adrenoceptor blocking properties and induces the release of histamine. Moreover, trimetaphan has direct vasodilator actions, although the mechanisms of this effect are not currently known. Although these additional actions are unlikely to account for these effects described in the study, unknown effects of the drug that alter the response of the vascular endothelium to RIPC cannot be excluded.[134]

In a further study to examine if brief anterior mesenteric artery occlusion (MAO) protects against myocardial infarction, involvement of a neurogenic pathway was suggested. The effect of ganglion blockade on the protection by MAO as well as CAO was examined. Ganglion blockade abolished the protection by 15-minute MAO during both normothermia and hypothermia but

had no effect on infarct size produced by 60-minute CAO and protection by IP at either temperature.[135] In addition, permanent MAO failed to limit myocardial infarct size produced by 60-minute CAO indicating that reperfusion of the small intestine was mandatory to activate the neurogenic pathway.

These results demonstrated the involvement of a neurogenic pathway in the protection by 15-minute MAO, indicating that protection by remote organ ischaemia may be different from that by IP. Because sustained MAO failed to produce cardioprotection it was suggested that the activation of the neurogenic pathway occurred during reperfusion after 15-minute MAO. The data could be interpreted to suggest that at reperfusion, substances released in the mesenteric bed (e.g. oxygen-derived free radicals, cytokines) stimulate afferent neurofibers.

A study on male Wister rats treated with an HOE-140 (bradykinin B<sub>2</sub> receptor antagonist) demonstrated an important role for bradykinin in RIPC by MAO. Results supported the hypothesis that RIPC acts through sensory nerve stimulation in the ischaemic organ.[136]

Further studies to support a neurogenic pathway include a study in rats preconditioned with a 15-min MAO that reduced infarct size produced by a 60-min CAO. Pretreatment with a ganglion blocker abolished the protection by MAO15 and the cardioprotection of an intramesenteric artery infusion of adenosine. The findings demonstrated that locally released adenosine during small intestinal ischaemia stimulates afferent nerves in the mesenteric bed during early reperfusion, initiating a neurogenic pathway that leads to activation of myocardial adenosine receptors.[137]

A study on rats found that RIPC significantly reduced infarct size which was

completely blocked by a PKC inhibitor. The study concluded that RIPC activates myocardial PKC $\epsilon$ , an essential mediator of classical IP, through a neuronal and bradykinin-dependent pathway. It was assumed that PKC $\epsilon$  activation is an important step in cardioprotection induced by remote preconditioning. [138]

## *2.7 Humoral Factors*

The combined body of knowledge to date suggests that either a neuronal or humoral trigger signal is released from distant ischaemic tissue, in effect preparing non preconditioned myocardium of the potential ischaemic insult to come. Isolation of the trigger signal responsible for preconditioning at a distance has obvious therapeutic potential as a stimulant of the patients own protective mechanisms. If the trigger signal responsible for preconditioning at a distance is humoral in nature then it should be transferable from one animal to another of similar species.

Humoral and neuronal mechanisms are not necessarily mutually exclusive. One can envision a combined neuronal/humoral signal such that a humoral signal released by ischaemic tissue is detected by the CNS, leading to protective neuronal output.

A study on rabbits paired by crossmatching and placed into preconditioned (P) or control sets and then further divided into preconditioned donor (PD) and preconditioned acceptor (PA) animals underwent a whole blood exchange between PD and PA animals before and after preconditioning [139]. The control rabbits underwent the same surgical procedures and time-sequenced transfusion without preconditioning. All animals then underwent prolonged

circumflex occlusion (60minutes) followed by reperfusion (30minutes). The percent infarct within the risk area was significantly lower in the PA and PD groups as compared with controls. There was no significant difference between the PA and PD groups. This study demonstrated the IP effect can be transferred to non-preconditioned animals via whole blood transfusion, suggesting a humoral mechanism for preconditioning at a distance.

### **3.0 Clinical Benefits of Preconditioning**

The demographics of patients presenting for cardiac surgery is progressively shifting towards increased risk and so a need for enhanced myocardial protection has arisen. Due to the potential complications of repeated aortic cross clamp application the inclusion of classical IP in the armamentarium of surgical myocardial protection has been frustrated. The possibility of a method of overcoming the hazards yet still harnessing the potential benefit of modifying ischaemia-reperfusion injury in the form of RIPC is warmly welcomed. It is advertised as a cheap, effective, non-invasive, practical method with the ability to improve outcome and recovery with health resource incentive and multiple applications.

#### *3.1 Clinical benefits of Ischaemic Preconditioning*

The evidence for utilising IP as a myocardial protective regimen in the clinical cardiothoracic setting began in 1993 when Yellon et al investigated whether it was possible to precondition the human heart in coronary artery bypass surgery [103]. 14 patients undergoing CABG were randomised equally into two groups; IP and control. After establishing cardiopulmonary bypass (CPB),

those in the preconditioning group underwent two 3-min periods of crossclamping interspersed with 2 min of reperfusion. A slowing of the rate of adenosine triphosphate (ATP) depletion in biopsy specimens of those patients who had prior controlled IP was observed demonstrating that it may be possible to precondition and protect the human myocardium with short controlled periods of intermittent ischaemia and reperfusion.

Further evidence for the myocardial protective properties of IP in humans was provided by a RCT into 33 patients with three vessel coronary artery disease and stable angina undergoing elective coronary artery bypass surgery [104]. After instituting CPB the IP group received a stimulus of two three-minute periods of myocardial ischaemia, by cross clamping the aorta, separated by two minutes of reperfusion. This was performed before the ischaemic period for the first coronary artery bypass graft distal anastomosis. All patients had an undetectable serum troponin T ( $<0.1$  microgram/l) before CPB which was raised postoperatively. At 72 hours the serum troponin T release was lower ( $P=0.05$ ) in the preconditioned group than in controls (median 0.3 vs. 1.4 micrograms.L<sup>-1</sup>). The direct application of a preconditioning stimulus in clinical practice had been shown to protect patients against irreversible myocyte injury.

The purported benefits of IP do not appear limited to coronary artery bypass surgery alone. In 1997 Lu et al published their study investigating the effects of preconditioning on myocardial ischemia-reperfusion injury in thirty patients with rheumatic heart disease requiring both aortic and mitral valve replacement undergoing prolonged cold crystalloid cardioplegic arrest [140]. Patients were randomly divided into two equal groups; preconditioned and

control. Preconditioning was achieved using two cycles of 2-minute occlusion of the vena cava and aorta followed by 3 minutes of reperfusion under CPB. All hearts were arrested with 4°C St. Thomas' Hospital cardioplegic solution. IP protected the human ischaemic myocardium by maintaining ATP content 90 minutes after ischemia ( $p < 0.05$ ), reducing the release of the myocardial-specific isoenzyme of creatine kinase, improving markers of myocardial contractility: first derivative of left ventricular developed pressure 30 minutes after reperfusion ( $1490 \pm 102$  vs.  $1250 \pm 97$  mmHg.s<sup>-1</sup>;  $p < 0.05$ ), and reducing the incidence of ventricular fibrillation, electric defibrillation time and elevation of ST segments.

More evidence for the benefit of IP during valve surgery was published in 1999 by Li et al. Forty patients requiring double valve replacement were randomised into two equal groups after the institution of CPB; IP and control. The IP stimulus was delivered as two cycles of 3 min ischaemia by occlusion of the vena cava and aortic crossclamping (effective left ventricular decompression by intracardiac drainage) followed by 2 min of reperfusion (removing all the occlusions) under CPB [141]. Hearts were arrested with 4°C cold-blood cardioplegia and sampling from the coronary sinus and radial artery measured calcitonin gene-related peptide (CGRP) and creatine kinase-MB (CK-MB) levels. Right atrial myocardial tissue was collected to measure superoxide dismutase/malondialdehyde (T-SOD/MDA) and to observe myocardial ultrastructure.

IP was observed to enhance cardioplegic protection by decreasing the production of oxygen free radicals. At 30 minutes after reperfusion IP reduced myocardial MDA formation ( $2.6 \pm 0.2$  vs.  $3.8 \pm 0.3$  nM.mg<sup>-1</sup>) and the

consumption of myocardial T-SOD ( $13.1 \pm 12.1$  vs.  $9.2 \pm 1.2$  IU.mg<sup>-1</sup>) as compared to controls. The production of the endogenous myocardial protective substance CGRP (protein kinase C pathway) was increased just after preconditioning ( $92.0 \pm 4.1$  vs.  $52.3 \pm 4.5$  pg.ml<sup>-1</sup>) and at the beginning of reperfusion ( $95.3 \pm 3.8$  vs.  $61.2 \pm 4.9$  pg.ml<sup>-1</sup>). Preconditioning reduced the release of CK-MB at 12 h post-reperfusion ( $77.5 \pm 9.2$  vs.  $136.5 \pm 8.9$  IU.l<sup>-1</sup>) and improved cardiac function at 30 min and 12 h (cardiac index  $2.8 \pm 0.3$  vs.  $2.3 \pm 0.2$  and  $2.9 \pm 0.1$  vs.  $2.4 \pm 0.2$  L.min<sup>-1</sup>.m<sup>-2</sup>).

As the evidence for the benefit of IP accrued, in a bid to refine myocardial protective strategy, a prospective randomised study compared IP with two established methods of protection, namely cold crystalloid cardioplegia and intermittent cross-clamp fibrillation (ICCF) [142]. 30 CABG patients were randomised to receive: (a) St Thomas' cardioplegia solution no. 2; (b) ICCF; or (c) IP. After establishing CPB, patients were preconditioned at normothermia using two 3-min periods of aortic cross-clamping with epicardial pacing, separated by 2 min of reperfusion (unpaced). The coronary grafts were then performed as in group (b).

IP was found to be superior at limiting myocardial necrosis but no difference was found to exist between cold crystalloid cardioplegia and ICCF. Mean troponin T at 72 h was significantly lower in the IP group compared with the cardioplegia and ICCF groups ( $0.5$  vs.  $2.1$  vs.  $1.3$  microg.L<sup>-1</sup>;  $p=0.05$ ).

Another technique designed as a myocardial protection strategy is off-pump surgery. Ghosh et al investigated whether IP was able to protect against myocardial tissue damage in patients undergoing CABG with CPB and on the beating heart [133]. 120 patients were divided into 3 groups: (1) CPB with

ICCF; (2) CPB with cardioplegic arrest using cold blood cardioplegia; and (3) surgery on the beating heart. In each group (n=40) patients were randomly subdivided (n=20/subgroup) to controls and IP (1 cycle of 5 minutes of ischaemia and 5 minutes of reperfusion before intervention). IP was induced by clamping the ascending aorta in groups (1) and (2) or by clamping the coronary artery in group (3).

IP was found to be protective in patients undergoing CABG on the beating heart but offered no additional benefit when associated with CPB regardless of the mode of cardioprotection used. Total troponin T release and the release profile was similar in the patients undergoing surgery with CPB (groups (1) and (2)); they were unaffected by IP. In contrast, the total troponin T release for the first 48 hours was significantly reduced by IP in the patients undergoing surgery without CPB (group (3)) ( $3.1 \pm 0.1$  to  $2.1 \pm 0.2$  ng.h.ml). Furthermore the release profile that peaked at 8 hours in the control group shifted to the left at 1 hour.

As further evidence for the lack of additional benefit of IP in patients on-CPB in vitro studies using atrial muscles obtained before CPB were found to be protected by IP however muscles obtained 10 minutes after CPB were already protected and IP did not result in further improvements.

### *3.2 Clinical Benefits of Remote Ischaemic Preconditioning*

Several clinical reports of the potential of RIPC to modify ischaemia-reperfusion have now been published.

In 37 children undergoing congenital heart defect repairs utilising CPB, lower limb RIPC (four 5min cycles blood pressure cuff inflation; n=17) has been

demonstrated to reduce troponin I release, inotrope requirements and airway resistance [118].

In abdominal aortic aneurysm surgery (RIPC n=41; Control n=41), RIPC, induced by 2 cycles of intermittent crossclamping of the common iliac arteries with 10mins ischaemia followed by 10mins reperfusion reduced the incidence of myocardial injury (troponin I) by 27% ( $p<0.01$ ), MI by 22% ( $p<0.01$ ) and renal impairment by 23% ( $p<0.01$ ) [143].

In adults undergoing CABG, intermittent upper limb ischaemia has been followed by reductions in post-operative release of lactate dehydrogenase [144] and troponin [103].

Further evidence that RIPC may improve myocardial protection in humans is provided in a single centre randomised trial investigating RIPC or no intervention in 45 patients undergoing CABG with or without concomitant aortic valve replacement (AVR) as an adjunct to antegrade±retrograde blood cardioplegia myocardial protection [145]. Patients with diabetes, renal, hepatic or pulmonary dysfunction were excluded as were those with unstable angina or myocardial infarction within 4 weeks of surgery. The remote preconditioning stimulus comprised of three 5-minute cycles of forearm ischaemia, induced by inflating a blood pressure cuff on the upper arm to 200mmHg with an intervening 5 min reperfusion. The control group had a deflated cuff placed on the upper arm for 30 min. On parametric analysis, RIPC was found to reduce AUC serum cTnT release by >40%. The magnitude of the effect was similar to that observed in a cohort of patients undergoing intermittent ischaemic arrest as a mode of myocardial protection reported by the same group previously [107]. Unfortunately, clinical outcomes are not reported.

A deeper examination of the study design by Venugopal et al, reveals blinding of treatment allocation was applied to patients and surgeons only; anaesthetists (who administer agents capable of preconditioning or affecting myocardial protection) and investigators were not blinded. Similar proportions of patients received isoflurane or sevoflurane for anaesthetic maintenance but dosages are not reported. As such volatile anaesthetic agents may induce a dose dependent conditioning effect [146] a potential for inadvertent bias arises. The study was small and contained only half of the estimated number of patients to detect the initially anticipated difference in AUC cTnT of  $15 \mu\text{g.L}^{-1} \cdot 72\text{hrs}$  (standard deviation  $25 \mu\text{g.L}^{-1} \cdot 72\text{hrs}$ ) quoted in the statistical methodology. Statistical significance was actually attained with a smaller mean difference and sample size and this is attributable to the lower than anticipated variance observed in the RIPC group. The study also included patients requiring AVR; whether RIPC was effective in the CABG alone patients is not reported. The larger number of combined AVR/CABG cases contributed to a longer mean bypass time in the control group and bypass time was an independent predictor of greater troponin release. Despite this potentially confounding effect, an inter-group statistically significant difference was maintained after correction for bypass time using a generalised linear model. Many of the important variables in the study e.g. bypass time, cross-clamp time and AUC cTnT had unequal variances yet were analysed parametrically unlike the authors' previous report. Although the drug history is reported, whether potentially relevant medications e.g. atorvastatin, potassium channel blockers [147][148] administered in the 24 hours pre-operatively is not clear.

Nevertheless, the effect of RIPC on troponin release was large and the data are very encouraging. In particular, the troponin effect was observed despite the use of halogenated anaesthetic gases in the majority of patients. Several studies have demonstrated that such volatile anaesthetic agents may reduce evidence of myocardial injury during CABG via what is thought to be a pre-conditioning mechanism [66][68][149]. Thus, this study is important as it suggests that the effect of RIPC is, at least additive to any protective effect afforded by iso- and sevo-flurane.

On the basis of this and other work, there is now a need to determine (i) the efficacy of RIPC in promoting protection in other forms of cardiac surgery (ii) to ascertain whether the changes in troponin release are reproducible in other studies and (iii) to establish if these changes are reflected in improved clinical outcomes and that RIPC independently reduces risk.

#### **4.0 Conclusion**

With the acknowledged shift to more severe disease within the patient population undergoing cardiovascular surgery, the cardiothoracic surgeon must reinforce his armoury with more robust, economically viable methods of myocardial protection. With the limitations of IP in mind, the initial experience of RIPC seems to suggest a novel method of protection that could be realistically harnessed and refined so as to meet the demands posed. A large randomised controlled study into the effects of RIPC in human cardiac surgery is thus demanded. In addition, a greater understanding of the mechanisms involved in IP and RIPC would offer a welcome hope for pharmacological agents to be developed and assist in the role of myocardial protection.

## **CHAPTER 3: CORE METHODOLOGY**

### **1.0 Introduction**

The work of this thesis reports the outcomes of a single centre, prospective, double blind, randomised, placebo-controlled trial and investigates the effects of intermittent upper limb ischaemia prior to cardiac ischaemia on the indices of myocardial injury (biochemical, functional and arrhythmic) and early renal and lung outcomes in patients undergoing first time isolated coronary artery bypass grafting (CABG) between January 2007 and March 2009.

This study was conducted at the Department of Cardiothoracic Surgery, University Hospital Birmingham NHS Trust with the assistance of the Wellcome Clinical Research Facility and local NHS and University Laboratories. Patient recruitment was from the operating lists of two Consultant Cardiothoracic Surgeons.

Research and ethics approval was sought (LREC reference number 06/Q2702/7) and funding was provided by the British Heart Foundation (BHF Project Grant PG/05/125). The trial was registered with the UKCRN (4659).

A sample of patient data collection, information and consent forms are included in Appendix A.

Procedures that have been standardised across a number of trials including protocols for departmental postoperative management of patients are referenced in Appendix B.

Below follows the study flow and methodology specific to the REMOTE Trial.

## *1.1 Study Flow*

1. Informed consent
2. Preoperative investigations (incl. Spirometry; echocardiography)
3. Final consideration of exclusion and inclusion criteria
4. >12 hrs preoperatively Continuous Holter ECG monitor applied. To continue until 48 hrs post-operatively
5. Randomisation
6. Anaesthesia according to protocol
7. PA catheter flotation in anaesthetic room
8. Baseline cardiac function and biochemical markers pre-sternotomy
9. Remote ischaemic preconditioning (RIPC) stimulus or placebo applied during the LIMA harvest (approx 45 mins prior to CPB, aortic clamping and cardioplegia arrest).
10. Cardiopulmonary bypass management according to protocol
11. Coronary artery bypass surgery conducted using intermittent antegrade cold blood cardioplegia administered according to protocol
12. Serial post-operative studies of haemodynamic performance and biochemical markers
13. Follow-up of post-operative events and timings

## **2.0 Recruitment**

### *2.1 Patient selection*

All patients undergoing elective or urgent isolated, first time coronary artery bypass grafts with intention to use cardiopulmonary bypass were considered for recruitment in this study.

Patients were invited to participate in the study at the preadmission clinic or on the ward in the case of in-patients. Patient Information sheet was provided (Appendix A) and those that agreed to the trial consented on a standard form (Appendix A). Patients were under the care of either Surgeon A or B.

Patient demographics and operative details were recorded in a standardised data collection sheet (Appendix A).

## *2.2 Inclusion and Exclusion Criteria*

Both elective and urgent patients (post-acute coronary syndrome) adult non-diabetic patients undergoing first time multi-vessel CABG utilising CPB were included in the trial.

Excluded patients were those >80 years, pregnant, renal impaired requiring pre-operative renal support therapy, diabetics, intended non-coronary surgery, episodes of angina or ischaemia within 48 hours of procedure, intention to use the left radial artery or cephalic vein as a bypass conduit and chronic AF.

Patients with ST-elevation myocardial infarction within 30 days were also excluded. All referred patients were considered for entry and all eligible patients were approached for consent.

Pre-medication, anaesthesia, perfusion, cardioplegia and surgical techniques were standardized.

## *2.3 Randomisation*

Prior to the start of the trial recruitment a computer generated block randomisation method with blocks of size 6 was used to prepare two boxes of envelopes labelled to 'Surgeon A' and 'Surgeon B'. Each box contained pre-

coded opaque envelopes containing instruction on treatment protocol (RIPC or control). Subjects were randomised on a 1:1 basis to receive RIPC (3 x 5min cycles of upper limb (or dummy arm) 9cm cuff inflation to 200mmHg separated by 5 min periods of cuff deflation. Trial staff, operating surgeons, anaesthetists and ITU personnel were all blinded to patient group allocation. The envelopes were accessed confidentially by the operating department practitioner (ODP) at anaesthetic induction.

The REMOTE technique ensured blinding of trial staff, anaesthetist and surgeons intraoperatively.

Each envelope contained a form which was signed by the ODP to confirm treatment had been performed successfully and then was sealed inside a pre-prepared envelope and returned to the Research Fellow.

### **3.0 Pre-operative medications**

All anti-anginal medication ( $\beta$ -blockers,  $\text{Ca}^{2+}$  channel blockers, nitrates) were continued up to and including the morning of surgery to prevent recurrence and to provide for a more stable anaesthetic course.

Antihypertensive medications were given the morning of surgery to prevent rebound hypertension and provide for a more stable anaesthetic course. As patients taking ACE inhibitors tend to have a lower systemic resistance on bypass and in the immediate postoperative period this medication was withheld the day prior to surgery. [150]

### 3.1 *Permitted drugs*

#### 3.1.1 *Statins*

Patients on a statin(oral) received their standard dose on the evening prior to surgery because of the beneficial effect (Chapter 1: Section2; 8.0)

#### 3.1.2 *Ca<sup>2+</sup> channel antagonists (oral)*

Patients receiving calcium channel antagonists on admission received a standard dose on the day of surgery.

#### 3.1.3 *β-receptor antagonists (oral)*

Patients receiving beta-blockers on admission received a standard dose on the day of surgery. The use of preoperative β-blockers has been shown to lower the mortality rate of coronary bypass surgery.[151][152]

#### 3.1.4 *Long acting nitrates (oral)*

Patients receiving long acting nitrates on admission received a standard dose on the day of surgery.

### 3.2 *Drugs to be omitted*

#### 3.2.1 *Anti-platelet agents*

Aspirin and other anti-platelet therapy were routinely stopped 7 days before admission or in the pre-admission clinic. The day that the aspirin was stopped was recorded. Failure to stop aspirin therapy did not exclude patients from this study but was recorded for data interpretation purposes.

In patient transfers with stable symptoms clopidogrel and aspirin was stopped for at least 1 week prior to surgery. If symptoms were unstable or there was significant (>50%) LMS stenosis aspirin was continued (or clopidogrel was converted to aspirin) for at least 7 days prior to surgery.

Aspirin has been shown to be associated with increased perioperative blood loss by superimposing impaired platelet function on numerous derangements in the clotting mechanism caused by CPB. Aspirin irreversibly acetylates platelet cyclooxygenase, impairing thromboxane A<sub>2</sub> formation and inhibiting platelet aggregation for the lifespan of the platelet (7-10 days) [150].

### *3.2.2 Angiotensin converting enzyme (ACE) inhibitors*

ACE inhibitors were stopped 24 hours prior to surgery or on admission in line with departmental policy.

### *3.2.3 Angiotensin-II receptor antagonists 'Sartans'*

Angiotensin-II receptor antagonists 'Sartans' were stopped for 24 hours prior to surgery or on admission.

### *3.2.4 Diuretics*

Diuretics were omitted on the day of surgery.

### *3.2.5 Potassium channel activators*

Nicorandil was not administered for 24 hours pre-operatively so as not to bias the results of the trial (Chapter 1; Section 2; 7.0).

### 3.3 *Scheduled drugs*

#### 3.3.1 *Premedication*

90 minutes before surgery Temazepam 20-30mg (oral), Ranitidine 150 mg (oral) and Metoclopramide 10 mg (oral) was administered

## 4.0 **Treatment Groups**

### 4.1 *Control Group*

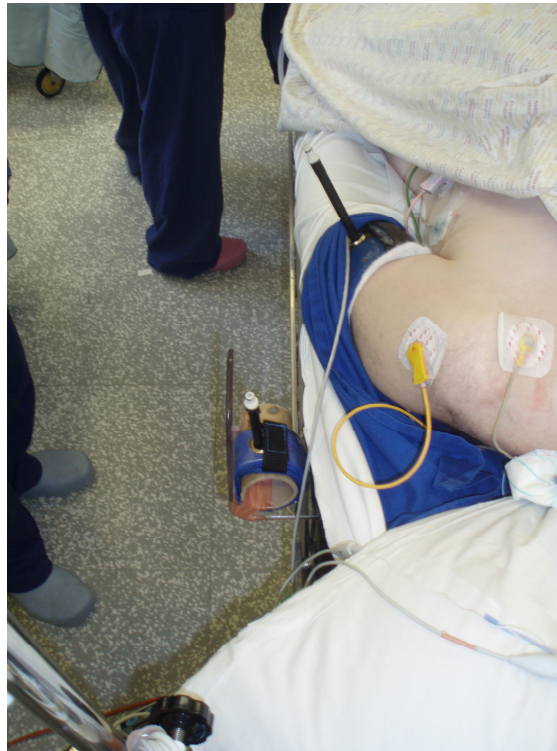
A dummy arm was placed beside the left upper arm. Both dummy arm and left upper arm were connected to a tourniquet machine with two independent tourniquet cuffs (Figures 3.1, 3.2).

Connection of the cuffs was conducted after the patient was anaesthetised, prepped and draped on the surgical table by the Operating Department Practitioner (ODP). The ODP would follow the instructions in the randomisation envelope when connecting the two cuffs and at all times would ensure blinding of trial staff, anaesthetists, surgeons and theatre staff remained.

**Figure 3.1: Tourniquet machine**



**Figure 3.2: Dummy arm and cuff adjacent to cuffed upper limb**



Dummy arm cuff inflation was commenced at left internal mammary artery (LIMA) harvest. 3 cycles of cuff inflation (5minutes) and deflation (5minutes) were performed.

A pulse oximeter placed on the left middle finger and connected to a concealed monitor below the draped operating table was checked to ensure no change in arterial trace took place.

#### *4.2 RIPC Group*

Both dummy arm and left upper arm were connected to a tourniquet machine with two independent tourniquet cuffs.

Connection of the cuffs was conducted after the patient was anaesthetised, prepped and draped on the surgical table by the ODP. The ODP would follow the instructions in the randomisation envelope when connecting the two cuffs

and at all times would ensure blinding of trial staff, anaesthetists, surgeons and theatre staff remained.

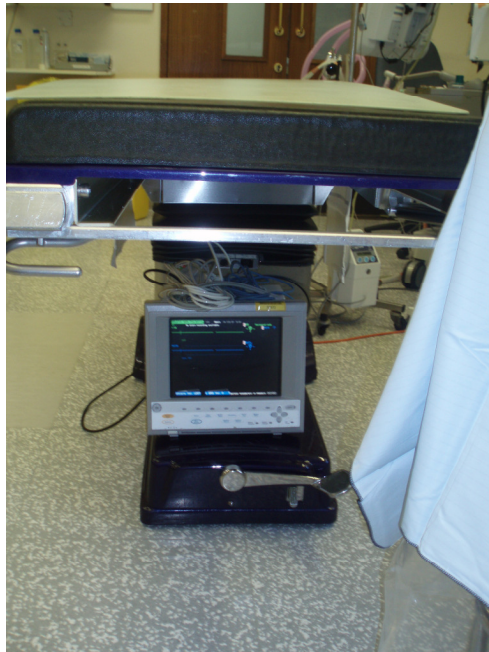
Upper arm cuff inflation was commenced at left internal mammary artery (LIMA) harvest. 3 cycles of cuff inflation (5minutes) and deflation (5minutes) were performed. In this study, we used an identical stimulus as previous reports that demonstrated a >40% reduction in cTnT release [107][145].

A pulse oximeter placed on the left middle finger and connected to a concealed monitor below the draped operating table was checked to ensure a change in arterial trace took place (Figure 3.3, 3.4, 3.5).

**Figure 3.3: Cuffed left upper limb with attached pulse oximeter probe**



**Figure 3.4: Pulse oximeter monitor concealed below operating table**



**Figure 3.5: Cuffs attached once patient prepped and draped**



## 5.0 Anaesthetic Protocols

### 5.1 Induction of anaesthesia

Anaesthesia was induced in the anaesthetic room with the following regimen:

- Fentanyl 10-15 mcg.kg<sup>-1</sup>
- Etomidate 0.1-0.2 mg.kg<sup>-1</sup>
- Pancuronium 0.1 mg.kg<sup>-1</sup>

### 5.2 Central venous cannulation

All patients received a:

- Quadruple lumen central venous line
- Pulmonary artery catheter and sheath, fully floated into the pulmonary artery prior to sternotomy.

Baseline haemodynamic measurements were performed prior to sternotomy.

### 5.3 Maintenance of anaesthesia

- Propofol 2% infused at 4-8 mg.kg<sup>-1</sup>.hr<sup>-1</sup>
- Alfentanil 25mg in 50ml used for maintenance infused at 50mcg.kg.h<sup>-1</sup>
- Enflurane 0-2% or Sevoflurane volatile anaesthesia was used to supplement the propofol-based anaesthesia (Figure 3.6).

n.b. isoflurane volatile anaesthesia was not permitted to supplement propofol-based anaesthesia as it has a potential pre-conditioning effect on the myocardium (Chapter 1: Section 2; 5.2).

**Figure 3.6: Anaesthetic machine**



#### 5.4 *Other medications*

- Mannitol 20%  $0.5\text{g.kg}^{-1}$  was administered into the central line and not the heart-lung machine reservoir, prior to cardiopulmonary bypass (CPB)
- GTN (50mg made up to 50ml) was administered intra-operatively at a minimum dose of  $0.1\text{ml.hr}^{-1}$
- Dopamine (low dose) as “renal dopamine” was not used in this study. However dopamine (high dose) was the first choice inotropic agent
- Isoflurane was NOT used for anaesthetic maintenance
- Tranexamic acid was used, not aprotinin, as the antifibrinolytic agent. Aprotinin has beneficial myocardial protective effects (Chapter 1:Section 2;9.0). Initial bolus of  $30\text{mg.kg}^{-1}$ ; pump prime  $2\text{mg.kg}^{-1}$ ; infusion

16mg.kg.h<sup>-1</sup> until the end of surgery. Tranexamic acid has a better risk benefit profile than aprotinin in low to moderate risk cases.[153]

- Patients with moderately impaired renal function (Cr 130-200) received frusemide 250mg in 25ml (10mg.ml<sup>-1</sup>) neat infused at 0.2 ml.hr<sup>-1</sup>

## 6.0 Perfusion Protocols

### 6.1 Bypass schedule

All patients underwent surgery utilising cardiopulmonary bypass (Table 3.1; Figure 3.7):

**Table 3.1: Bypass schedule**

Prime	2 L Hartman's saline + 10000 units heparin
Pump	Roller Pump
Oxygenator	Capiiox.SX Membrane Oxygenator
Flow range	2.4 L.min <sup>-1</sup> .m <sup>-2</sup>
Mean perfusion pressure	Radial artery 50-60 mmHg
Vasoactive drugs	Phenylephrine (1mg in 10ml. N/Saline) administered in 0.5 1.0ml aliquots and titrated to effect. Not metariminol (aramine). If phenylephrine fails to maintain perfusion pressure, noradrenaline may be used
Cooling/rewarming	Blood/water temperature gradient to be $\leq 7^{\circ}\text{C}$ $\alpha$ -stat standardisation of the blood gases
Cooling	active cooling to 34 <sup>o</sup> C in all cases
Protection	Antegrade intermittent cold blood cardioplegia

Perfusionists were requested to enter the time and dose of each administration of phenylephrine onto the perfusion chart. The effect of each test solution on haemodynamic stability during CPB was assessed as part of the study.

**Figure 3.7: Cardiopulmonary bypass machine**



## 6.2 *Cardioplegia composition*

- St. Thomas A stock solution:
  - 1L of Ringer's compound sodium chloride
  - 102 ml of cardioplegia infusion
  - $16.6 \text{ MgCl}_2 \cdot \text{L}^{-1}$
  - $6.0843 \text{ KCl} \cdot \text{L}^{-1}$
  - Procaine hydrochloride  $1391.28 \text{ mg} \cdot \text{L}^{-1}$
- St. Thomas B stock solution:
  - 1L of Ringer's compound sodium chloride
  - 32 ml of cardioplegia infusion
  - $5.2 \text{ MgCl}_2 \cdot \text{L}^{-1}$
  - $1.9 \text{ KCl} \cdot \text{L}^{-1}$
  - Procaine hydrochloride  $435.68 \text{ mg} \cdot \text{L}^{-1}$

### 6.3 *Cardioplegia administration* (Table 3.2)

Intermittent antegrade cold blood St Thomas' cardioplegia (Martindale pharmaceuticals Essex, UK) was used for myocardial protection with an induction dose of  $12\text{ml.kg}^{-1}$  followed by maintenance administration each 15-20 minutes

**Table 3.2: Cardioplegia protocol**

Temperature	4° C
Delivery	Aortic root
Pressure	120-150 mmHg
Induction	St Thomas A stock at AXC at $12\text{ ml.kg}^{-1}$ (800 ml blood + 200 ml stock A solution)
Maintenance	St Thomas B stock after max 30 minutes or between anastomoses at surgical discretion 300ml doses (400 ml blood + 100 ml stock B solution). Hot shot was not permitted

### 6.4 *Discontinuation of CPB*

On rewarming once the nasopharyngeal temperature reached 35°C the heater/chiller temperature was set to 37°C

CPB separation occurred at 36°C-37°C nasopharyngeal temperature using atrial or dual-chamber epicardial pacing to achieve a target heart rate of  $90\text{min}^{-1}$ .

## 7.0 **Surgical Protocol**

### 7.1 *Conduct of operation*

The technique to be adopted is as follows in the operating theatre:

- Institution of cardiopulmonary bypass

- Aortic cross-clamping and completion of all proximal and distal anastomoses during a single clamp period. Planned bypass conduits would be internal mammary artery and saphenous vein.
- Rewarming should commence during the penultimate anastomosis.

## 7.2 Surgical technique (Table 3.3)

**Table 3.3: Surgical technique**

Heparinisation timing	Heparin should be requested and administered following procurement of all conduit just prior to commencing the aortic purse string insertion
Heparin dose	300 units.kg <sup>-1</sup>
Cannulation	Standard cannulation
Cooling	Active cooling to 34°C in all cases

## 7.3 Myocardial protection (Table 3.4)

**Table 3.4: Myocardial protection**

Technique	Antegrade intermittent cold blood cardioplegia
Induction dose	St Thomas 'A' stock at AXC at 12 ml.kg <sup>-1</sup> (800 ml blood + 200 ml 'A' solution)
Maintenance dose	St Thomas 'B' stock in all cases after 20 minutes or between anastomoses at surgical discretion (400 ml blood + 100 ml 'B' solution) for intermittent administration of 300ml doses. Hot shot is not permitted
De-airing	

## 7.4 Myocardial Biopsy

Patients underwent myocardial biopsy studies. Biopsies (LV) were full thickness and taken from a non-scarred area of the anterior wall.

Biopsies (x1 core) were taken from:

- Right atrium ↔ at cannulation within boundaries of the atrial purse string
- LV 1 (pre-ischaemia) ↔ just before AXC on CPB using fibrillation if preferred
- LV2 (ischaemia) ↔ immediately before AXC release
- LV3 (reperfusion) ↔ 10 minutes after the AXC release

Atrial and trucut biopsies were snap frozen in liquid nitrogen and stored at -70°C. The biopsy sites required 5'0' prolene suture oversewn to ensure haemostasis.

## 8.0 Operating department practitioner protocol

### 8.1 Checklist prior to commencing (Table 3.5)

**Table 3.5: ODP checklist**

Anaesthetic drugs as per protocol	Placebo group: Dummy arm
Quad-lumen and Swan-Ganz catheter + injectate coil	Stimulus group: Patient LEFT arm
Air splitter device	Portable saturation monitor
Tourniquet machine with two cuffs	Stopwatch

### 8.2 Conduct of ODP

1. ODP action randomisation - boxes Surgeon A or Surgeon B
2. Prepare anaesthetic drugs as per protocol
3. Insert RIGHT radial arterial line and ABG
4. 20 ml blood for 2x yellow top bottles (cTnT and routine biochemistry), 1x purple and 1x blue (haematological indices) top withdrawn
5. Quad-lumen and Swan-Ganz catheter + injectate coil insert and float

prior to draping

6. Urine (for ACR and  $\alpha$ -1 microglobulin)
7. Connect air splitter to AIR port in theatre
8. Air splitter device in AIR port in operating theatre to supply anaesthetic and cuff inflation machine
9. Apply tourniquet cuffs to LEFT arm and dummy arm. Keep tourniquet hoses detached
10. Apply Pulse oximeter to LEFT index finger from separate monitor (blinded to all except ODP). Check trace
11. Drape patient
12. Connect tourniquet hose A and hose B to either LEFT arm or dummy arm (as per randomisation instructions)
13. Ensure RED LIGHT on tourniquet machine (cuff is deflated)
14. Document 1<sup>st</sup> systolic blood pressure (SBP) from either RIGHT radial or RIGHT femoral arterial line
15. At beginning of LIMA harvest, blinded anaesthetist to inflate tourniquet A to 200mmHg (1<sup>st</sup> cycle of STIMULUS). Start stopwatch for 5 mins.
16. GREEN LIGHT will show on tourniquet machine (cuff is inflated)
17. ODP check pulse oximeter trace lost if patient arm inflated.
18. After 5 mins of ischaemia deflate tourniquet A. RED LIGHT will show
19. Time 5 mins of reperfusion with stopwatch
20. Document 2<sup>nd</sup> SBP
21. Reinflate cuff to 200mmHg for 5 mins (2<sup>ND</sup> cycle of STIMULUS). GREEN LIGHT will show.

22. Deflate cuff for 5 mins reperfusion. RED LIGHT will show
23. Document 3<sup>rd</sup> SBP
24. Reinflate cuff to 200mmHg for 5 mins (3<sup>rd</sup> cycle of STIMULUS).  
GREEN LIGHT will show
25. Deflate cuff for reperfusion. RED LIGHT will show
26. ODP to check pulse oximeter trace returned once patient arm  
deflated and document on sheet  
  
Stimulus now complete.
27. Disconnect tourniquet hoses from cuffs and remove dummy arm
28. On de-draping, safeguard the Holter monitor leads and monitor on the  
patient

## **9.0 Biochemical monitoring protocol**

### *9.1 Cardiac Troponin T [154]*

Biochemical estimation of myocyte injury was deduced from cardiac Troponin T (cTnT). Samples were taken pre-sternotomy (in anaesthetic room), 6h, 12h, 24h and 48h after aortic cross clamp release and sent for analysis to the Department of Biochemistry at the University Hospital Birmingham NHS FT. A commercially available assay (Elecsys 2010; Roche Diagnostics, UK) was utilised in conjunction with an automated clinical analyser (Roche Modular E170).

Troponin T (TnT) is a component of the contractile apparatus of the striated musculature. Although the function of TnT is the same in all striated muscles, TnT originating exclusively from the myocardium (cardiac TnT, molecular weight 39.7kD) clearly differs from skeletal muscle TnT. As a result of its high

tissue-specificity, cTnT is a cardio specific, highly sensitive marker for myocardial damage.

The Elecsys Troponin T assay employs two monoclonal antibodies specifically directed against human cardiac troponin T. The antibodies recognise two epitopes (amino acid position 125-131 and 136-147) located in the central part of the cardiac troponin T protein, which consists of 288 amino acids. Elecsys Troponin T assay detects free troponin T as well as binary and ternary complexes of troponin.

## 9.2 Test Principle – Sandwich principle [154]

The total duration of the assay was 18 minutes.

- 1<sup>st</sup> incubation: 15µl of sample, a biotinylated monoclonal troponin T-specific antibody and a monoclonal troponin T-specific antibody labelled with ruthenium complex react to form a sandwich complex.
- 2<sup>nd</sup> incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (5-point calibration) provided by the reagent barcode.

The measuring range defined by the lower detection limit and the maximum of the master curve is 0.010-25.00  $\mu\text{g.L}^{-1}$  or  $\text{ng.mL}^{-1}$ . Values below the detection limit are reported as  $<0.010 \mu\text{g.L}^{-1}$  ( $\text{ng.mL}^{-1}$ ). Values above the measuring range are reported as  $>25.00 \mu\text{g.L}^{-1}$  or  $\text{ng.mL}^{-1}$  (or up to  $250 \mu\text{g.L}^{-1}$  or  $\text{ng.mL}^{-1}$  for 10-fold diluted samples).

## **10.0 Haemodynamic monitoring**

### *10.1.1 Initial pre-bypass studies (Baseline/pre-sternotomy)*

Time was taken for a good wedge trace. Full cardiac output studies were obtained prior to sternotomy but after cleaning and draping and with the legs horizontal and the patient flat in the neutral position.

### *10.2 Post-bypass haemodynamic studies (pre-protamine)*

Cardiac output studies were performed following discontinuation of bypass after volume and pressor adjustment was complete but prior to protamine administration.

Volume adjustment was at discretion of the surgeon. The aim was to establish comfortable filling pressures with a MAP of 65-85mmHg at the time of cardiac output studies.

### *10.3 Post-protamine haemodynamic studies*

Cardiac output studies were repeated between 5 and 15 minutes after the completed administration of protamine but at an appropriate time convenient to the surgeon performing haemostasis. The patient was flat. The chest was open, preferably without the retractor in position

#### *10.4 Post-sternal closure haemodynamic studies*

Cardiac output studies were repeated 2 minutes following sternal re-approximation

#### *10.5 Haemodynamic studies on the intensive care unit*

Haemodynamic studies were performed 2, 4, 6, 9 and 12 hours post-reperfusion.

During this 12 hour period, need for IABP and the usage and dosage of any inotropic support was noted.

#### *10.6 Low cardiac output episodes*

Low cardiac output episodes (defined as a  $CI \leq 2.2 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$  with  $CVP \geq 12\text{mmHg}$  and/or  $PCWP \geq 14\text{mmHg}$  in the presence of a native or paced synchronised rhythm and heart rate of  $90-110\text{min}^{-1}$ ) were recorded.

### **11.0 Protocol for post-operative inotropic support and vasoconstrictor**

The haemodynamic aims were as follows:

- M.A.P. of 65-85 mmHg
- $C.I. \geq 2.2 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$
- Sinus rhythm (Pacing)

In the ideal situation inotropes were not administered until post-bypass haemodynamic studies were complete but sometimes this was not possible.

Inotropic support, initially with dopamine ( $200\text{mg}\cdot 50 \text{ ml}^{-1} \text{ D5\%W}$ ) at  $5-10\text{ml}\cdot\text{hr}^{-1}$  was commenced if the cardiac index(CI) was  $\leq 2.2 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$  in the

presence of a central venous pressure(CVP) of  $\geq 12\text{mmHg}$ , pulmonary capillary wedge pressure(PCWP) of  $\geq 14\text{mmHg}$ , and heart rate of  $\geq 90\text{min}^{-1}$  in a coordinated rhythm(sinus, AAI pacing or DDD pacing in order of preference). Escalation of inotrope therapy with adrenaline or noradrenaline or intra-aortic balloon pump (IABP) insertion was then at clinician discretion. Introduction of support was also permitted if the operating surgeon identified poor contractility at separation of CPB or if marginal haemodynamics were noted by attending physicians on the intensive care unit (ICU).

Intra-operative boluses of phenylephrine were used to maintain a mean arterial pressure of  $\geq 55\text{mmHg}$  during CPB and  $\geq 65\text{mmHg}$  following separation in the context of a low systemic vascular resistance. Phenylephrine was also used as a post-operative vasoconstrictor at  $0\text{-}0.4\mu\text{g.kg}^{-1}.\text{min}^{-1}$  and substituted as necessary noradrenaline.

Extubation, ICU and hospital discharge criteria and post-operative atrial fibrillation management were standardised.

## **12.0 Echocardiography**

A comparison was made between left ventricular function assessed on echocardiography 1-3 days before surgery with post-operative results 5-7 days after surgery in patients undergoing first time coronary artery bypass surgery with or without RIPC. Patients were studied using a Vivid 7 ultrasound machine (GE Vingmed Ultrasound, Horten, Norway) with a multifrequency transducer (3V2c) and second harmonic imaging with presets available for contrast and tissue Doppler imaging. Left ventricular dimensions and function were assessed by standard M-mode and 2D according to current American

Society and European Society of Echocardiography Guidelines [155]. Studies were performed by two accredited echocardiographers but analysed off-line by a single observer (Ectopic PC Version 108.x.x, GE Medical System, Horten, Norway).

### *12.1 Contrast Echocardiography for EF*

The current method recommended for quantification of LV volumes and function is the Simpsons biplane estimation [155]. Accuracy of this technique is reduced if there is difficulty in endocardial border definition and in obtaining true perpendicular views of the LV in apical four and two chamber views. Inter-study variability may be up to 10% ejection fraction points between two studies as a result of these technical issues [156]. Difficulty in obtaining an optimal acoustic window becomes more acute in the post-operative period due to pain from the median sternotomy and as patients may not be able to lie in a left lateral decubitus position. The use of contrast opacification overcomes the issue of endocardial border definition and enables the echocardiographer to identify the true apex of the LV more reliably. As a consequence, reproducibility of LV volumes and function using contrast opacification during 2D echocardiography is equivalent to that of cardiovascular magnetic resonance imaging [157]. Contrast-enhanced 2D Simpson's biplane was therefore used as the main method for estimation of LV volumes and ejection fraction in this study. SonoVue (Bracco, Italy) was used as the transpulmonary contrast agent, which is a suspension of microspheres filled with perfluorocarbon gas that has a similar size to red blood cells. 3D echocardiography was not used in this study as the

technology available at our institution requires the stitching of pyramids of data acquired over 4-7 RR intervals to cover larger volume ventricles, which may be inaccurate in irregular post-operative rhythms such as atrial fibrillation. Patients were cannulated before study with a 20 gauge cannula located in the left antecubital fossa. A three-way tap was attached to the cannula with a Luer lock syringe containing 10mls saline and another Luer lock syringe containing 1-4 ml Sonovue contrast. After optimisation of the 2D image in the apical four chamber view, a pre-specified contrast pre-set was activated to deliver low mechanical index imaging (MI 0.2) and contrast was injected with a prompt saline flush. Focus, compression and gain settings were optimised to delineate LV endocardial borders.

### *12.2 Isovolumic Acceleration*

Although widely used, LV ejection fraction is of most use when studying populations or identifying individual risk at any one point in time. LV ejection fraction is a method for assessment of LV contractility that is altered significantly by change in loading conditions. As a result of this, it is less useful as a method of tracking outcomes in an individual patient before and after CABG surgery - whether 2D, contrast-enhanced or 3D. In order to attempt to overcome this, load-independent indices of LV function were studied. In experimental models, myocardial acceleration during isovolumic contraction (IVA) is a sensitive index of left ventricular contractile function which is unaffected by changes in loading conditions within a physiological range [158].

After acquisition of standard 2D and M-mode measurements and before injection of LV opacification contrast, the tissue Doppler preset was activated. Tissue Doppler presets are required to introduce low pass filters to exclude low amplitude, high velocity blood signal, thereby optimising image acquisition to high amplitude, low velocity signal from myocardium. The image sector was narrowed to the wall of interest to optimise frame rate. During suspended respiration following exhalation, images were acquired of the inferoseptal, anterolateral, inferior and anterior walls after optimisation of baseline and scale to exclude aliasing. IVA was derived by measurement of the slope of the time-velocity profile from tissue Doppler data acquired by a sample volume placed within 1 cm of the insertion point of the mitral valve at the base of all 4 walls. Images were optimised to minimise the angle between the direction of annular motion and the ultrasound beam. Post-processing of this signal enabled calculation of the slope of the acceleration during isovolumic contraction, that part of the cardiac cycle when the LV is contracting to increase pressure without change in volume before aortic valve opening. [159] The  $\Delta$ isovolumic acceleration ( $\Delta$ IVA) was an average from basal septal (A4C), basal lateral (A4C), anterior (A2C) and inferior (A2C) tissue Doppler images demonstrating change postoperatively as compared to before surgery.

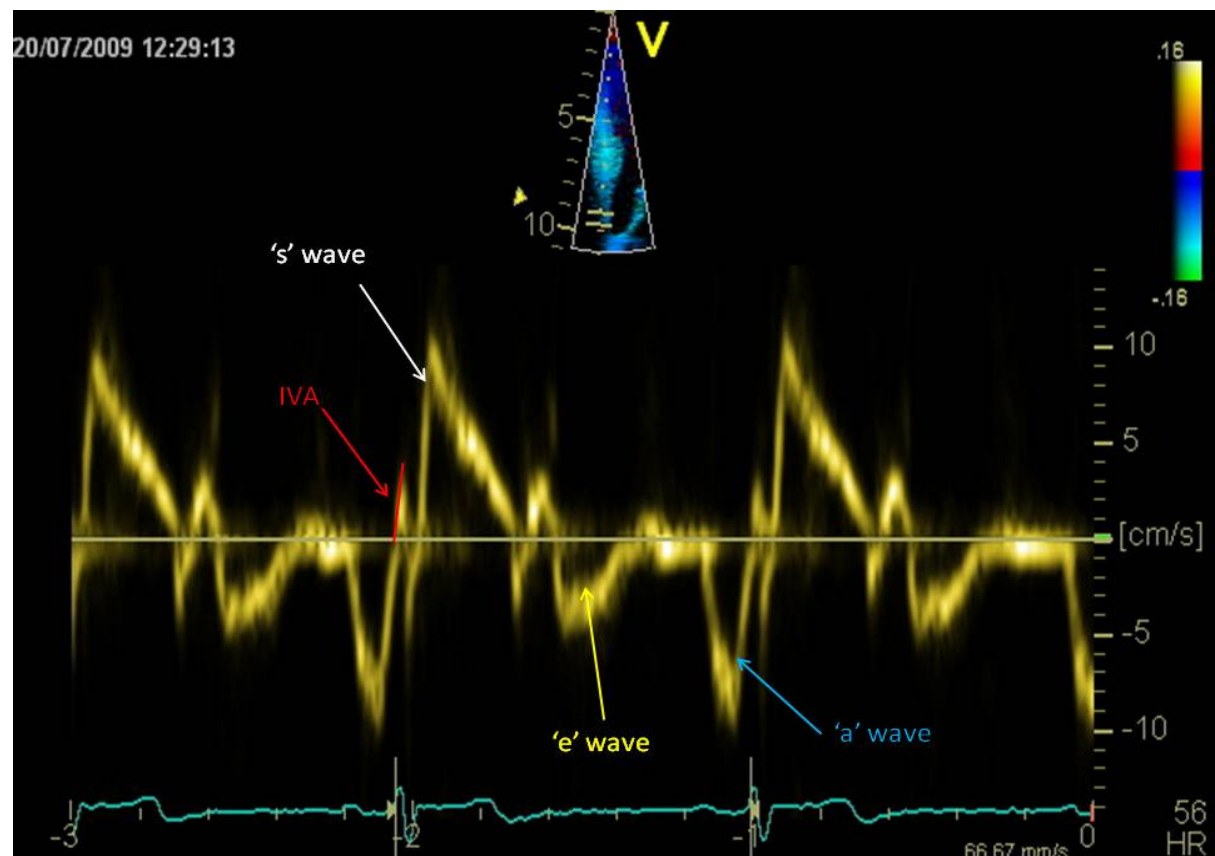
### *12.3 Tissue Velocity Imaging*

Left ventricular function is now thought to depend upon both radial and longitudinal function in a fashion analogous to the wringing of a cloth. Histopathological studies have clearly demonstrated that longitudinally-orientated fibres predominate in the endocardium, while both the mid- and

epicardial layers share longitudinal, helical and circumferential fibres [160]. As the myocardium is perfused from epicardium to endocardium, the longitudinal fibres of the endocardium are most at risk from hypoperfusion. Longitudinal function therefore declines before fall in radial function in ischaemia [161]. There is good evidence that the fall in longitudinal function with ischaemia is initially compensated for by an increase in radial contraction, leading to apparent hyperdynamic EF. Hence both basal systolic myocardial velocities ( $s'$ ) and early myocardial relaxation velocities ( $e'$ ) both provide incremental prognostic information to ejection fraction in ischaemic heart disease [162]. Moreover, early myocardial relaxation velocities can be used to give an estimate of LV filling pressure in patients with normal EF and assessment of transmitral to mitral myocardial relaxation velocity ratio ( $E/e'$ ) is a strong prognosticator in ischaemic heart disease.

After acquisition of standard 2D and M-mode measurements and before injection of LV opacification contrast, the tissue Doppler preset was activated. The image sector was narrowed to the wall of interest to optimise frame rate. During suspended respiration following exhalation, the pulsed Doppler sample volume was placed at the base of the inferoseptal, anterolateral, inferior and anterior walls after optimisation of baseline and scale to exclude aliasing. The size of the sample volume was altered to cover longitudinal displacement of the annulus (5-10mm) and placed within 1 cm of the insertion point of the mitral valve. Images were optimised to minimise the angle between the direction of annular motion and the ultrasound beam. Gain was then set to provide clear tissue velocity traces from which  $s'$ ,  $e'$  and  $a'$  were recorded (Figure 3.8).

**Figure 3.8: Tissue velocity imaging**



### **13.0 Arrhythmia Assessment**

Three lines of investigation were employed to assess and compare perioperative arrhythmias between the groups:

#### *13.1 Continuous Holter ECG recording*

A continuous Holter ECG recording device was attached by three leads to the patient  $\geq 12$  hours prior to aortic crossclamping and was kept in-situ until 48 hours postoperatively. Leads were applied to the right shoulder, left shoulder and left lower chest in the anterior axillary line. Leads ran behind the patient, and not across the chest, so as not to obstruct the operative field (median sternotomy). (Figures 3.9, 3.10, 3.11, 3.12).

Ventricular tachyarrhythmia quantitation was undertaken at the following time periods; pre-operative 12 hours (Pre-12h), the 10 minute period after reperfusion (Post-10min), the next 2 hours of reperfusion (Post-120min), 2-24 hours post-operative (Post2-24h) and 24-48 hours (Post 24-48h).

**Figure 3.9: Continuous Holter ECG Monitor**



**Figure 3.10: Anterior view of applied Holter leads**



**Figure 3.11: Lateral view of applied Holter leads**



**Figure 3.12: Posterior view of applied Holter leads**

**Note leads do not obstruct operative (median sternotomy) field**



### *13.2 12 lead electrocardiograms (ECGs)*

12 lead ECGs were recorded in patients preoperatively, immediately postoperatively, on postoperative day 1 and day 4.

Perioperative myocardial infarction (PMI) defined by the presence of new left bundle branch block (LBBB) or new Q waves of 2mm in depth in 2 contiguous leads by postoperative day 4 was assessed by an independent cardiologist.

### *13.3 Atrial Fibrillation – Treated*

The incidence of atrial fibrillation (AF) that required treatment with either/or amiodarone, digoxin, DCCV during the postoperative period was recorded.

## **14.0 Renal outcomes protocol**

Changes in serum creatinine, urinary albumin:creatinine ratio, urinary  $\alpha$ -1 microglobulin, and requirement for diuretics or haemofiltration were used as outcome measures to assess the effect of renal protection by RIPC on peri-operative renal injury in this double-blind, randomised controlled trial.

### *14.1.1 Creatinine*

Post-operative creatinine was measured on days 0 and 4.

In muscle metabolism, creatinine is synthesized endogenously from creatine and creatine phosphate. Under conditions of normal renal function, creatinine is excreted by glomerular filtration. Serum creatinine measurements are used as a marker of glomerular filtration in diagnosis and monitoring of acute and chronic renal disease as well as for the monitoring the efficacy of renal replacement therapies such as haemofiltration, haemodialysis and chronic ambulatory peritoneal dialysis.

Because urine excretion of creatinine is constant unless there is an acute change in renal function, it can be used to correct for variations in urine flow rate by expressing analytes such as urine albumin or alpha 1 microalbumin as the urine protein/creatinine ratio. The method used is based on the Jaffe reaction as described by Popper et al., Seelig and Wust and modified by Bartels [163][164]. This modified version has a higher sensitivity and better precision than the original Jaffe method. Serum and urine creatinine were measured by the Clinical Biochemistry Department using a kinetic, blanked and compensated method using Roche Modular automated clinical chemistry analysers.[165][166][167][168][169]

#### 14.1.2 Test principle – Kinetic colorimetric assay

In alkaline solution, creatinine forms a yellow-orange complex with picrate. The colour intensity is directly proportional to the creatinine concentration and can be measured photometrically. Assays using rate-blanking minimize interference with bilirubin.

- Sample and addition of R1 (Sodium hydroxide: 0.20 mol/L)
- Addition of R2 (Picric acid: 25 mmol.L<sup>-1</sup>) and start of reaction:

creatinine + picric acid  $\longrightarrow$  creatinine-picric acid complex

The reference range for 24 hr urine creatinine is 9-21 mmol.24h<sup>-1</sup> (1040-2350 mg.24h<sup>-1</sup>) and for women is 7-14 mmol.24h<sup>-1</sup> (740-1570 mg.24h<sup>-1</sup>).

#### 14.2.1 Albumin

Albumin is a non-glycosylated protein with a molecular weight of 66,000 daltons. Albumin is quantitatively the most important protein component in plasma normally representing about 50% of the total protein content. However, in normal urine albumin only contributes <30mg.24h<sup>-1</sup> of the total urine protein which is normally <150 mg.24h<sup>-1</sup>. Low level but pathologically significant albuminuria, undetectable by chemical dipstick, called 'microalbuminuria' is a sensitive marker of renal injury, and may reflect glomerular or renal tubular dysfunction.

A variety of methods, such as radial immunodiffusion, nephelometry and turbidimetry, are available for the determination of albumin.

[170][171][172][173][174] Immunoturbidimetric assay for the in vitro

quantitative determination of albumin in human urine on Roche Modular automated clinical chemistry analyzers was performed in this series.

#### *14.2.2 Test principle – Immunoturbidimetric assay*

Urine albumin was measured by automated immunoturbidimetry using an antibody to human albumin.

- Sample and addition of R1 (buffer; TRIS (Tris(hydroxymethyl)-aminomethane) buffer: 50mmol.L<sup>-1</sup>, pH 8.0 (25°C); PEG: 4.2%; EDTA: 2.0mmol.L<sup>-1</sup>; preservative)
- Addition of R2 (anti-albumin antibodies; Polyclonal anti-human albumin antibodies (sheep): dependent on titre; TRIS buffer: 100mmol.L<sup>-1</sup>, pH 7.2 (25°C); preservative) and start of reaction: Anti-albumin antibodies react with the antigen in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically.
- Addition of R3 (human albumin; Albumin (human): 200mg.L<sup>-1</sup>; phosphate buffer: 50mmol.L<sup>-1</sup>, pH 7.0 (25°C); sodium chloride: 150mmol.L<sup>-1</sup>; preservatives): Human albumin is added and the resulting additional turbidity change is evaluated for detection of antigen excess.

The 24 hr urine expected values in adults are <20 mg.L<sup>-1</sup> (0.304 µmol.L<sup>-1</sup>) albumin or <30 mg.24h<sup>-1</sup> (0.456 µmol.24h<sup>-1</sup>) albumin.

#### *14.3.1 Urine ACR*

Urine was collected pre-sternotomy (in anaesthetic room), 4h, 8h, 12h and 24h after aortic cross clamp release and sent to the Department of Biochemistry for analysis. Urinary albumin-creatinine ratios (ACR) were assessed at 0, 12 and 24 hours. The normal urine ACR reference range for women is <3.5 and men is < 2.5 mg.mmol<sup>-1</sup>.

#### *14.4.1 Urine for $\alpha$ -1 microglobulin*

Urine was collected pre-sternotomy (in anaesthetic room), 4h, 8h, 12h and 24h after aortic cross clamp release and sent to the Department of Biochemistry for storage of aliquots that were then sent to East Kent Hospitals for analysis.

Measurement of alpha-1-microglobulin in urine aids in the diagnosis of kidney tubular damage. A1M reagent when used in conjunction with the Beckman Coulter IMMAGE<sup>®</sup> Immunochemistry Systems and Urine Protein Calibrator was utilised for quantitative determination of Alpha-1-Microglobulin (A1M) in human urine by rate nephelometry.

#### *14.4.2 Test principle – Rate nephelometry*

The A1M test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

Alpha-1-microglobulin(sample) + Antibody  $\longrightarrow$  [Alpha-1-microglobulin(sample)-

Antibody (aggregates)]

The 24 h urine expected values are <1.28 mg.dL<sup>-1</sup>.

## 15.0 Lung outcomes protocol

### 15.1 Spirometry

Patients underwent preoperative simple spirometry to assess FEV<sub>1</sub>, FVC and FEV<sub>1</sub>/FVC ratio (Figure 3.13).

**Figure 3.13: Spirometer**



### 15.2 Positive End Expiratory Pressure

As Positive End Expiratory Pressure (PEEP) has been shown to increase right and left ventricular stroke volume variation both during open and closed chest conditions in addition to reducing right ventricular end-diastolic volume indicating a preload reductive effect, [175] its use in ventilator management was standardised to 5cm H<sub>2</sub>O. Changes in arterial blood partial pressure of oxygen (PaO<sub>2</sub>) / fractional inspired oxygen (FiO<sub>2</sub>) ratio and intubation times were used as outcome measures.

### *15.3 Arterial PaO<sub>2</sub> / fractional inspired oxygen ratio (PaO<sub>2</sub>:FiO<sub>2</sub> ratio)*

Arterial blood was sampled preoperatively from a right sided radial arterial line in the anaesthetic room prior to induction and assessed for PaO<sub>2</sub> (kPa) (Bayer RapidLab865 Analyser). PaO<sub>2</sub>:FiO<sub>2</sub> ratio was recorded and the process repeated for measurements at 6hr and 12hr postoperatively.

### *15.4 Intubation time*

Intubation time (or time to extubation) was recorded as the time from arrival to the Intensive Care Unit to extubation (minutes). Extubation was nurse-led and performed according to standard departmental criteria (>36°C, normal and stable acid base balance, chest output <100ml.h<sup>-1</sup>).

## **16.0 Analysis of myocardial samples**

During a small mechanistic study in patients undergoing on-pump coronary artery bypass grafting randomised to RIPC (three 5 min cycles left upper limb ischaemia) and Control groups, left ventricular myocardial Tru-cut biopsies were collected: (a) 'pre-ischaemia' ('LV1' – just prior to application of aortic cross clamp); (b) 'end-ischaemia' ('LV2' – just prior to removal of aortic cross clamp); (c) 'post-reperfusion' ('LV3' – 10 minutes after removal of aortic cross clamp). Biopsies were snap frozen in liquid N<sub>2</sub> and stored at –80°C for batch analysis using standard techniques.

Myocardial samples were transported on dry ice to the University of Oxford for analysis.

The left ventricular apical biopsy samples, taken 10 minutes after the cessation of the extra-corporeal circuit, were homogenised and protein extracted for Immunoblotting (see below). Quantification was done by densitometry and all samples were normalised to beta-tubulin. Comparisons between control (n=6) and RIPC (n=7) was by unpaired t-testing.

#### *16.1 Tissue homogenization and protein quantification*

All samples were stored at -80°C until use. Tissue samples were homogenized using a Polytron rotor (GlenMills) in RIPA buffer (50 mM TrisHCl pH7.6, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS) containing 1x complete protease inhibitor tablet (Roche Applied Sciences), 1x PhosSToP phosphatase inhibitor cocktail tablet (Roche Applied Sciences) and 0.5mM PMSF.

Protein content was quantified using the colorimetric BCA protein assay kit (Thermo Scientific). Briefly assay reagents A and B supplied were mixed in a ratio of 50:1. Bovine serum albumin at concentrations of 0, 50, 100, 200, 400 and 800µg.ml<sup>-1</sup> was used to create a standard curve. 10µl of each sample of homogenate was diluted in 40µl of ddH<sub>2</sub>O. 1ml of mixed assay reagent was then added to each sample and then samples were incubated at 37°C for 30mins.

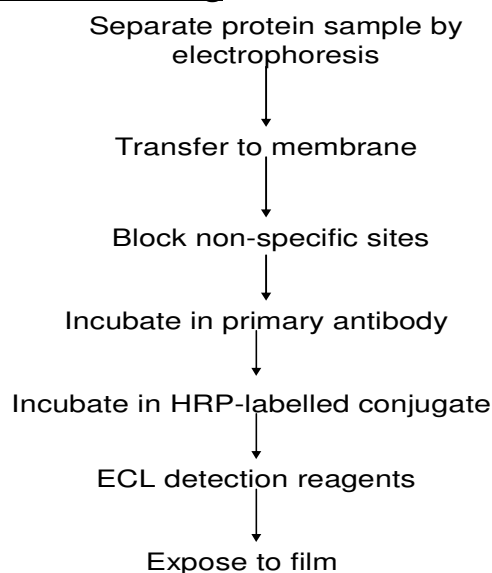
Samples were transferred to cuvettes and then colorimetric density was assessed using a spectrophotometer (Ultrospec 2000, Pharmacia Biotech) set at 562nm. By comparison against the standard curve all samples were normalised to 200µg.ml<sup>-1</sup> before the addition of SDS-Page running buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromphenol blue, 0.125

M Tris HCl) to give a 6:1 dilution. All samples were subsequently heated to 95°C for 5 minutes prior to immunoblotting.

## 16.2 Western Blotting

### **Protocol for ECL Western blotting**

#### **Flow diagram**



Pre-cast 4-12% 1.5mm Bis-Tris Gels were utilised (Invitrogen) in a standard electrophoresis tank containing MOPS buffer (200mM MOPS free acid, 50mM sodium acetate, 10mM EDTA, and 10mM EGTA) and 500 µl of NuPage antioxidant (Invitrogen) in the central section only.

Into each well was loaded 20µl of sample with 12 µl of Full Range Rainbow Marker (GE Healthcare) in the outside lane.

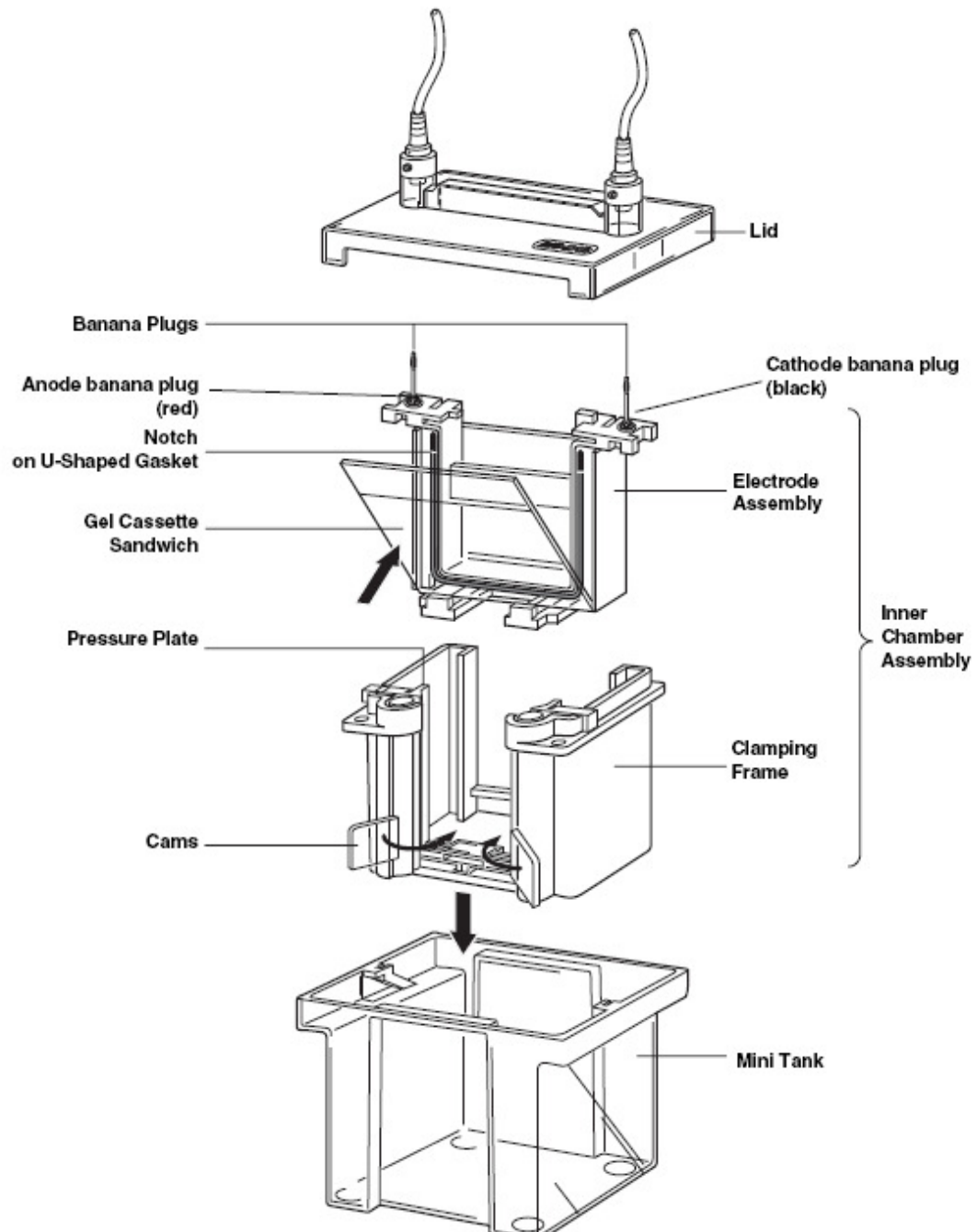
Gels were run at 200V for a minimum of 50mins or until the forward protein marker was seen to have run off the gel. Once completed, gels were removed from the tank and placed into cooled transfer buffer (4°C) (48mM Tris Base, 390mM Glycine, 0.1% SDS, 20% Methanol). Transfer stacks were formed with a layer of fibre pad and pre-cut blotting paper (both soaked in transfer buffer) as the base for the gel. A PVDF membrane (Bio-Rad) (activated by

soaking in 100% methanol for 10 min prior to rinsing in transfer buffer) was placed on top of the gel and the stack completed with further layers of blotting paper and fibre pad, prior to rolling to remove any air bubbles. Transfers were conducted overnight at 20mA, in a 4°C cold room with constant stirring using a magnetic flea.

After transfer PVDF membranes were placed in 50ml Falcon tubes and incubated with constant rolling for 1 hour at room temperature in 5% fat free milk in TBST (50 mM tris-HCl, 10 ml 0.5 M tris-HCl, 150 mM NaCl, 75 ml 2 M NaCl and 0.05 % Tween 20) as a blocking agent. The blocking solution was then replaced with 10ml of the same solution containing the primary antibody at a dilution of 1:1000. After incubation at room temperature for 2 hours, the antibody was removed and the PVDF membrane washed 3 times for 5 minute periods in 15ml TBST. Secondary antibody in the 5% milk and TBST solution at a dilution of 1:4000 was then added and the membrane incubated at room temperature for 40 min, prior to a final 3 washes in TBST as above.

ECL advance solution (Amersham Biosciences) reagents A+B were mixed in a 1:1 ratio and pipetted onto the PVDF membrane, allowing it to incubate at room temperature for 5 mins. The resulting chemoluminescence was detected in a Chemi-Doc molecular imaging system (Bio-Rad) and band densitometry performed using the QuantityOne software package. (Figure 3.14; Table 3.6).

**Figure 3.14: Gel tank assembly for SDS-polyacrylamide gel electrophoresis**



**Table 3.6: Antibodies used in Western Blotting**

<b>Antibody</b>	<b>Species/Ig class</b>	<b>Dilution</b>	<b>Manufacturer/catalogue No.</b>
Phospho-Akt (Ser 473)	Rabbit/Ig G/polyclonal	1:1000	Abcam/ab28821
Pan-Akt	Rabbit/Ig G/polyclonal	1:1000	Abcam/ab8805
Phospho-GSK3 beta (S9)	Rabbit/Ig G/polyclonal	1:1000	Abcam/ab30619
GSK3 beta	Rabbit/Ig G/polyclonal	1:1000	Abcam/ab31366
$\beta$ -catenin	Rabbit/Ig G/polyclonal	1:1000	Abcam/ab6302
$\beta$ -tubulin	Rabbit/Ig G/polyclonal	1:1000	Abcam/ab6046
Rabbit IgG (HRP)	Goat/ Ig G/polyclonal	1:4000	Abcam/ab6721

## 17.0 Statistics

### 17.1 Primary outcome measure and estimation of sample size

If RIPC is an effective adjunct to myocardial protection it would be expected to reduce evidence of myocardial ischaemic injury. The optimal biochemical measurement of myocyte injury remains unclear [176][177]. It is not known whether a peak, timed or serial measure (ANOVA, AUC) is the most appropriate predictor of biochemical injury but serial measures contain greater information about the total injury and until further comparative studies become available we believe AUC represents the best predictor of mid-term survival post CABG.

At first, cTnI was considered as the biochemical measurement of myocyte injury.

#### 17.1.1 Estimation of sample size using cTnI

Single time point comparisons and cumulative area-under-the-curve comparisons of creatine kinase MB fraction (CK-MB) [149] and Troponin I (cTnI) [178] are currently accepted standards [179][180][181]. In our previous studies GIK therapy reduced cTnI by 3.07ng.ml<sup>-1</sup>. (population SD 6.905) [182].

If this difference were replicated in the current study this will be detectable with a total sample size of 162 patients ( $\alpha$  0.05.  $1-\beta$  0.8). Previous studies of ischaemic preconditioning in myocardial protection have suggested that the reduction in peak or AUC CK-MB or cTnl approximates  $\geq 0.5SD$ . For instance, Belhomme et al. reported a difference of  $250\text{ng.ml}^{-1}.\text{h}^{-1}$  (population SD 404) for AUC CKMB, a difference of  $80\text{ ng.ml}^{-1}.\text{h}^{-1}$  (population SD 140) for cTnl AUC, a difference of  $29\mu\text{g.L}^{-1}$  (control SD 33) for 6 hour CKMB and a difference of  $1.9\text{ng.ml}^{-1}$  (population SD 3.64) for 6 hour cTnl [149]. Replication of these differences for the proposed study with a total sample size of 162 patients would yield the following power and alpha (Table 3.7).

**Table 3.7: cTnl sample size calculations**

<b>Parameter &amp; study</b>	<b>Estimated difference</b>	<b>Population standard deviation</b>	<b>Total sample size</b>	<b><math>\alpha</math></b>	<b><math>1-\beta</math></b>
6h cTnl [183]	3.07	6.905	162	0.05	0.8
*6h cTnl [183]	3.453	6.905	162	0.05	0.85
6h cTnl [149]	1.9	3.64	162	0.05	0.9
6h CK-MB [149]	29	33	162	0.01	>0.95
AUC CK-MB [149]	250	404	162	0.025	0.95
AUC cTnl [149]	80	140	162	0.05	0.9
AUC cTnl [183]	105	210	162	0.05	0.85

\*GIK study (postulated 0.5SD difference)

Thus, using cTnl the primary end-point of 6 hour and 48 hour AUC cTnl estimation with sampling at 0, 6, 12, 24 and 48 hours post-operatively [181][184] would be achieved with a total sample size of 162 patients.

### *17.1.2 Estimation of sample size using cTnT*

48h AUC cTnT release was finally selected as the primary endpoint biochemical measurement of myocyte injury for the following reasons: (1) more available assay; (2) only one assay exists from a single provider; (3) as used in previous similar studies this study would then be comparable.

Previous studies suggested a large treatment effect; a standardised difference of 0.8. We hypothesised that RIPC would reduce cTnT AUC by a standardised difference of 0.6 at 90% power and 5% alpha. This required a sample size of 120 subjects which we increased by 33% to accommodate withdrawal or missing data points.

### *17.2 Secondary outcome measures*

Secondary cardiac end points were 6hour and peak cTnT, incidence of myocardial injury on 12-lead ECG, serial cardiac and left ventricular stroke work indices (CI, LVSWI), low cardiac output episodes (LCOE) incidence, inotrope usage and dosage, reperfusion ventricular fibrillation and peri-operative ventricular tachyarrhythmia incidence and functional echocardiographic change, ICU and hospital length of stay(LOS) and the renal and lung data listed above.

### *17.3 Statistical analysis for cardiac, renal and lung outcomes*

Data were analysed with statistical package (SPSS 15.0, Chicago, Ill). Categorical or ordinal data were compared by using  $\chi^2$  tests or Kendall tau b, respectively. Continuous data are presented as mean $\pm$ standard deviation or median(interquartile range). Normally distributed data were compared using

independent two-sided  $t$  tests. Repeated-measures analysis of variance (RMANOVA) was used for serial measurements. Skewed data were either logarithmically transformed or analysed non-parametrically (Mann-Whitney  $U$  test).

To assess cTnT AUC, we estimated missing troponin values (6% measurements) from the group and sampling time using a general linear model.

#### *17.4 Statistical analysis of echocardiographic parameters*

The primary end-point for the echocardiographic assessment of the functional outcome from RIPC was IVA. With a sample size of 73 patients in each group, this study would be able to detect a difference in IVA  $\geq 0.5SD$  between groups ( $\alpha$  0.05,  $1-\beta$  0.85) [158][159][185][186]. Secondary end-points were change in contrast-enhanced LVEF and change in myocardial tissue systolic and early myocardial relaxation velocities.

Data were analysed with statistical package (SPSS 15.0, Chicago, Ill). Categorical or ordinal data were compared by using  $\chi^2$  tests or Kendall tau b, respectively. Continuous data are presented as mean $\pm$ standard deviation or median(interquartile range). Normally distributed data were compared using independent two-sided  $t$  tests. Repeated-measures analysis of variance (RMANOVA) was used for serial measurements. Skewed data were either logarithmically transformed or analysed non-parametrically (Mann-Whitney  $U$  test).

### **18.0 Demographic and intraoperative variables**

Both groups were well matched (Table 3.8).

**Table 3.8: Pre-operative and intra-operative patient characteristics. CCS = Canadian Cardiovascular Society; NYHA = New York Heart Association; TIA = Transient ischemic attack; CVA = Cerebrovascular accident; ACE-I = Angiotension converting enzyme inhibitor; IABP = intra-aortic balloon pump.**

	<b>Control (n=82)</b>	<b>RIPC (n=80)</b>	<b>p</b>
Age	65(60-73)	63(57-71)	0.19
Male gender (n(%))	72(88)	71(89)	0.59
CCS Angina Class	2(1-2)	1(1-2)	0.54
NYHA Class	2(1-2)	2(1-3)	0.25
Body mass index (kg.m <sup>-2</sup> )	28(25-31)	27.5(26-30)	0.80
Elective (n(%))	38(46)	42(52)	0.53
Urgent (n(%))	44(54)	38(48)	0.53
Current Smokers (n(%))	7(9)	12(15)	0.15
Ex-smokers (n(%))	52(63)	39(49)	
Never smoked (n(%))	23(28)	29(36)	
High risk lung status (n(%))	17(21)	22(28)	0.36
Hypercholesterolemia (n(%))	59(72)	61(76)	0.59
Hypertension (n(%))	52(63)	44(55)	0.34
Past history of TIA (n(%))	4(5)	3(4)	1.00
Past history of CVA (n(%))	1(1)	1(1)	1.00
Statins (n(%))	71(87)	76(95)	0.10
Aspirin (n(%))	70(85)	73(91)	0.33
Diuretics (n(%))	16(20)	10(13)	0.29
Potassium channel blocker (n(%))	18(22)	12(15)	0.31
Angiotensin II Antagonist (n(%))	7(9)	6(8)	1.00
ACE I (n(%))	57(70)	48(60)	0.25
β-blocker (n(%))	68(83)	63(79)	0.55
Oral Nitrates (n(%))	30(37)	18(23)	0.06
Ca <sup>2+</sup> channel blockers (n(%))	31(38)	26(33)	0.51
Pre-CPB IABP (n(%))	1(1)	0(0)	1.00
Fasting glucose >7mmol.L <sup>-1</sup> (n(%))	0(0)	3(4)	0.33
Fasting triglycerides (mmol.l <sup>-1</sup> )	1.4(1.1-1.7)	1.3(1.0-1.7)	0.81
Fasting cholesterol(mmol.l <sup>-1</sup> )	3.6(2.9-4.3)	3.6(3.2-4.4)	0.20
Creatinine (mg.dL <sup>-1</sup> )	1.09±0.18	1.11±0.18	0.44
Pre-operative pO <sub>2</sub> :FiO <sub>2</sub> ratio	53(47-61)	55(48-63)	0.31
Euroscore	3(2-5)	3(2-4.5)	0.10
Logistic Euroscore	2.5(1.5-4.3)	1.9(1.3-3.4)	0.78
<b>Intra-operative factors</b>			
Enflurane (n(%))	65(79)	64(80)	0.46
Sevoflurane (n(%))	15(18)	15(19)	
Propofol (n(%))	2(2)	1(1)	
Bypass time (min)	96±22	100±23	0.25
Cross-clamp time (min)	71±18	76±21	0.08
Number of grafts	3.5(3-4)	3(3-4)	0.88

## **CHAPTER 4      THE EFFECT OF RIPC ON CARDIAC OUTCOMES IN PATIENTS   UNDERGOING   CORONARY   ARTERY BYPASS SURGERY**

### **1.0 Introduction**

In remote ischemic pre-conditioning (RIPC) short cycles of repeated limb (or other organ) ischemia provoke a protective effect that can halve the mass of infarction caused by substantive vessel occlusion and reperfusion [70]. In coronary artery bypass grafting (CABG), adverse outcomes predominantly relate to cardiomyocyte injury due to myocardial protection failure and there is experimental and clinical evidence that RIPC could attenuate such injury [187]. The adequacy of myocardial protection can be assessed using an array of measurements that are indicative of cardiomyocyte death, injury or dysfunction [188]. These include cardiac troponin (cTn) release, the incidence of post-operative low cardiac output episodes and ventricular arrhythmias, the need for inotropic support and functional assessment by haemodynamic monitoring and echocardiography. These measures contribute to an assessment of reversible and irreversible myocardial injury.

Experimental models have demonstrated a first window (1-4 hours) followed by a second window (24-72 hours) of protection against infarction afforded by ischaemic preconditioning. In rabbit models the second window also protects against infarct and arrhythmias [189] and this has been corroborated by rat models where repeated left coronary artery occlusions have been shown to reduce infarct size by >40% [190] and the incidence of ventricular fibrillation [191].

Human ventricular myocytes [192] and atrial appendages taken during CABG [193] have also been shown to exhibit protection at both windows with preconditioning protocols followed by prolonged ischaemia and reperfusion. Suppression of proinflammatory gene transcription in leukocytes within 15 minutes and 24 hours of an intermittent forearm ischaemic stimulus highlights evidence for first and second windows in human models following RIPC protocols [194].

In elective percutaneous coronary intervention(PCI), RIPC has been associated with reduced cTnI release, less electrocardiographic ST elevation and a reduced incidence of major adverse cardiovascular events [195]. In abdominal aortic aneurysm repair, RIPC induced by intermittent iliac artery occlusion reduces the incidence of myocardial injury and may reduce post-operative renal impairment [143].

In cardiac surgery, the first clinical report of a protective RIPC effect was in children. RIPC, induced by four 5-min cycles of lower limb ischemia-reperfusion resulted in lower cTnI release, reduced inotrope requirements and lower airway resistance implying a possible protective effect on myocardial injury, function and bypass-related lung injury, a finding also demonstrated in experimental models [107][118][196].

Two studies in CABG patients, one investigating RIPC in CABG undertaken using predominantly intermittent ischemic arrest and a second utilising cardioplegia have reported [107][145]. Both were single blinded, used a 3-cycle upper arm RIPC stimulus and both demonstrated a significant reduction of cTnT area-under-the-curve(AUC) release of over 40%. Clinical and hemodynamic outcomes were however not reported. Much of the cardiac

injury associated with CABG appears to be a reversible phenomenon manifest as transiently subnormal cardiac output and a temporary need for inotropic support. The role of RIPC in affording protection against such post-ischemic dysfunction is far less clear [197].

The aims of this study were to confirm the role of RIPC in reducing troponin release and to ascertain if this is accompanied by an enhanced protection against post-operative cardiac, renal and pulmonary dysfunction.

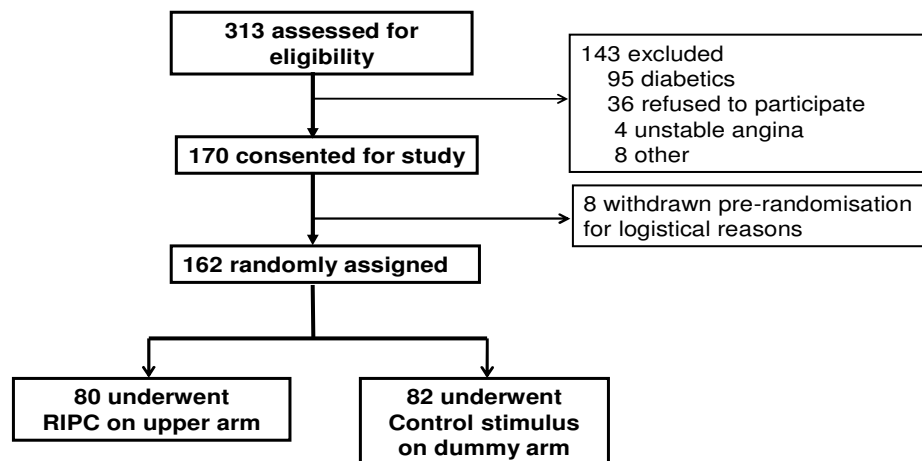
## **2.0 Methods**

Biochemical and haemodynamic assessment of cardiac outcomes followed methodology set out in Chapter 3; Section 9.0 to 11.0. Statistical analysis was performed as per Chapter 3; Section 17.0 to 17.2.

## **3.0 Results**

Of 313 possible subjects, 206 met the inclusion criteria, 170 consented and were initially enrolled, 8 of which were withdrawn pre-randomisation for logistical reasons (Figure 4.1) leaving a study population of 162. All patients had the appropriate signal on pulse oximetry during RIPC or placebo stimulus delivery with no inadvertent crossover. The stimulus was applied in the  $74 \pm 16$  minutes between incision and aortic cross clamping (AXC). Demographic and intra-operative variables were similar (Table 3.8).

**Figure 4.1: Consort diagram of trial recruitment.**



All patients survived 30 days. There was one in-hospital death in the control group at day 109 due to pneumonia, a hospital mortality of 0.6%. There was no difference in ITU or hospital length of stay (Table 4.1).

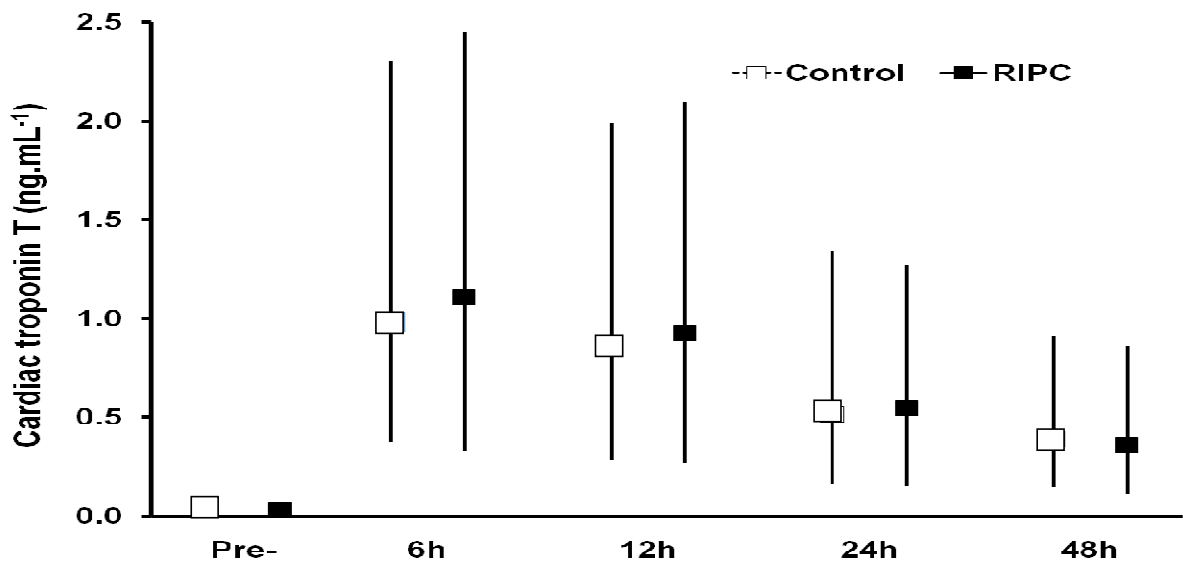
**Table 4.1: Post-operative data. cTnT = Cardiac Troponin T; IABP = intra-aortic balloon pump; LCOE = low cardiac output episode; LOS = length of stay.**

	<b>Control (n=82)</b>	<b>RIPC (n=80)</b>	<b>p=</b>
6 hour cTnT (ng.ml <sup>-1</sup> )	0.93(0.59-1.35)	1.01(0.72-1.43)	0.522
Cmax cTnT (ng.ml <sup>-1</sup> )	1.02(0.74-1.44)	1.04(0.78-1.51)	0.789
Postoperative IABP (n(%))	7(9)	6(8)	0.52
12h LCOE incidence (n(%))	20(24)	27(34)	0.225
Inotrope usage (n(%))	32(39)	40(50)	0.155
Vasoconstrictor usage (PhE or NE) (n(%))	54(66)	51(64)	0.870
ITU LOS (days)	3(2-4);	3(2-5)	0.287
Hospital LOS (days)	8(6-11);	8(6-12)	0.392

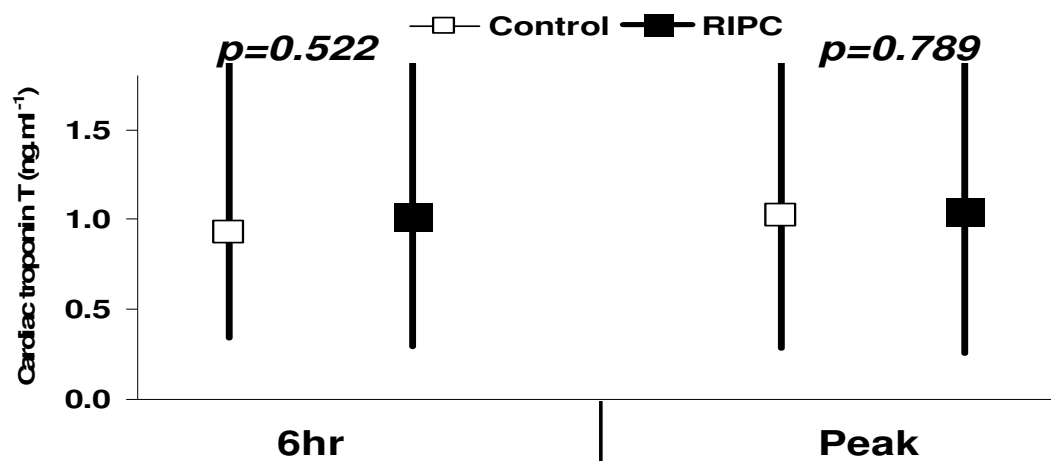
### 3.1 Myocardial Injury

Of 810 possible cTnT measurements, 758(94%) were assayed. Using a generalised linear model, there was no difference in 48 hour AUC cTnT (RIPC 30.1(22.2-38.1) vs. 27.7(18.9-39) ng.48h.ml<sup>-1</sup>; p=0.721) (Figure 4.2). When completely sampled patients (n=123; 76%) were considered, the results were similar (RIPC 29.3(20.8-39.2) vs. 27(17.4-39.6) ng.48h.ml<sup>-1</sup>; p= 0.451). Other cardiac injury indices were not different (Table 4.1; Figure 4.3). There was no difference between the groups when AUC cTnT release was considered for elective (RIPC 31.7(23.4-40.9) vs. 27.6(17.4-39.5) ng.48h.ml<sup>-1</sup>; p= 0.260) or urgent patients (RIPC 28.0(18.1-35.3) vs. 28.4(19.4-36.3) ng.48h.ml<sup>-1</sup>; p= 0.765).

**Figure 4.2: Cardiac troponin T release over 48 hours. Medians and interquartile ranges are presented. No difference in release profiles was identified on area-under-the-curve analysis (p=0.721)**



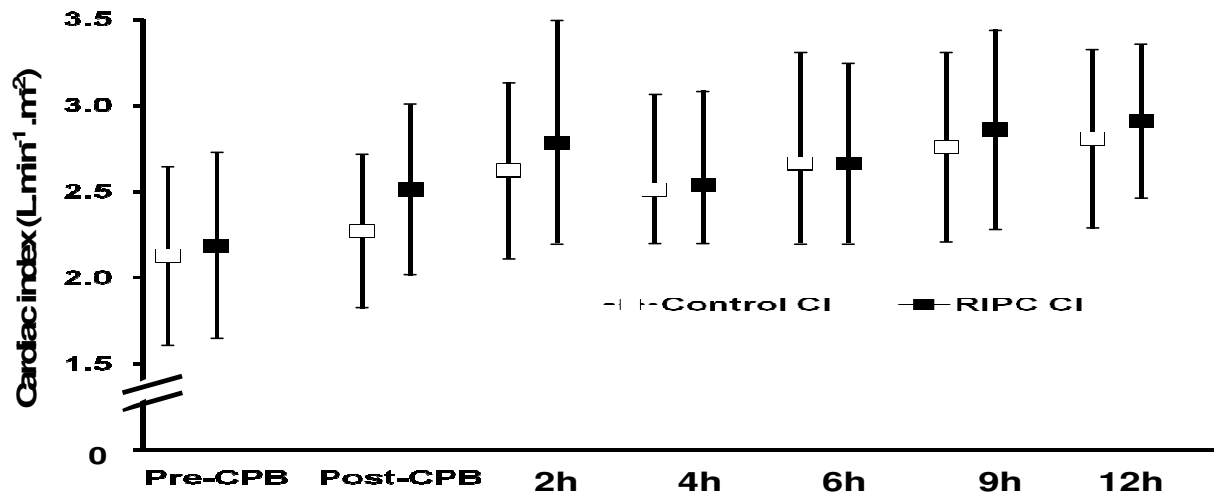
**Figure 4.3: 6 hour and Peak Cardiac troponin T release. Medians and interquartile ranges are presented. No difference in release profiles was identified.**



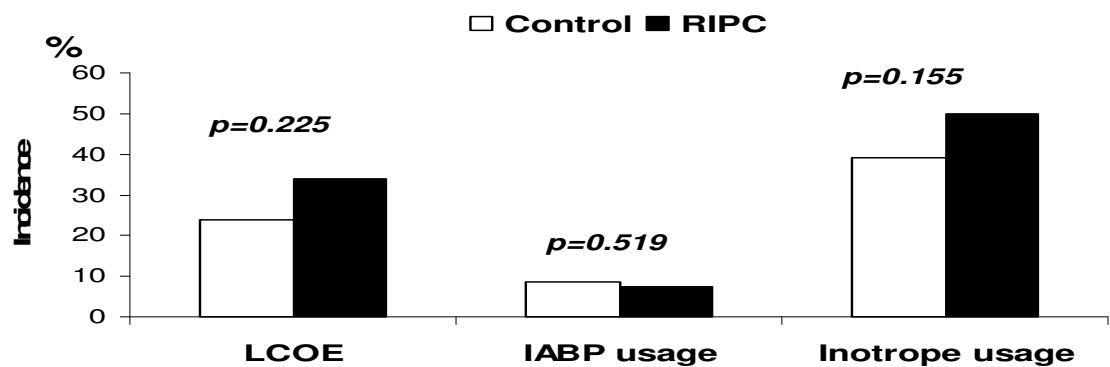
### 3.2 Hemodynamic Effects

Over the measurement period, the CI increased in both groups but there was no significant difference between groups (Figure 4.4). Similar numbers in each group experienced LCOEs or required IABP (Table 4.1; Figure 4.5). Serial LVSWI was not different ( $p=0.844$ ; RMANOVA; Figure 4.6).

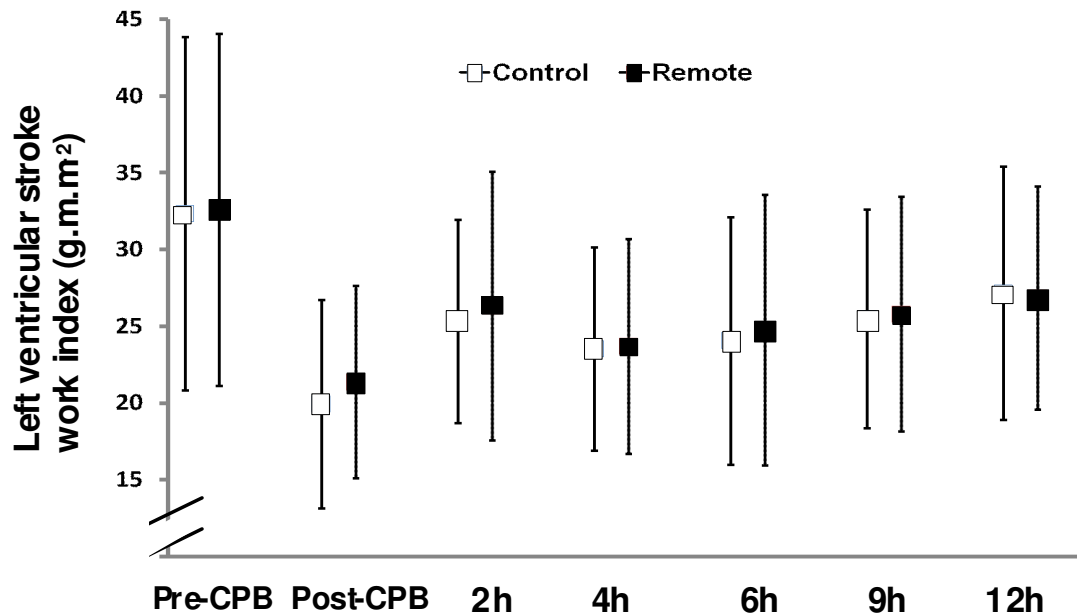
**Figure 4.4: Serial cardiac index measurements in each group**  
(mean $\pm$ SD). Cardiac index increased from pre-operative values in both groups ( $p<0.001$ ) but was not different between groups ( $p=0.620$ ; RMANOVA)



**Figure 4.5: Incidence of Low Cardiac Output Episode (LCOE), Intra-aortic balloon pump (IABP) and Inotrope usage.**



**Figure 4.6: Serial left ventricular stroke work index in each group**  
(mean $\pm$ SD).



### 3.3 Inotrope and vasoconstrictor requirements

Intra-operatively all patients required phenylephrine administration at similar dosage (RIPC 0.36(0.22-0.63) vs. 0.43(0.25-0.67) mg.kg<sup>-1</sup>; p=0.622). A similar number of patients in each group required inotropes (Table 4.1; Figure 4.7) at equivalent dosages (Figure 4.8).

Figure 4.7: Incidence of individual inotrope usage in the first two 6 hour periods. Dopa. = dopamine, Epi.= epinephrine, NorE. = norepinephrine.

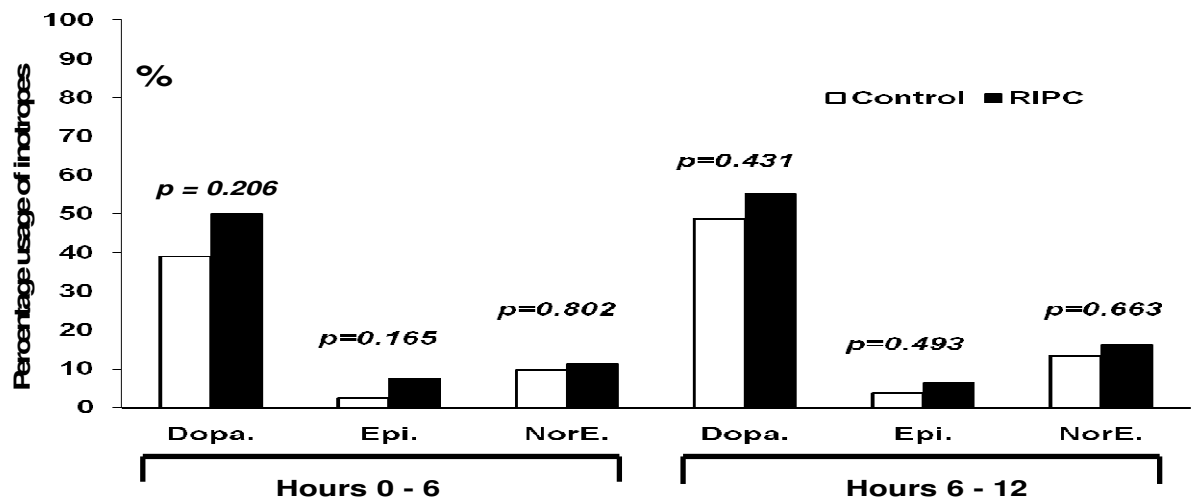
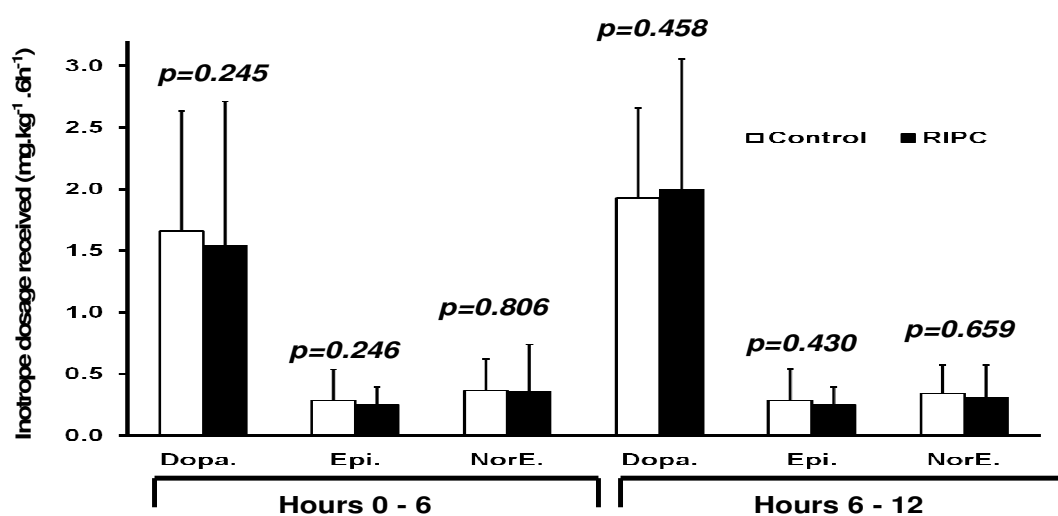


Figure 4.8: Cumulative dose(SD) of inotrope received over 6 hours by group. For presentation, the doses of epinephrine (Epi.) and norepinephrine(NorE.) are multiplied by  $10^{-1}$ .



## 4.0 Discussion

This double-blind study using an identical RIPC stimulus and cTnT assay has not corroborated previous smaller single blind studies that found RIPC to reduce troponin release following on-pump CABG. This absence of effect on measurable myocardial injury is matched by a failure to demonstrate any advantage of RIPC in terms of cardiac performance and inotrope requirement. Our study suggests that RIPC, like its predecessor classical cardiac ischaemic preconditioning (IP), has not fulfilled the promise of a practically useful form of improved myocardial protection. While early studies using classical IP reported reduced troponin release [103][104], improvement in high energy phosphate conservation and reduced inotrope requirement these effects were replicated in some but not all studies [141][142][133][198]. The clinical effect was less obvious and in some reports detrimental[106] and this frustrated the use of classical IP as surgical myocardial protective adjunct.

There is debate regarding the site of origin and significance of troponin release during on-pump CABG. Troponin release may be indicative of myofibrillar damage and myocyte necrosis or changes in sarcolemmal permeability with leakage from cytosolic pools [199][200]. RIPC may protect against necrosis-related but not cytosolic release. CPB may itself affect sarcolemmal permeability or may have a pre-conditioning effect that precludes further protection by RIPC [106].

For assessment of perioperative myocardial infarction and heart failure a range of cardiac biochemical and inflammatory markers have been identified. Although cardiac troponin I (cTnI) is more sensitive and specific than CK-MB[201][202] it has not shown itself to be a better discriminator when

compared to cardiac troponin T (cTnT)[203]. The release of markers in on-CPB surgery is now well established and known to be higher than those off-CPB with the associated sequelae[204][205][206][207].

Postoperative cardiac troponin is an independent predictor of in-hospital death and high concentrations are associated with a cardiac cause of death and major postoperative outcomes[208][209]. The magnitude of release has been shown to be related to the type of cardiac surgical procedure[210] In one series perioperative elevation of cTnI reliably predicted mortality in infants and children undergoing surgical repair of congenital heart defects.[211]

In this RCT cTnT, as a marker of perioperative myocardial infarction and myocardial protective intervention efficacy [212][213][214], has shown no difference between groups when collated up to 48 hours after reperfusion. Thus, we have no evidence to suggest the benefit of a 'first window' of RIPC protection.

To corroborate the evidence all secondary endpoints of myocardial injury: peak cTnT, 6hr cTnT; LCOE incidence; serial cardiac indices; IABP usage; inotrope and vasoconstrictor usage failed to show any advantage for RIPC. To accommodate for changes in systemic vascular resistance left ventricular stroke work indices was compared between groups but again failed to show advantage to those receiving RIPC.

Post-CABG myocardial injury often manifests as a transient period of reversible dysfunction, treatable by hemodynamic optimisation and temporary inotropic support. This implies a cardiac stunning phenomenon and the role of RIPC as a protective measure against stunning is unclear. During clinical PCI, RIPC has been shown to not attenuate post-ischemic stunning [197]. Our

hemodynamic data suggests there is similar absence of effect on reversible dysfunction in CABG.

In this study, we used an identical stimulus and cTnT assay as previous reports that demonstrated a >40% reduction in cTnT release [107][145]. To remove inadvertent bias, we developed a scrupulous technique to blind the surgeon, anaesthetist and investigators from the group allocation. The stimulus was administered within an appropriate time-frame and delivery verified by observing disappearance of the oximetry pulse signal. In the single-blind studies, statistical significance was attained by the much smaller variances observed in cTnT levels in the RIPC group. In the current study, the cTnT release in both groups was similar to that of the control groups in the previous reports. The study design also had important similarities; most patients received a volatile halogenated anaesthetic agent peri-bypass and high percentages of patients received pre-operative statins. Isoflurane, a known pre-conditioning agent was specifically avoided in the current study.

When re-examining the data in this study no subtle evidence appears to exist to explain the comparability between the two groups. Volatile anaesthetics have a known preconditioning effect yet there was no significant difference between groups in the incidence of Enflurane (RIPC 64/80(80%) v 65/82(79%) or Sevoflurane (RIPC 15/80(19%) v 15/82(18%) used. In three cases intravenous propofol alone was used as the anaesthetic agent (RIPC 1/80(1%) v 2/82(2%). No difference between the groups was observed when cTnT AUC was adjusted for calculated CrCl or elective/urgent cases.

Other differences exist between our study and those previously reported. We noted a higher pre-operative usage of beta-blockers continued until the

morning of surgery. Our bypass and cross-clamp times were slightly longer as we used a single clamp technique and we included non-elective patients who had angina or an acute coronary syndrome within 30-days of surgery. However, no patients had experienced angina within 48 hours of surgery and we observed no differences between those admitted electively and more urgent cases.

By excluding the harder to precondition group of diabetics the opportunity to observe a real change from RIPC has been improved. And by further exclusion of patients who have suffered with recent angina (<48hours) there can be confidence that changes observed in primary and secondary endpoints can be attributable to the beneficial effects of our treatment.

As to why the present study did not replicate similar recent studies the answer is more difficult. An initial challenge may be the ischaemic stimulus itself. Interestingly not only the same stimulus was used by Haunseloy et al where a 43% decrease in cTnT AUC was observed but also in the present study verification of the ischaemic stimulus by loss of pulse oximeter trace was confirmed by the ORT. Differences in protocols such as lack of blinding of anaesthetists and investigators; lack of data on dosages of volatile anaesthetics with proven conditioning effects; concomitant AVR with antegrade and retrograde cardioplegia may offer lines of reasoning attributable to the purported benefit of RIPC in one study over the other. Finally, and perhaps most strongly, the statistical difference in the power generated by a study with nearly four times as many patients investigated may offer an explanation [111].

Thus, we conclude, that RIPC fails to augment myocardial protection in patients undergoing CABG. Whether it may have a specific role in higher risk patients undergoing surgery with prolonged cross-clamp times or even cardiac transplantation warrants further study.

Future clinical applications of IP and RIPC will depend on the clarification of the underlying biochemical mechanisms, the development of pharmacological methods to induce preconditioning, and controlled trials in humans showing improved outcomes.

## **5.0 Conclusion**

In contrast to prior smaller studies, RIPC did not reduce troponin release or improve post-operative haemodynamics protection in this double-blind study.

## **CHAPTER 5                    THE FUNCTIONAL IMPACT OF RIPC ON PATIENTS UNDERGOING FIRST TIME CABG**

### **1.0     Introduction**

Coronary artery bypass grafting (CABG) is highly effective in improving life expectancy and quality of life in symptomatic patients with multivessel coronary artery disease [215]. In patients without left main stem stenosis, the major benefit of surgical revascularisation has been demonstrated in those with concomitant LV dysfunction. As a result, echocardiographic estimation of left ventricular ejection fraction (LVEF) has become the main method used in clinical practice to guide surgical management of patients with coronary artery disease [216][217].

### **2.0     Effects of Coronary Artery Surgery on Myocardial Function**

#### *2.1     Coronary Artery Surgery and LV Function*

Increased survival following CABG has only been observed in those with left main stem stenosis and in those with three-vessel coronary artery disease, particularly in the context of impaired LV function [218][219][220][221]. While reduction in LVEF identifies those with most to benefit from revascularisation, it is also a predictor both of in-hospital morbidity [222] and mortality [223]. Although LVEF above 40% is associated with reduced peri-operative complications [224], those with lower values suffer higher in-hospital mortality, longer cardiopulmonary bypass time, and longer hospital stays.

Surgical revascularisation may improve LV function. Hibernation is the presence of resting LV dysfunction due to a reduction in myocardial perfusion

sufficient to prevent efficient contraction but without cell death. In patients with impaired LV function and evidence of myocardial hibernation, CABG increases LVEF and improves regional LV function [225]. Furthermore, surgical revascularisation of hibernating myocardium reduces mortality compared to medical therapy [226]. The improvement in LV systolic function following surgical revascularisation of hibernating myocardium is greatest in those patients with the lowest preoperative LVEF [227].

Although CABG may result in an improvement in LVEF in those with preoperative LV dysfunction, standard on-pump CABG for multi-vessel disease in patients with normal LV function at surgery does not alter LVEF. This has been established in studies comparing multi-vessel arterial revascularisation utilising either off-pump CABG (OP-CABG) or on-pump CABG (ON-CABG). In the standard ON-CABG surgical cases assessed with cine MRI, mean preoperative LVEF was  $62 \pm 12\%$  and mean postoperative LVEF was  $59 \pm 11\%$  at a median 6 days following surgery. Surgical technique however, can affect post-operative LVEF, since there was an absolute difference in post-operative EF between off-pump and on-pump groups [180].

## *2.2 Coronary Artery Surgery and RV Function*

There is growing evidence that CABG is associated with right ventricular (RV) dysfunction. RV function is a major determinant of outcome following on-pump CABG and RV dysfunction is associated with a high mortality [228]. RV function post-CABG is markedly reduced 3 months after on-pump surgery compared with preoperative measurements [229]. Reduction in RV systolic longitudinal function does not improve within the first 3 months after CABG

and recovers partially at 1 year, whereas diastolic tissue velocities show no improvement [230]. This fall in RV long axis contraction occurs in patients who have no obstructive disease within the right coronary artery and who do not have surgery on the right heart [229]. RV depression also occurs after bypass grafting in patients with a moderate stenosis of the right coronary artery that is not revascularized. Revascularization of a more severe stenosis of the right coronary artery in this context however, appears to preserve postoperative RV function [231].

Reduction in RV function does not appear to be related to the type of cardioplegia used during CABG [230][232][233][234]. The use of on-pump or off-pump surgical techniques does not reduce the impact of CABG on RV function. RVEF falls significantly whichever technique is used, with a relative reduction of 12% in the OPCABG group and 7% in the ONCABG group compared to pre-operative values without significant intergroup difference. [235]. In this series, preoperative RVEF was  $66\pm 6\%$  in the OPCABG group and  $65\pm 8\%$  in the ONCABG group. After surgery, early RVEF decreased significantly in both groups ( $P<0.05$ ) with a relative reduction of 12% in the OPCABG group and 7% in the ONCABG group, but without significant intergroup difference ( $p=0.46$ ). This early reduction in RV function recovered completely by 6 months. Because of the number of independent variables involved, a model-building strategy was employed to assess the potential association between baseline variables and early RV function. First a simple regression analysis was performed to examine any potential association between baseline variables (e.g. age, sex, body mass index, preoperative LVEF and RVEF) and early RV function. Variables with  $P<0.1$  were then

included in a multiple linear regression by stepwise selection to assess the 'best' subset in predicting early RV function. This analysis indicated the only predictors of postoperative RV function to be preoperative RV and LV function [235].

Assessment of RV ejection fraction is difficult using 2D echocardiography due to the complex anatomical shape, which means that standard geometric formula for calculation of volumes do not readily apply. Furthermore, the position of the ventricle immediately behind the sternum means that full 2D visualisation of the RV endocardial border cannot be achieved from a single acoustic window. Assessment of RV function has therefore come to rely on alternative methods. The RV myocardium differs in structure and function to that of the LV [236]. There is a greater preponderance of longitudinal fibres within the RV and contraction in this plane is of relatively greater importance to RV function [160]. Methods that rely on assessment of RV long axis function such as tricuspid annular systolic plane excursion (TAPSE) and systolic tissue velocity imaging of the RV lateral annulus (s') therefore have a close correlation with gold standard measurement of RV function, including radionuclide ventriculography and cardiovascular magnetic resonance [237][238].

### *2.3 Effect of IP and RIPC on LV and RV function*

Early reports of the functional benefit of IP and RIPC have been promising. In a porcine model (n=17) RIPC induced by intermittent hindlimb ischaemia (four cycles 5 mins tourniquet inflation and 5 mins deflation; n=9) reduced extent of myocardial necrosis on histology induced by 40 min balloon occlusion of the

LAD compared to controls (n=8) (area of infarction RIPC  $26\pm 9\%$  vs. Control  $53\pm 8\%$ ;  $p<0.05$ ) and calculated mass of myocardial infarction (RIPC 3.5g vs. Control 7.5g,  $p<0.05$ ). The effect on LV function was assessed invasively by real time left ventricular (LV) pressure-volume loops generated by 8 polar conductance catheters with integrated micromanometer. During ischaemia, there was a greater fall in LVEF in controls compared to those with RIPC ( $p=0.02$ ) and a greater increase in the time constant of ventricular relaxation (Tau) in controls ( $p=0.02$ ). At reperfusion however, this difference was abolished and late effects were not studied. [117]

Several clinical reports of RIPC in cardiovascular surgery have been published. In children undergoing congenital heart defect repairs using cardiopulmonary bypass, lower limb RIPC has been shown to reduce troponin release and inotrope requirement [118]. In adults undergoing CABG, intermittent upper limb ischaemia has been followed by reductions in postoperative release of lactate dehydrogenase [144] and troponin T [107]. None of these studies assessed the functional consequences of RIPC on LV and RV function assessed by echocardiography. To-date there has been only one small study of 40 patients listed for CABG for angina assessing the functional consequence of RIPC, recruiting 20 patients randomised prospectively to Control and 20 to IP arms. Those patients who had experienced angina within 48-72 hours (not  $<48$ hours) were included [239]. After establishing CPB and running the pump to empty the heart, the ascending aorta was occluded by cross clamping for 2 min, following 3 min reperfusion, the procedure being repeated once. In the control group, the pump was also running for 10min before routine operation. The temperature

was kept in normothermia during this period. Blood cardioplegia was delivered first antegradely and then retrogradely at temperature of 6-9°C. Warm cardioplegia (37°C) was given retrogradely before release of the aortic cross clamp. CI and RVEF were measured using a fast-response volumetric thermister-tipped pulmonary artery catheter and a microprocessor which allowed measurement of the diastolic washout plateaus of a thermodilution cardiac output curve using exponential curve analysis. Prior to surgery there was no difference in demographics and functional parameters between groups (IP CI  $2.63 \pm 0.29$  vs.  $2.75 \pm 0.58$  L.min<sup>-1</sup>.m<sup>-2</sup>, p=0.51; IP RVEF ( $41.54 \pm 8.22$  vs.  $44.11 \pm 10.8\%$ , p=0.52). After declamping, CI improved in the IP group at 1 hour compared to controls (106% vs. 88% of baseline; p=0.03) but this advantage was not maintained at 6 hours and the 1<sup>st</sup> postoperative day (POD) (123% vs. 116% and 121% vs. 114% of baseline). Similarly RVEF fared better in the IP group at 1 hour (103% vs. 81%; p=0.02) but not at 6 hours and the 1<sup>st</sup> POD (90% vs. 80%; p=0.27 and 94% vs. 81%; p=0.10). There was no difference in CI between the groups after the operation (p=0.14) but the fall in RVEF was less falling CABG in those exposed to IP (p=0.03). Cardiac troponin I and CK-MB levels remained the same.

In summary, outcome from CABG is dependent upon LV function and RV function. Surgical revascularization may improve LVEF in those with pre-operative dysfunction, dependent in part on surgical technique. CABG is associated with a prolonged fall in RV function, predominantly governed by pre—operative status. The effect of IP and RIPC on outcome following CABG has mainly been studied using biochemical surrogate markers and there are no data on an effect on ventricular function beyond the very early post-

operative period. The primary aim of the present study was to assess the effect of remote ischaemic preconditioning during cardiac surgery on left ventricular function assessed by transthoracic echocardiography.

### **3.0 Methods**

Echocardiographic assessment of cardiac outcomes followed methodology set out in Chapter 3; Section 12.0 to 12.3. Statistical analysis was performed as per Chapter 3; Section 17.4.

### **4.0 Results**

Of the 162 patients (Control n=82; RIPC n=80) recruited to the trial 76 patients underwent preoperative echocardiography, the remaining 86 patients did not undergo echocardiographic studies for logistical reasons or resource availability. Of the 76 patients that did undergo preoperative echocardiography 4 patients did not have post-operative echocardiography (Prolonged ITU stay n=2; Logistical reasons n=2) and thus preoperative and postoperative data was available on 72 patients (Control n=38; RIPC n=34) for comparative analysis. Echocardiography was performed with ventricular opacification contrast (SonoVue) (Control n=29/38(76%); RIPC n=26/34(76%)) to assess left and right ventricular size and function. Both groups who did have (n=72) and did not have echocardiography (n=90) were well matched for preoperative and intraoperative variables (Table 5.1). Control (n=38) and RIPC (n=34) groups who did undergo echocardiography were also well matched at baseline (Table 5.2). There were no significant differences in LV geometry, LV systolic function or LV diastolic relaxation at baseline

between the patients assigned to Control and RIPC (Table 5.3). There were no differences in RV size and function at baseline between Control and RIPC (Table 5.3).

Following surgery, there was no change in LV geometry or function within Control or RIPC groups (Table 5.4 and 5.5). The only difference between pre-operative and post-operative echocardiography was the fall in TAPSE but with no other change in RV dimensions or function. There was no difference between the groups following surgery or difference in the change (postop-preop) between Control and RIPC groups (Table 5.6 and 5.7).

When examining all patients (Control and RIPC) as a group, no markers of echo injury was found to correlate with 48 hour cTnT release, (Table 5.8), incidence of inotrope at 0-6 hours (Table 5.9) and at 6-12 hours postoperatively (Table 5.10).

**Table 5.1: Comparison for preoperative demographics and intraoperative variables of those who did and did not undergo echocardiography**

	Echocardiography (n=72)	No Echocardiography (n=90)	p
Age	65(61-71)	64(56-72)	0.28
Male gender (n(%))	82	61	0.31
Body mass index (kg.m <sup>-2</sup> )	28(26-31)	27(25-30)	0.59
Elective (n(%))	34	46	0.64
Urgent (n(%))	38	44	0.64
Euroscore	3(2-5)	3(2-5)	0.27
Logistic Euroscore	2.5(1.5-4.1)	2.1(1.3-3.5)	0.21
Bypass time (min)	98.2±23.5	96.3±19.7	0.58
Cross-clamp time (min)	75.3±18.4	73.4±18.0	0.58

**Table 5.2: Comparison for preoperative demographics and intraoperative variables of Control and RIPC groups of those who underwent echocardiography**

	Control (n=38)	RIPC (n=34)	p
Age	68(63-74)	63(59-68)	0.09
Male gender (n(%))	33	28	0.75
Body mass index (kg.m <sup>-2</sup> )	28(25-31)	29(26-30)	0.91
Elective (n(%))	19	15	0.64
Urgent (n(%))	19	19	0.64
Euroscore	4(2-5)	3(2-5)	0.33
Logistic Euroscore	2.7(1.5-4.3)	2.1(1.4-3.8)	0.28
Bypass time (min)	98.4±20.1	98.0±26.9	0.93
Cross-clamp time (min)	74.5±14.4	76.2±22.3	0.71

**Table 5.3: Analysis of preoperative echocardiographic parameters of LV and RV size and function between Control and RIPC groups. IVSd = Intraventricular septum in diastole; LVIDd = Left ventricular internal dimension in diastole; LVPWd = Left ventricular posterior wall dimension in diastole; LVEDV(i) = Left ventricular end-diastolic volume (index); LVESVi = Left ventricular end-systolic volume index; LVEF = Left ventricular ejection fraction; IVA = Isovolumic acceleration; RVIDd = Right ventricular internal dimension in diastole; TAPSE = Tricuspid annular plane systolic excursion.**

	Control	RIPC	p
<b>LV Size</b>			
IVSd (cm)	1.6±0.4	1.5±0.4	0.25
LVIDd (cm)	4.7±0.6	4.8±0.6	0.51
LVPWd (cm)	1.1±0.2	1.1±0.3	0.82
LVEDV Contrast (ml)	117±45	111±40	0.59
LVEDVi Contrast(ml.m <sup>-2</sup> )	58.5±19.6	55.3±18.4	0.54
LVESVi Contrast(ml.m <sup>-2</sup> )	22.9±13.8	24.2±15.6	0.75
<b>LV Function</b>			
LVEF Contrast (%)	61.6±13.4	58.5±13.3	0.40
Average IVA	2.0±0.7	2.0±0.7	0.56
LVTei	0.6±0.2	0.7±0.3	0.58
E/A	0.9±0.2	1.0±0.3	0.07
Deceleration Time (ms)	227±68	203±46	0.09
e'	0.07±0.04	0.07±0.04	0.37
E/e'	11.6±8.8	10.9±6.2	0.34
LA Volume (ml)	33±19	34±17	0.73
<b>RV Size</b>			
RVIDd (cm)	2.7±0.6	2.7±0.4	0.95
<b>RV Function</b>			
TAPSE (mm)	21±5	23±5	0.15
RVTei	0.6±0.3	0.5±0.1	0.17

**Table 5.4: Within Control group analysis preoperative and postoperatively of echocardiographic parameters of LV and RV size and function. IVSd = Intraventricular septum in diastole; LVIDd = Left ventricular internal dimension in diastole; LVPWd = Left ventricular posterior wall dimension in diastole; LVEDV(i) = Left ventricular end-diastolic volume (index); LVESVi = Left ventricular end-systolic volume index; LVEF = Left ventricular ejection fraction; IVA = Isovolumic acceleration; RVIDd = Right ventricular internal dimension in diastole; TAPSE = Tricuspid annular plane systolic excursion.**

	Preop	Postop	P
<b>LV Size</b>			
IVSd (cm)	1.6±0.4	1.7±0.3	0.19
LVIDd (cm)	4.7±0.6	4.7±0.7	0.27
LVPWd (cm)	1.1±0.2	1.2±0.3	0.42
LVEDV Contrast (ml)	117±45	111±38	0.27
LVEDVi Contrast (ml.m <sup>-2</sup> )	58.5±19.6	55.4±17.1	0.24
LVESVi Contrast(ml.m <sup>-2</sup> )	22.9±13.8	23.0±15.4	0.24
<b>LV Function</b>			
LVEF Contrast (%)	61.6±13.4	61.8±17.0	0.30
Average IVA	2.0±0.7	2.2±0.7	0.24
LVT <sub>ei</sub>	0.6±0.2	0.7±0.2	0.24
E/A	0.9±0.2	1.1±0.3	0.21
Deceleration Time (ms)	227±68	171±48	0.24
e'	0.07±0.04	0.07±0.04	0.12
E/e'	11.6±8.8	12.4±7.6	0.24
LA Volume (ml)	33±19	37±22	0.26
<b>RV Size</b>			
RVIDd (cm)	2.7±0.6	2.7±0.5	0.32
<b>RV Function</b>			
TAPSE (mm)	21±5	12±3	<0.01
RVTei	0.6±0.3	0.6±0.3	0.25

**Table 5.5: Within RIPC group analysis preoperative and postoperatively of echocardiographic parameters of LV and RV size and function. IVSd = Intraventricular septum in diastole; LVIDd = Left ventricular internal dimension in diastole; LVPWd = Left ventricular posterior wall dimension in diastole; LVEDV(i) = Left ventricular end-diastolic volume (index); LVESVi = Left ventricular end-systolic volume index; LVEF = Left ventricular ejection fraction; IVA = Isovolumic acceleration; RVIDd = Right ventricular internal dimension in diastole; TAPSE = Tricuspid annular plane systolic excursion.**

	Preop	Postop	P
<b>LV Size</b>			
IVSd (cm)	1.5±0.4	1.5±0.2	0.25
LVIDd (cm)	4.8±0.6	4.9±0.7	0.24
LVPWd (cm)	1.1±0.3	1.1±0.3	0.21
LVEDV Contrast (ml)	111±40	101±37	0.09
LVEDVi Contrast(ml.m <sup>-2</sup> )	55.3±18.4	51.4±19.9	0.24
LVESVi Contrast(ml.m <sup>-2</sup> )	24.2±15.6	22.0±14.0	0.24
<b>LV Function</b>			
LVEF Contrast (%)	58.5±13.3	58.7±16.7	0.26
Average IVA	2.0±0.7	2.2±0.7	0.24
LVTei	0.7±0.3	0.7±0.2	0.24
E/A	1.0±0.3	1.2±0.4	0.33
Deceleration Time (ms)	203±46	154±33	0.26
e'	0.07±0.04	0.07±0.05	0.19
E/e'	10.9±6.2	13.4±13.1	0.24
LA Volume (ml)	34±17	35±18	0.20
<b>RV Size</b>			
RVIDd (cm)	2.7±0.4	2.7±0.5	0.21
<b>RV Function</b>			
TAPSE (mm)	23±5	12±3	<0.01
RVTei	0.5±0.1	0.5±0.2	0.24

**Table 5.6: Analysis of postoperative echocardiographic parameters of LV and RV size and function between Control and RIPC groups. IVSd = Intraventricular septum in diastole; LVIDd = Left ventricular internal dimension in diastole; LVPWd = Left ventricular posterior wall dimension in diastole; LVEDV(i) = Left ventricular end-diastolic volume (index); LVESVi = Left ventricular end-systolic volume index; LVEF = Left ventricular ejection fraction; IVA = Isovolumic acceleration; RVIDd = Right ventricular internal dimension in diastole; TAPSE = Tricuspid annular plane systolic excursion.**

	Control	RIPC	P
<b>LV Size</b>			
IVSd (cm)	1.7±0.3	1.5±0.2	0.05
LVIDd (cm)	4.7±0.7	4.9±0.7	0.32
LVPWd (cm)	1.2±0.3	1.1±0.3	0.23
LVEDV Contrast (ml)	111±38	101±37	0.36
LVEDVi Contrast(ml.m <sup>-2</sup> )	55.4±17.1	51.4±19.9	0.46
LVESVi Contrast(ml.m <sup>-2</sup> )	23.0±15.4	22.0±14.0	0.82
<b>LV Function</b>			
LVEF Contrast (%)	61.8±17.0	58.7±16.7	0.52
Average IVA	2.2±0.7	2.2±0.7	0.89
LVTei	0.7±0.2	0.7±0.2	0.79
E/A	1.1±0.3	1.2±0.4	0.07
Deceleration Time (ms)	171±48	154±33	0.09
e'	0.07±0.04	0.07±0.05	0.52
E/e'	12.4±7.6	13.4±13.1	0.70
LA Volume (ml)	37±22	35±18	0.60
<b>RV Size</b>			
RVIDd (cm)	2.7±0.5	2.7±0.5	0.91
<b>RV Function</b>			
TAPSE (mm)	12±3	12±3	0.37
RVTei	0.6±0.3	0.5±0.2	0.31

**Table 5.7: Analysis of  $\Delta$ (Postop-Preop) echocardiographic parameters of LV and RV size and function between Control and RIPC groups. IVSd = Intraventricular septum in diastole; LVIDd = Left ventricular internal dimension in diastole; LVPWd = Left ventricular posterior wall dimension in diastole; LVEDV(i) = Left ventricular end-diastolic volume (index); LVESVi = Left ventricular end-systolic volume index; LVEF = Left ventricular ejection fraction; IVA = Isovolumic acceleration; RVIDd = Right ventricular internal dimension in diastole; TAPSE = Tricuspid annular plane systolic excursion.**

	Control	RIPC	P
<b>LV Size</b>			
$\Delta$ IVSd (cm)	0.0 $\pm$ 0.4	0.1 $\pm$ 0.3	0.89
$\Delta$ LVIDd (cm)	0.0 $\pm$ 0.6	0.0 $\pm$ 0.7	0.70
$\Delta$ LVPWd (cm)	0.1 $\pm$ 0.3	0.02 $\pm$ 0.3	0.24
$\Delta$ LVEDV Contrast (ml)	-10 $\pm$ 30	-14 $\pm$ 39	0.69
$\Delta$ LVEDVi Contrast(ml.m <sup>-2</sup> )	-4.8 $\pm$ 15.3	-4.2 $\pm$ 16.2	0.90
$\Delta$ LVESVi Contrast(ml.m <sup>-2</sup> )	-0.4 $\pm$ 9.3	-2.4 $\pm$ 9.9	0.46
<b>LV Function</b>			
$\Delta$ LVEF Contrast (%)	-0.4 $\pm$ 16.7	0.0 $\pm$ 15.0	0.64
$\Delta$ Average IVA	0.1 $\pm$ 0.7	0.2 $\pm$ 0.7	0.74
$\Delta$ LVTei	0.0 $\pm$ 0.3	0.0 $\pm$ 0.3	0.42
$\Delta$ E/A	0.2 $\pm$ 0.3	0.2 $\pm$ 0.4	0.67
$\Delta$ Deceleration Time (ms)	-63 $\pm$ 55	-48 $\pm$ 50	0.25
$\Delta$ e'	0.00 $\pm$ 0.03	0.00 $\pm$ 0.03	0.73
$\Delta$ E/e'	0.4 $\pm$ 9.0	1.3 $\pm$ 9.9	0.36
$\Delta$ LA Volume (ml)	4 $\pm$ 13	-1 $\pm$ 20	0.22
<b>RV Size</b>			
$\Delta$ RVIDd (cm)	-0.1 $\pm$ 0.7	0.0 $\pm$ 0.5	0.63
<b>RV Function</b>			
$\Delta$ TAPSE (mm)	-9 $\pm$ 5	-11 $\pm$ 6	0.08
$\Delta$ RVTei	-0.1 $\pm$ 0.4	0.0 $\pm$ 0.2	0.33

**Table 5.8: Correlation of echo injury with 48 hour AUC cTnT release (all patients)**

	Value	Correlation coefficient	p
ΔLVEF(Contrast)	-0.2±15.7	-0.13	0.36
ΔLV Tei	0.0±0.3	-0.02	0.89
ΔRV Tei	-0.1±0.3	0.16	0.21
ΔTAPSE	-10±6	-0.09	0.53
ΔAverage IVA	0.1±0.7	0.17	0.17

**Table 5.9: Correlation of echo injury with 0-6hrs inotrope incidence (all patients)**

	Value	Correlation coefficient	p
ΔLVEF(Contrast)	-0.2±15.7	-0.08	0.49
ΔLV Tei	0.0±0.3	-0.01	0.91
ΔRV Tei	-0.1±0.3	-0.01	0.90
ΔTAPSE	-10±6	-0.03	0.78
ΔAverage IVA	0.1±0.7	-0.13	0.20

**Table 5.10: Correlation of echo injury with 6-12 hours inotrope incidence (all patients)**

	Value	Correlation coefficient	p
ΔLVEF(Contrast)	-0.2±15.7	-0.03	0.82
ΔLV Tei	0.0±0.3	0.10	0.32
ΔRV Tei	-0.1±0.3	0.08	0.43
ΔTAPSE	-10±6	0.07	0.55
ΔAverage IVA	0.1±0.7	-0.14	0.16

## 5.0 Discussion

This double-blind study is the first to demonstrate that no functional benefit on left or right ventricular function assessed by transthoracic echocardiography arises from RIPC during on-pump CABG.

Early evidence for a benefit from RIPC on LV function had been promising. Kharbanda et al in a porcine model undergoing intermittent hindlimb ischaemia observed a greater fall in LV function, measured invasively, during ischaemia in controls. Unfortunately this was not maintained at reperfusion and late effects were not studied perhaps suggesting that benefit in LV function is short-lived (minutes) and long term functional benefit is unlikely.

In the present study markers of left heart function (LVEF with contrast; LV Tei) remained comparable between groups when measured following surgery. The primary endpoint selected was the load-independent IVA which remained unchanged but there were no changes in LVEF using contrast or in global measures of systolic and diastolic relaxation. Due to logistical reasons, the study did not recruit the planned 146 patients (73 patients in each group) so as to achieve the planned power to detect a difference in IVA  $\geq 0.5SD$  ( $\alpha$  0.05,  $1-\beta$  0.85). Therefore, the lack of effect in this study could have been due to a lack of statistical power arising from the failure to perform echocardiography in all subjects recruited. With the achieved sample size of 72 patients however (Control  $n=38$ ; RIPC  $n=34$ ), this study would still have the power to detect a difference in IVA  $\geq 0.675SD$  ( $\alpha$  0.05,  $1-\beta$  0.80). The observed difference in  $\Delta IVA$  between the two groups was non-significant ( $p=0.74$ ) and the 95% confidence interval of the difference was small (-0.4 to 0.3). From this, it is

suggested that should a difference between the two groups exist as a result of RIPC, this is likely to be small and offer minimal clinical benefit.

Another potential reason for the failure to demonstrate an effect could be the degree of variability between pre-operative and post-operative measurements on echocardiography. Image acquisition is dependent not only on observer seniority but also patient morphological variation. Intra-patient variability exists particularly in the post-operative setting where “poor acoustic windows” are commonplace. Poor pain control, less patient compliance, post-operative fluid and ventilation requirements result in a difficulty in obtaining a “good acoustic window” postoperatively. This variability may be reflected in the wide standard deviations for the LVEF values recorded, albeit similar in both groups. The inter-study variability in our population was minimised as far as possible by using a single blinded observer to analyse all studies.

Intraoperative transoesophageal echocardiography was not performed and so comment on any immediate, albeit short lived, benefit on contractility is not possible. From a clinical perspective data on longer term functional improvement is more relevant and it is clear RIPC failed to achieve this. This failure should not be due to inadequate RIPC stimulus, since an identical stimulus to that which in previous reports was associated with a >40% reduction in cTnT release. The RIPC stimulus was administered in this study within an appropriate time-frame and delivery was verified by observing disappearance of the oximetry pulse signal. One possibility is that a greater effect of RIPC would be documented in patients with abnormal LVEF prior to CABG, in whom improvement following revascularisation of hibernating segments might be more subject to variation in peri-operative conditions. This

study did not directly address the issue of whether RIPC could afford additional *benefit* to improvement in LV function in those undergoing CABG with impaired LV function and hibernation, and was restricted to patients with normal EF at entry.

At present there is no data in the literature that has investigated the effects of RIPC on RV function however the effect of IP has been studied. In a clinical trial Wu et al showed that an IP stimulus (crossclamping of the aorta) with antegrade and retrograde cold blood cardioplegia followed by a hot shot improves cardiac index and RVEF 1 hour after declamping. Yet again though this immediate advantage was not replicated at 6 hours nor on the first postoperative day. Indeed multiple samples of cardiac troponin I and CKMB, markers of myocardial necrosis, up to the second postoperative day were similar between groups. Our study has corroborated the findings that preconditioning does not preserve late RV function (TAPSE and RV Tei).

Within groups, markers of load-dependent (LVEF with contrast; LV Tei) and load-independent (IVA) heart function was unchanged following surgery. Conversely, reduced TAPSE but unchanged RV Tei in both groups would suggest a reduction in RV longitudinal function that is compensated for circumferentially as global function remains unchanged. Current myocardial protection techniques would appear to be effective in the LV but are sub-optimal in the RV. This reduced RV function was not related to peri-operative myocardial necrosis or inotrope use. Indeed deleterious echocardiographic change post-CABG is not predicted by cTnT release or inotrope requirement up to 12 hours postoperatively.

## **6.0 Conclusion**

In this double-blind study, RIPC has failed to afford benefit in LV or RV contractile function 5-7 days following CABG in spite of manoeuvres designed to overcome inherent difficulties: intravenous ultrasound contrast; measurement of load-independent IVA. RV longitudinal function is reduced post-CABG but overall global RV function is maintained.

## **CHAPTER 6                    THE EFFECTS OF RIPC ON ARRHYTHMIAS ON PATIENTS UNDERGOING FIRST TIME CORONARY ARTERY BYPASS SURGERY**

### **1.0    Introduction**

In a porcine model of remote ischaemic preconditioning (RIPC), short cycles of repeated limb ischaemia during myocardial ischaemia have reduced myocardial infarction by >20% and protected against malignant arrhythmia during the reperfusion phase through a K(ATP) channel-dependent mechanism [240]. Partial hindlimb occlusion has also delayed the development of ischemia-induced ventricular arrhythmias induced by coronary artery occlusion [241] in experimental models.

Although as yet no study of the effects of RIPC on arrhythmias in humans has been reported, IP has been investigated. In one series preinfarction angina, a clinical form of preconditioning, has been positively associated with protection from the development of sustained ventricular tachycardia and ventricular fibrillation (VT/VF) after reperfusion (by PCI) following STEMI. [242] VT/VF is a significant determinant of long-term major adverse cardiac events. Continuous Holter ECG monitoring of patients with variant angina has demonstrated that preconditioning by transient ischemia induces protection against ischemia-induced complex ventricular arrhythmias [243].

Ischaemic heart disease carries an increased risk of malignant VF and VT and sudden death [244][245][246][247][248][249] and post-CABG both VF and VT have been identified as potential life threatening complications. In a RCT of 86 patients undergoing CABG only (three vessel disease) IP has been

shown to reduce postoperative VF (48.8% vs. 79.1%;  $p < 0.01$ ) and VT, the mechanical ventilation period and need for inotropes [250]. IP has also been observed to reduce the QT dispersion provoked by coronary artery occlusion and reperfusion during coronary angioplasty.[251] Preconditioning with adenosine, which has been implicated in the IP pathway, has been shown to suppress the frequency of pre- and post-ischaemic tachyarrhythmia's against an ischaemia-reperfusion insult in human myocardium harvested from CABG patients [252].

The timing of the conditioning stimulus does not appear to influence effect. In a rat model of postconditioning ventricular arrhythmia after regional ischemia was markedly attenuated [253].

However analyses of the effects of preconditioning on ischaemia and reperfusion-induced rhythm disturbances have yielded controversy. In a porcine model no changes were observed in the refractory duration, ventricular vulnerability or defibrillation energy requirements up to 90 min after ventricular ischaemic preconditioning [254].

In a prospectively randomised trial (n=35) Ascione et al observed antegrade cold blood cardioplegia to be associated with less ischaemic stress and myocardial injury than antegrade warm blood cardioplegia in hypertrophic hearts of patients undergoing aortic valve surgery. Troponin I release and the total incidence of postoperative arrhythmias was higher in the warm blood group. As the evidence pointed towards myocardial injury being responsible for the occurrence of supraventricular arrhythmias [178], this endpoint following reperfusion may be an important index of myocardial protection.

The aims of this study were to confirm the role of RIPC in reducing perioperative MI (LBBB or new onset Q waves), ventricular tachyarrhythmia and treated atrial fibrillation rates in non-diabetic adults undergoing first time CABG.

## 2.0 Methods

Three lines of investigation were employed to assess and compare perioperative arrhythmias between the groups as set out in Chapter 3; Section 13.0 to 13.3.

## 3.0 Results

### 3.1 Perioperative myocardial infarction (PMI) outcomes

148 patients (Control n=75; RIPC n=73) underwent 12 lead electrocardiography preoperatively through to postoperative day 4. No difference in the incidence of LBBB or new onset Q waves 2mm depth in 2 leads by postoperative day 4 was found between the two groups (Table 6.1).

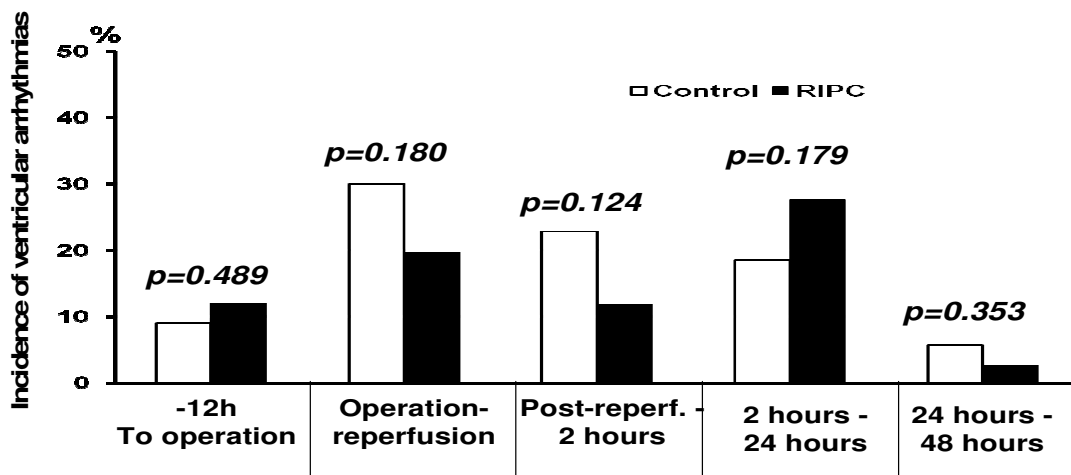
**Table 6.1: Post-operative dysrhythmias. For 12 lead ECG data Control n=75, RIPC n=73. AF = atrial fibrillation; LBBB = left bundle branch block.**

	<b>Control (n=82)</b>	<b>RIPC (n=80)</b>	<b>p=</b>
New onset LBBB (n(%))	3/75(4%)	0/73(0%)	0.25
New onset Q wave (n(%))	4/75(5.3%)	4/73(5.5%)	1.00
Incidence of treated AF (n(%))	30(37%)	28(35%)	0.87

### 3.2 Continuous ECG recording Outcomes

Continuous ECG monitoring was performed in 146 patients (RIPC n=76 vs. Control n=70). Initial heart rate at the start of monitoring in both groups was comparable (RIPC 47(43-51) vs. Control 47(43-50) bpm). There was no difference in the incidence of reperfusion ventricular fibrillation during the 10 minutes after AXC release (RIPC 15.8% vs. Control 11.4%;  $p=0.43$ ). After the reperfusion period there were no episodes of sustained ventricular tachyarrhythmia requiring treatment. Brief non-sustained tachyarrhythmia's occurred in 48/76(63.2%) RIPC and 43/70(61.4%) Control patients in the first post-operative 24 hours. There was no difference in tachyarrhythmia incidence at different time points between groups (Figure 6.1).

**Figure 6.1: Rates of detected non-sustained ventricular tachyarrhythmia's in each group: -12h to operation ( $p=0.489$ ); operation to reperfusion( $p=0.180$ ); post-reperfusion to 2 hours ( $p=0.124$ ); 2-24hours( $p=0.179$ ); 24-48 hours ( $p=0.353$ )**



### 3.3 *Atrial fibrillation - treated*

The overall incidence of treated atrial fibrillation was 58(36%) (RIPC 28(35%) vs. Control 30(37%);  $p=0.87$ ) (Table 6.1).

## 4.0 Discussion

This double-blind study is the first to demonstrate that RIPC has no role in reducing arrhythmia rates during on-pump CABG in non-diabetic adults.

No previous studies have investigated the anti-arrhythmic potential of RIPC in humans but reports from experimental models had been promising. In a porcine model four 5 minute cycles of lower limb ischaemia by tourniquet inflation had reduced myocardial infarction by >20% and protected against malignant arrhythmia. The present study, however, has failed to replicate this advantage. All markers of arrhythmia: perioperative MI (12 lead ECG); ventricular tachyarrhythmia incidence (continuous Holter monitor); treated AF incidence have afforded no anti- or pro-arrhythmic potential in humans.

A difference in protocols may have prevented change. The porcine model employed one more cycle of 5 minutes limb ischaemia than the present study and selected the lower limb. It is possible that this may have conferred extra benefit but seems unlikely as this study utilised an identical stimulus as previous reports that demonstrated a >40% reduction in cTnT release on the upper limb [107][145]. Whether response to RIPC is species-dependent is possible and identical protocols would have to be used as part of a trial to deduce the answer.

The benefit afforded by IP in humans has been documented. In a randomised controlled trial by Wu et al postoperative ventricular tachyarrhythmia's have

been reduced by >30% in patients undergoing on-pump CABG following two cycles of cross-clamping the aorta for 2 minutes followed by 3 minutes of reperfusion[250]. The present study however has been unable to replicate these positive findings.

An explanation in the differences observed may lie in the different cardioplegic protocol employed by Wu et al. Using both an antegrade and retrograde delivery system for the cold blood cardioplegia and then completing the myocardial protection with a further 3 minutes of warm cardioplegia (37°C) prior to release of the aortic cross clamp may have offered additional protective benefit. A further possibility for the difference between the IP and RIPC patients may lie within the level of ischaemia generated by the preconditioning stimuli. Crossclamping the ascending aorta may generate a more substantial conditioning stimulus as compared to the 3 cycles of 5min inflation/deflation peripherally used in the RIPC protocol. This in turn may be sufficient to generate a functional change which the RIPC stimulus, although able to activate pathways, is simply not strong enough to match and thus bring about functional change.

A much lower overall incidence of reperfusion fibrillation and a lower overall rate in the post-operative 48 hour period was found. This suggests important differences in protection efficacy between studies but the lower arrhythmia incidence in the current study makes it under-powered to exclude a lack of protection by RIPC for this end-point.

Nonetheless at present the evidence is not in favour of any anti-arrhythmic potential afforded by RIPC to humans. A randomised controlled trial using a

different endpoint with higher quantitation of arrhythmia incidence may be more suitable in detecting change between treatment groups.

## **5.0 Conclusions**

RIPC does not offer any anti-arrhythmic benefit in non-diabetic adult humans undergoing first time coronary artery bypass surgery. It has not shown any potential for pro-arrhythmia also.

## **CHAPTER 7            THE EFFECT OF RIPC ON RENAL OUTCOMES IN PATIENTS UNDERGOING FIRST TIME CORONARY ARTERY BYPASS SURGERY**

### **1.0    Introduction**

Acute kidney injury (AKI) is a common and prognostically important complication of cardiac surgery. It is independently associated with in-hospital mortality, even after adjustment for comorbid disease and other complications, all degrees of AKI are associated with increased mortality and other adverse outcomes. [255].

In a series of 817 patients undergoing on-pump CABG, a discrete alteration in renal function (AKI) defined as an increase of  $0.3 \text{ mg.dL}^{-1}$  or 50% in baseline creatinine was found in 52% of patients and independently predicted death within 30 days ( $p < 0.01$ ). These patients were observed to have higher 30 day mortality (12.6% vs. 1.4%;  $p < 0.01$ ), longer intensive care unit (ICU) stay (median 2(2-3) vs. 3(2-5) days;  $p < 0.01$ ) and a larger proportion of patients with prolonged ICU stay ( $>14$  days) (14 vs. 2%;  $p < 0.01$ ) [256].

In a larger series of 2672 patients undergoing CABG, 7.9% patients developed acute renal failure (ARF), defined as a rise in serum Cr  $>1.0 \text{ mg/dl}$ , and 0.7% developed acute renal failure requiring some form of dialytic therapy (ARF-D). ARF and ARF-D patients were observed to have increased mortality (14% vs. 1%;  $p < 0.01$  and 28% vs. 1.5%;  $p < 0.01$  respectively). Increased age, elevated preoperative Cr, duration of CPB, carotid artery bruit and diabetes were associated with development of AFR and ARF-D by multivariate analysis. [257] Other risk factors identified for AKI development in

a multicenter cohort of 3500 patients undergoing cardiac surgery were left ventricular dysfunction, presence of IABP, urgent surgery, preoperative anaemia, perioperative RBC transfusions and postoperative re-exploration[255].

The aetiology of renal injury has been linked strongly to preoperative and intraoperative factors, in those presenting for cardiac surgery, that result in renal cellular ischaemia induced tubular epithelial and vascular endothelial injury and activation [258][259].

Preoperatively, patients tend to have impaired autoregulation due to pre-existing comorbidities, (advanced age, atherosclerosis, chronic hypertension or chronic kidney disease), renal-injurious medication (NSAIDS, ACEI, Angiotensin receptor blockers) or a pro-inflammatory state. Normal renal perfusion is autoregulated such that the glomerular filtration rate is maintained until the mean arterial blood pressure (MABP) falls below 80mmHg [259]. During surgery MABP is often at the lower limits of normal autoregulation and especially during periods of haemodynamic instability these patients are vulnerable to ischaemic renal injury.

Intraoperatively, proinflammatory events such as operative trauma, contact of the blood components with the artificial surface of the CPB circuit, ischaemia-reperfusion injury, and endotoxaemia [260][261][262] contribute to injury. The generation of free haemoglobin and iron from haemolysis during CPB [260] has also been linked.

Early experimental and human models have reported positively to the application of RIPC in cardiovascular surgery. Temporary infrarenal aortic occlusion in an animal model, during open thoracoabdominal aneurysm repair

has been shown to decrease IR renal injury caused by subphrenic aortic cross-clamping [263] and patients undergoing elective open abdominal aortic aneurysm repair demonstrate a 23% reduction in the incidence of renal impairment with intermittent common iliac artery cross clamping[143].

More recently, in a RCT of patients undergoing endovascular repair (EVAR) for abdominal aortic aneurysms (n=40), 18 patients undergoing sequential lower limb ischaemia had a reduced increase in urinary markers of renal injury (urinary retinol binding protein and urinary albumin:creatinine ratio(ACR) [264] however no differences in the rates of renal impairment or major adverse cardiac events were observed.

Reports of ischaemic preconditioning in experimental models have been equally encouraging. Intermittent renal artery clamping (renal IP) has been shown to ameliorate ischaemic renal injury as assessed by functional, metabolic and morphological methods [265][266][267][268][269][270]. Attenuation of NF-kappa B activity, downregulation of IL-18 expression, attenuation of NGAL and IKKbeta have been implicated in the renoprotective pathways [159][270]. Ischemic postconditioning has also been shown to decrease apoptosis and improve renal function, effects possibly related to activation of Akt and ERK $\frac{1}{2}$  activation.[271]

In the assessment of renal injury both proteinuria and microalbuminuria have an established role in diagnosis and management. The glomerular membrane selectively allows passage of water and low molecular weight solutes into the tubule and restricts passage of larger molecular weight plasma proteins, based on a combination of molecular size, shape and charge. Despite the protein-retaining properties of the glomerular membrane, some protein does

pass into the proximal tubular fluid. Only a very small proportion of plasma proteins with a molecular weight >50 000 Da normally reaches the urine. This is primarily due to retention of large molecular weight proteins by the glomeruli, and tubular reabsorption of lower molecular weight proteins that pass through the glomerular membrane more easily. Proteins such as albumin which do reach the glomerular filtrate are reabsorbed in the proximal tubules by a low capacity high affinity mechanism.

The National Kidney Foundation Guidelines recommends urine albumin as a sensitive marker for chronic renal disease due to diabetes, glomerular disease and hypertension. Urinary albumin:creatinine ratio (ACR) may also be used as a sensitive and early marker of primary and secondary renal disease[272][273][274]. A reduction in the 24 h urinary ACR by Walsh et al following intermittent lower limb ischaemia (median 5 vs. 8.8;  $p=0.06$ ) in patients undergoing EVAR reconfirms the relevance of this test in a cardiovascular setting [264].

The glomeruli filter larger amounts of low molecular weight proteins (<50 000 Daltons). Measurement of the urinary content of low molecular weight proteins such as  $\alpha$ 1-microglobulin can be used as sensitive markers of proximal tubular function because. Under normal conditions filtered low molecular weight proteins are almost completely reabsorbed by the proximal tubule, but even subtle tubular damage is associated with increased urinary excretion[275].

The aims of this study were to confirm the role of RIPC in reducing markers of renal injury (peak creatinine, significant increase in creatinine ( $>44 \mu\text{mol.L}^{-1}$ ),

$\Delta$ -creatinine (day 0-4), 24h area under the curve (AUC) ACR, 24 h  $\alpha_1$ - $\mu$ globulin) in non-diabetic adults undergoing first time CABG.

## **2.0 Methods**

Changes in serum creatinine, urinary albumin:creatinine ratio, urinary  $\alpha_1$ -microglobulin, and requirement for diuretics or haemofiltration were used as outcome measures to assess the effect of renal protection by RIPC on peri-operative renal injury in this double-blind, randomised controlled trial. Protocols for assessment of renal outcomes are set out in Chapter 3; Section 14.0 to 14.4.2. Statistical analysis was performed as per Chapter 3; Section 17.3.

## **3.0 Results**

Of 162 patients (89% males) 80 were randomized to treatment and 82 to placebo. The groups were well matched for pre-operative renal function (Serum creatinine: Control  $96 \pm 16$  vs. RIPC  $98 \pm 16$   $\mu\text{mol.L}^{-1}$ ). The mean time on bypass ( $96 \pm 22$  vs.  $100 \pm 23$ ;  $p=0.25$ ) mins and aortic cross-clamp times ( $71 \pm 18$  vs.  $76 \pm 21$ ;  $p=0.08$ ) mins were not significantly different (Table 3.8).

Peak creatinine, day 4 creatinine levels and  $\Delta$ -creatinine between days 0-4 were not different. Similar numbers of patients had increases  $>44$   $\mu\text{mol.L}^{-1}$ . Three RIPC (3.8%) and one (1.2%) control patient required temporary dialysis. There was no difference in 24h AUC urine ACR or 24 hour urine  $\alpha$ -microglobulin levels (Table 7.1).

**Table 7.1: Post-operative renal outcomes. For creatinine Control n=77, RIPC n=75. ACR = albumin:creatinine ratio.**

	<b>Control (n=82)</b>	<b>RIPC (n=80)</b>	<b>p=</b>
Peak creatinine ( $\mu\text{mol.L}^{-1}$ )	106(97-123)	106(88-123)	0.61
>44 $\mu\text{mol.L}^{-1}$ increase in creatinine (n(%))	8/77(10)	5/75(7)	0.56
$\Delta$ - creatinine (day 0-4) ( $\mu\text{mol.L}^{-1}$ )	-0.88(-10.6-7.9)	-2.6(-8.8-7.9)	0.67
24h AUC ACR	69(40-112)	58(32-85)	0.08
Pre-op. $\alpha_1$ - $\mu\text{globulin}$ ( $\text{mg.L}^{-1}$ )	8(4-16)	8(5-13)	0.75
24 h $\alpha_1$ - $\mu\text{globulin}$ ( $\text{mg.L}^{-1}$ )	51(38-71)	45(28-74)	0.42
Temporary dialysis (n(%))	1(1)	3(4)	0.36
Intravenous frusemide infusion (n(%))	6(7)	8(10)	0.59

#### **4.0 Discussion**

This double-blind study is the first to demonstrate that RIPC has no role in reducing rates of acute renal injury following on-pump CABG in non-diabetic adults.

No previous studies have investigated the renal protective effects of RIPC following cardiac surgery. Following three relatively small experimental and human studies: Lazaris et al (n=26); Walsh et al (n=40); Ali et al (n=82), the evidence for a beneficial effect of RIPC on aortic aneurysmal surgery (including endovascular) has been encouraging.

However, by failing to reduce 24h AUC urinary ACR, RIPC has delivered no detectable protection at the glomerular level from the insult of cardiac surgery.

There is also no suggestion of protection against damage to tubular reabsorptive function as 24 h  $\alpha_1$ -microglobulin levels between the groups were similar. A difference in protocol between the aneurysmal studies and the present study may offer a clue to the lack of the purported effect. In the rat

model of Lazaris et al a vascular clamp was applied on the infra-renal aorta just above its bifurcation for 15 minutes and in Ali et al's human model the right iliac vessel was crossclamped for 10 minutes and then released and applied to the left iliac vessel for 10 minutes. These invasive methods of applying a remote ischaemic stimulus were both longer in duration and arguably more likely to provide a significant ischaemic stimulus. Walsh et al used two sequential periods of lower limb ischaemia using a tourniquet inflated for 10 minutes on one leg and then repeated on the other leg. Whether the three five minute cycles of non-invasive tourniquet cuff inflation on an upper limb is just as likely to produce an equal strength or sufficient strength stimulus to effect change is difficult to deduce. Certainly this study utilised an identical stimulus as previous reports that demonstrated a >40% reduction in cTnT release on the upper limb [107][145] and so evidence for robustness of ischaemic stimulus exists. A large scale trial comparing the effects of intermittent upper limb ischaemia (three 5 min cycles tourniquet inflation/deflation) on similar aneurysmal surgical procedures would be able to clarify whether the shorter non-invasive stimulus is just as effective as the reported longer noninvasive and invasive stimuli. Such a trial, if positive, would also stimulate discussion on whether similar to reports of IP [133] RIPC offers no additional benefit when associated with CPB regardless of the mode of cardioprotection used, because CPB per se induces preconditioning.

Other surrogate markers of renal injury: peak serum creatinine concentration, change in serum creatinine, incidence of intravenous frusemide requirement; incidence of dialysis requirement also failed to support the improvement of renal function by intermittent upper limb ischaemia (RIPC).

A sub-analysis of the incidence of those patients who suffered a more severe acute kidney injury as defined by  $>44 \mu\text{mol.L}^{-1}$  increase in creatinine once again demonstrated no benefit afforded in this group by RIPC (Control 8/77(10%) vs. RIPC 5/75(7%);  $p=0.56$ ).

In spite of early suggestions that RIPC may offer benefit against IR injury of the kidneys during coronary surgery this study has failed to offer evidence. The morbidity and mortality from renal injury post-CABG remains a concern for the surgical community and the search for a more effective treatment strategy must continue.

## 5.0 **Conclusion**

In this double blind randomised controlled trial RIPC does not offer any renal protection in non-diabetic adult humans undergoing first time coronary artery bypass surgery.

## **CHAPTER 8                    THE EFFECT OF RIPC ON LUNG OUTCOMES IN PATIENTS UNDERGOING FIRST TIME CORONARY ARTERY BYPASS SURGERY**

### **1.0    Introduction**

Acute lung injury, presenting as pulmonary oedema and pulmonary gas exchange abnormalities such as postoperative hypoxaemia is common after cardiac surgery. Pulmonary dysfunction is a frequent complication of CABG and is associated with a longer duration of mechanical ventilation, difficulty weaning the patient, prolonged hospitalization, major morbidity, mortality and health care expenditure [276][277][278][279]. The pathophysiology reflects the combined effects of general anaesthesia, surgical injury, median sternotomy and CPB to produce hypoxia, atelectasis, pleural effusion and dysfunction of the diaphragm [280].

CPB has been implicated in decreased oxygen and nutrient delivery to the alveoli due to a number of factors: microemboli from the ascending aorta may become dislodged during placement of cannula [281][282][283][284]; haemodilution by crystalloid fluids used to prime the pump reduces oxygen carrying capacity[285][286]; hypothermia increases blood viscosity increasing the risk of sludging in microvessels and impairing oxygen transfer from erythrocytes to alveolar cells; nonpulsatile flow, compared to physiologic pulsatile flow, decreases microvascular blood flow and increases the expression of inflammatory mediators in blood and vascular cells [287][288][289].

However CPB itself may not be the major contributor to the development of post-operative pulmonary dysfunction. In a series of 175 patients undergoing CABG with CPB (n=150) and without CPB (n=25) changes in postoperative gas exchange was similar between groups. These findings were in spite of higher levels of markers of systemic inflammatory response in CPB patients [280]. These findings have been replicated in other studies [276]

In a series of 16,847 patients undergoing on-pump CABG, valve, or CABG-valve surgery patients receiving transfusion (red blood cells; fresh frozen plasma) had more risk-adjusted pulmonary complications: respiratory distress, respiratory failure, longer intubation times, acute respiratory distress syndrome and reintubation [290].

Early evidence in experimental models has been reassuring for the existence of lung IP. Clamping of the lung hilum (lung IP) in a rabbit model [291] has improved markers of lung ischaemia-reperfusion injury. Activation of adenosine A(1) receptors, alteration of the pulmonary vasodilator response to histamine, sarcolemmal K(ATP) channels, preservation of endothelial-dependent vasodilator responses and restoration of surfactant properties have been identified in the mediation of the protective properties of lung IP on ischemia-reperfusion injury[292][293][294]. Postconditioning has also been shown to attenuate lung ischemia-reperfusion injury through upregulating the protein expression of HO-1 that leads to reduced post-ischemic oxidative damage[295].

In small scale human models RIPC has been shown to benefit the lungs whether the upper or lower limbs undergo intermittent ischaemia. In a prospective RCT of infants undergoing repair of ventricular septal defects

those randomised to 3 cycles of 5 min cuff inflation of the left upper limb at 24 h and 1 h before surgery (RIPC n=30; Control n=30) were found to have higher calculated static lung compliance, dynamic lung compliance and lower respiratory index than controls postoperatively [296]. An attenuation of the systemic inflammatory response syndrome and increase in systemic tolerance to ischaemia-reperfusion injury enhanced lung preservation. A RCT in children undergoing repair of congenital heart defects with four 5 minute cycles of tourniquet cuff inflation to the lower limb (RIPC n=17; Control n=20) resulted in lower airway resistance 6 hours postoperatively [118]. Cardiac ischemic preconditioning has also been shown to improve lung preservation in patients having valve replacement, whereby reduction in the accumulation of polymorphonuclear leukocytes in lung tissue and the formation of oxygen free radicals has been suggested[297].

Small study experimental models have also produced encouraging results. In a sheep model of repeated coronary artery occlusion and reperfusion (mimicking multi-vessel off-pump CABG), three episodes of five-minute occlusion of the external iliac artery (RIPC n=9; Control n=5) improved gas exchange ( $\text{PaO}_2$ ;  $\text{PaO}_2:\text{FiO}_2$  ratio) and preserved pulmonary vascular resistance [298]. A porcine model (n=18) where the external iliac arteries were isolated bilaterally and simultaneously clamped for three cycles of 5 minutes before a prolonged ischemic period reduced markers of systemic inflammation and acute lung injury [299]. Furthermore lower limb ischaemia have been observed to reduce lipid peroxidation and lung injury [300][301] also when CPB has been used[196].

The ratio of the partial pressure of oxygen in arterial blood ( $\text{PaO}_2$ ) to the inspired oxygen fraction ( $\text{FiO}_2$ ) has been utilised as a tool for quantification of the degree of abnormality in pulmonary gas exchange[280][302]. The ratio has been used in experimental models to quantify pulmonary gas exchange before and after therapeutic intervention [303][304][305] and in clinical models has aided in the classification of patients' pulmonary gas exchange status, including the definitions of acute lung injury (ALI) ( $27\text{kPa} \leq \text{PaO}_2:\text{FiO}_2 < 40\text{kPa}$ ) and of adult respiratory distress syndrome (ARDS) ( $\text{PaO}_2:\text{FiO}_2 < 27\text{kPa}$ ) [306][307]. In the experimental model mimicking CABG by Xia et al RIPC increased  $\text{PaO}_2$  and  $\text{PaO}_2:\text{FiO}_2$  reconfirming the relevance of this test in the setting of RIPC. Time to extubation according to standardised departmental protocols also allows comparative analysis of improvement from lung injury.

Coronary artery bypass grafting (CABG) is established as a highly effective treatment strategy in improving life expectancy and quality of life of patients with ischaemic heart disease[215] but its success has been tarnished by a 'postperfusion syndrome' implicated in dysfunction of the kidneys, lungs, heart and clotting system frequently found in patients undergoing extracorporeal circulation[308]. The activation of the clotting, kallikrein, complement systems and neutrophils and monocytes together with other blood elements produce the vasoactive and cytotoxic substances that cause myocardial and respiratory injury[309].

The aims of this study were to confirm the role of RIPC in reducing markers of lung injury (6h and 12h  $\text{PaO}_2:\text{FiO}_2$  ratio; intubation time) in non-diabetic adults undergoing first time CABG.

## 2.0 Methods

As Positive End Expiratory Pressure (PEEP) has been shown to increase right and left ventricular stroke volume variation both during open and closed chest conditions in addition to reducing right ventricular end-diastolic volume indicating a preload reductive effect, [175] its use in ventilator management was standardised to 5cm H<sub>2</sub>O. Changes in arterial blood partial pressure of oxygen (PaO<sub>2</sub>) / fractional inspired oxygen (FiO<sub>2</sub>) ratio and intubation times were used as outcome measures as set out in Chapter 3; Section 15.0 to 15.4. Statistical analysis was performed as per Chapter 3; Section 17.3.

## 3.0 Results

Of 162 patients (89% males) 80 were randomized to treatment and 82 to placebo. The groups were well matched for incidence of Forced expiratory volume in one second (FEV<sub>1</sub>) / Forced vital capacity (FVC) ratio <0.7 (RIPC 12/80(15%) vs. Control 13/82(16%); p=0.51), preoperative PaO<sub>2</sub>:FiO<sub>2</sub> ratio (RIPC 55(48-63) vs. Control 53(47-61); p=0.31) and smoking history (p=0.15); current smokers (RIPC 12/80(15%) vs. Control 7/82(9%)), ex-smokers (RIPC 39/80(49%) vs. 52/82(63%)), and never smoked (RIPC 29/80(36%) vs. Control 23/82(28%)). Mean bypass times (RIPC 100±23 vs. Control 96±22; p=0.25)mins and aortic cross-clamp times (RIPC 76±21 vs. Control 71±18; p=0.08)mins were not significantly different (Table 3.8). Postoperatively times to extubation were not different (RIPC 895(675-1180) vs. 938(766-1403)min; p=0.28) and PaO<sub>2</sub>:FiO<sub>2</sub> ratios at 6h (RIPC 37(28-45) vs. 36(29-43); p=0.85) and 12h (RIPC 35(28-41) vs. 33(26-43); p=0.56) were also comparable (Tables 8.1). Incidence of high risk lung status (preop FEV<sub>1</sub>:FVC ratio <0.7

and/or current smokers) was comparable and no difference postoperatively in 6h (RIPC 36(32-43) vs. Control 30(23-38);  $p=0.70$ ), 12 h (RIPC 35(27-40) vs. Control 32(23-40);  $p=0.50$ )  $\text{PaO}_2\text{:FiO}_2$  ratios and time to extubation (RIPC 900(713-1940) vs. Control 838(574-1112)mins;  $p=0.29$ ) was observed.

**Table 8.1: Post-operative lung outcomes. LOS = length of stay.**

	<b>Control (n=82)</b>	<b>RIPC (n=80)</b>	<b><i>p</i>=</b>
Extubation time (min)	937(766-1402)	895(675-1180)	0.28
ITU LOS (days)	3(2-4)	3(2-5)	0.29
Hospital LOS (days)	8(6-11)	8(6-12)	0.39
6h $\text{PaO}_2\text{:FiO}_2$ ratio	36(29-43)	37(28-45)	0.85
12h $\text{PaO}_2\text{:FiO}_2$ ratio	33(26-43)	35(28-41)	0.56

#### **4.0 Discussion**

This double-blind study is the first to demonstrate that RIPC has no role in reducing rates of acute lung injury following on-pump CABG in non-diabetic adults.

No previous studies have investigated the pulmonary protective effects of RIPC following CABG in humans. In three relatively small human studies, Wenwu et al (n=60), Cheung et al (n=47) and Li et al (n=40) presented assuring evidence for the beneficial effect of RIPC in cardiac surgical procedures. However in this study, RIPC did not improve lung preservation as assessed by 6 and 12 hour  $\text{PaO}_2\text{:FiO}_2$  ratio and time to extubation.

Small sample size, age of population, differences in remote stimulus delivery and cardioplegia protocols may help explain why RIPC did not produce effect.

All three human studies investigated a considerably younger population group than the present study (median 65 years): Wenwu et al (mean 5 months); Cheung et al (mean 1 year); Li et al (mean 36 years). Whether the lung is less likely to respond to RIPC with age would be suggested from the evidence but could only be truly answered with trials using similar protocols in older age groups. Certainly this would be less practical in the case of repairs of congenital defects but may be more so in the case of valve replacements.

Perhaps more significantly a case for the difference in findings attributable to strength of ischaemic stimulus used could be presented. Wenwu et al used 3 cycles of 5 min cuff inflation of the left upper limb twice (at 24 h and 1 h) before surgery, Cheung et al employed an extra 5 minute cycle of lower limb cuff inflation than the three cycles in the present study and Li et al executed two 3 minute cycles of cross-clamping the aorta. Both non-invasive protocols were longer in total duration and the invasive stimulus although shorter arguably produced a more powerful ischaemic stimulus than the present study. At present no test exists to ascertain the 'strength' of a stimulus but certainly this study utilised an identical stimulus as previous reports that demonstrated a >40% reduction in cTnT release [107][145] and so evidence for robustness of ischaemic stimulus exists.

All three positive human studies were conducted on CPB. Cardioplegia administered may have played an integral part of the purported benefit of RIPC seen but unfortunately lack of information on protocols makes comment difficult. Wenwu et al does not qualify what 'cardioplegic arrest' was used, Cheung et al does not expand on 'blood cardioplegia' used. Li et al uses a similar 'intermittent cold blood (4<sup>0</sup>C) cardioplegic arrest' in valve replacements

as the antegrade cold blood (4°C) cardioplegic arrest used in the present coronary surgery but operative details are limited.

Experimental models also have observed improvement in markers of lung function. Both Xia et al (n=14) and Harkin et al (n=18) occluded the external iliac arteries for three 5 minute cycles unilaterally and bilaterally respectively. Again these invasive protocols may produce stimuli that the present study is not able to match. However, like repeated cross-clamping of the aorta, it is unlikely that these invasive procedures will be taken up by the cardiothoracic surgical community due to the potential for atheroembolism.

A sub-analysis did not observe RIPC to improve lung preservation (6h and 12h PaO<sub>2</sub>:FiO<sub>2</sub> ratio; time to extubation) in those considered as high risk lung patients (preoperative FEV<sub>1</sub>:FVC ratio <0.7 and/or current smokers).

In spite of early suggestions that RIPC may offer benefit against IR injury of the lungs during coronary surgery this study has failed to offer evidence. The morbidity and mortality from lung injury post-CABG remains a challenge for the cardiothoracic fraternity and the search for a more effective treatment strategy must continue.

## **5.0 Conclusion**

In this double blind randomised controlled trial RIPC does not offer any lung protection in non-diabetic adult humans undergoing first time coronary artery bypass surgery.

## **CHAPTER 9                      PILOT STUDY INTO THE EFFECTS OF RIPC ON GENES      AND      PATHWAYS      IN      PATIENTS UNDERGOING FIRST TIME CORONARY ARTERY BYPASS SURGERY**

### **1.0      Introduction**

Changes in gene expression and signal transduction pathways have been reported to be involved in ischaemic conditioning mediated myocardial protection.

In experimental models RIPC has been shown to modify myocardial gene expression by upregulating cardioprotective genes and suppressing proinflammatory genes potentially involved in the pathogenesis of ischemia-reperfusion injury [311]. Genes encoding key proteins involved in cytokine synthesis, leucocyte chemotaxis, adhesion and migration, exocytosis, innate immunity signalling pathways, and apoptosis have been shown to be suppressed within 15 mins of the RIPC stimulus and these findings are more pronounced after 24 hours (second window)[194]. In one model, 24 hours following RIPC, the expression of genes known to be associated with the stress response, redox regulation, growth and metabolism, DNA repair and other cellular functions was increased [312].

Hypoxic conditioning of atrial trabeculae harvested from patients undergoing cardiac surgery has been observed to protect myocardium following prolonged ischaemia-reperfusion. Protection has been shown to be dependent on activation of the Reperfusion Injury Salvage Kinase (RISK) pathway [313].

The RISK-pathway represents a group of pro-survival protein kinases, including Akt and Erk $^{1/2}$ , and has been seen to be recruited by IP to mediate cardioprotection in a series of animal models.

In an isolated perfused rat heart model two 5 min cycles of global ischaemia (IP) prior to prolonged ischaemia-reperfusion increased prosurvival kinase (Akt, Erk $^{1/2}$ , p70S6K) phosphorylation and reduced histological infarct risk-to-volume ratio [314] In an isolated rabbit heart model, one 5 min period of ischaemia followed by 10 min of reperfusion (IP) prior to lethal ischaemic insult reduced histological infarct risk-to-volume ratio and binding of adenosine (A $_1$  and/or A $_2$ B) receptors during reperfusion was observed to initiate the protective signal transduction cascade [315]. IP-mediated activation of prosurvival kinases has been replicated in other studies [316].

Akt and Erk $^{1/2}$  are activated through phosphorylation by PI3K and MEK $^{1/2}$  respectively and seem to reduce the cellular damage specifically caused by the event of reperfusion [314][317]. Anti-apoptotic pathways are activated by phosphorylated Akt and Erk $^{1/2}$  but one downstream target of activated Akt has been found to be of particular importance in this regard: Glycogen Synthase Kinase-3 $\beta$  (GSK-3 $\beta$ ) and its association to the mitochondrial permeability transition pore (mPTP).

GSK-3 $\beta$  is inhibited by site-specific phosphorylation and has previously been shown to be involved in cardioprotection in rat models [318]. Some evidence suggests that GSK-3 $\beta$ -inhibition is the effector mechanism of mPTP-inhibition preventing uncoupling of oxidative phosphorylation and mitochondrial swelling in the reperfusion period [319]. Moreover GSK-3 $\beta$ -inhibition is associated with accumulation of cytoplasmic  $\beta$ -catenin in the cytosol and nucleus.

Cytoplasmic  $\beta$ -catenin is a transcriptional regulator of several genes involved in cell survival and proliferation [320][321] and has been implicated in the process of preconditioning and healing after myocardial infarction (MI). Adenovirus mediated gene transfer of  $\beta$ -catenin in a rat model has been shown to decrease apoptosis in cardiomyocytes and cardiac fibroblasts. It has been suggested that  $\beta$ -catenin plays an important role in the healing process after MI by promoting survival and cell cycle in cardiomyocytes and cardiac fibroblasts with its differentiation into myofibroblasts [322].

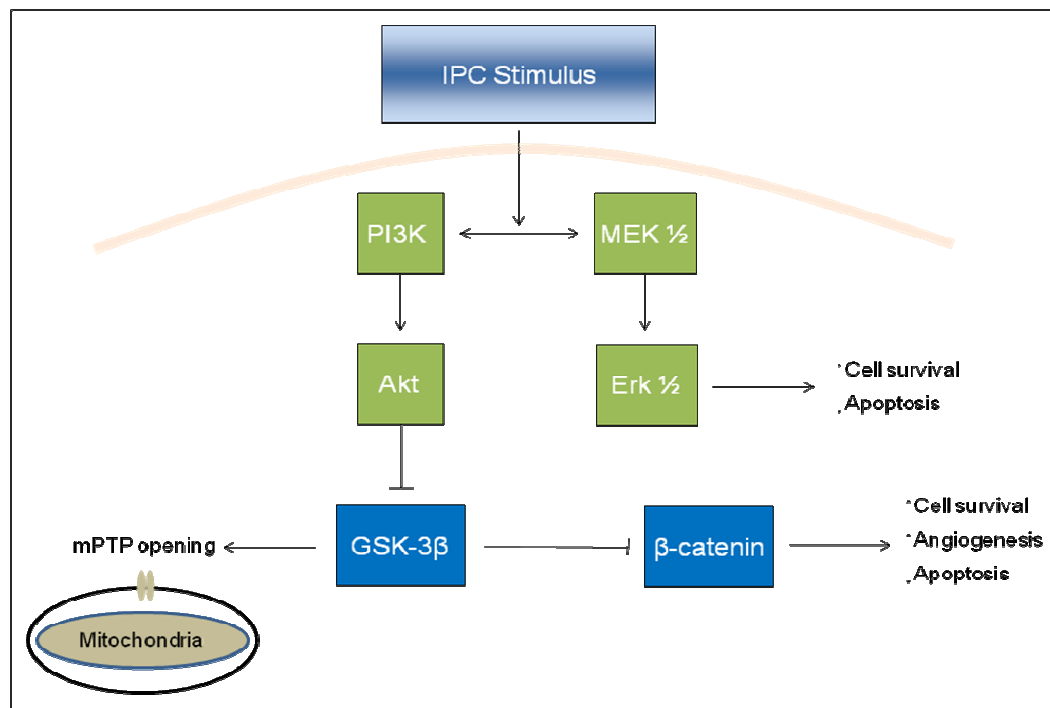
Activation of PI3K-Akt, a part of the Risk-pathway, inhibits GSK-3 $\beta$  through site-specific phosphorylation. GSK-3 $\beta$  inactivation allows  $\beta$ -catenin accumulation and inhibits opening of the mPTP. Together with Erk1/2 activation these mechanisms seem to underlie the cytoprotective effects of preconditioning.

Thus the PI3K/AKT/GSK-3  $\beta$ / $\beta$ -catenin cell survival pathway has been proposed as key in conditioning pathways (Figure 9.1).

Invasive and non-invasive models of RIPC in rats have demonstrated nitric oxide [323], free radical pathways, heat shock proteins, antioxidant enzymes [324] and protein kinase C to be involved in these protective pathways [325].

The aim of this pilot study was to generate hypotheses: RIPC exerts its cardioprotective effect through activation of the PI3K/AKT/GSK-3  $\beta$ / $\beta$ -catenin cell survival pathway in myocardial specimens of non-diabetic adults undergoing first time CABG.

**Figure 9.1: Activation of PI3K-Akt, a part of the Risk-pathway, inhibits GSK-3 $\beta$  through site-specific phosphorylation. GSK-3 $\beta$  inactivation allows  $\beta$ -catenin accumulation and inhibits opening of the mitochondrial Permeability Transition Pore (mPTP). Together with Erk1/2 activation these mechanisms underlie the cytoprotective effects of preconditioning.**



## 2.0 Methods

Assessment of acquired myocardial specimens followed methodology set out in Chapter 3; Section 16.0 to 16.2.

## 3.0 Results

Adult non-diabetic patients for CABG were recruited into a trial. All patients had three myocardial biopsies harvested intraoperatively (LV1, LV2, LV3). Of the 162 patients recruited to the trial 80 were randomised to RIPC and 82 to Control groups. From the Control arm of the trial 6 'post-reperfusion' biopsy samples were randomly selected and from the RIPC arm of the trial 7 'post-

reperfusion' biopsy samples were randomly selected and analysed (Control n=6; RIPC n=7). RIPC increased expression of p-Akt ( $p=0.05$ ; Figure 9.2) and pan-Akt ( $p<0.01$ ; Figure 9.3). Total GSK-3 $\beta$  was increased by RIPC ( $p<0.01$ ; Figure 9.4) but expression of p-GSK-3 $\beta$  was comparable between groups ( $p=0.95$ ; Figure 9.5).  $\beta$ -catenin was significantly increased by RIPC ( $p<0.01$ ) (Figure 9.6).

Figure 9.2: Relative expression of p-Akt between groups (p=0.05).

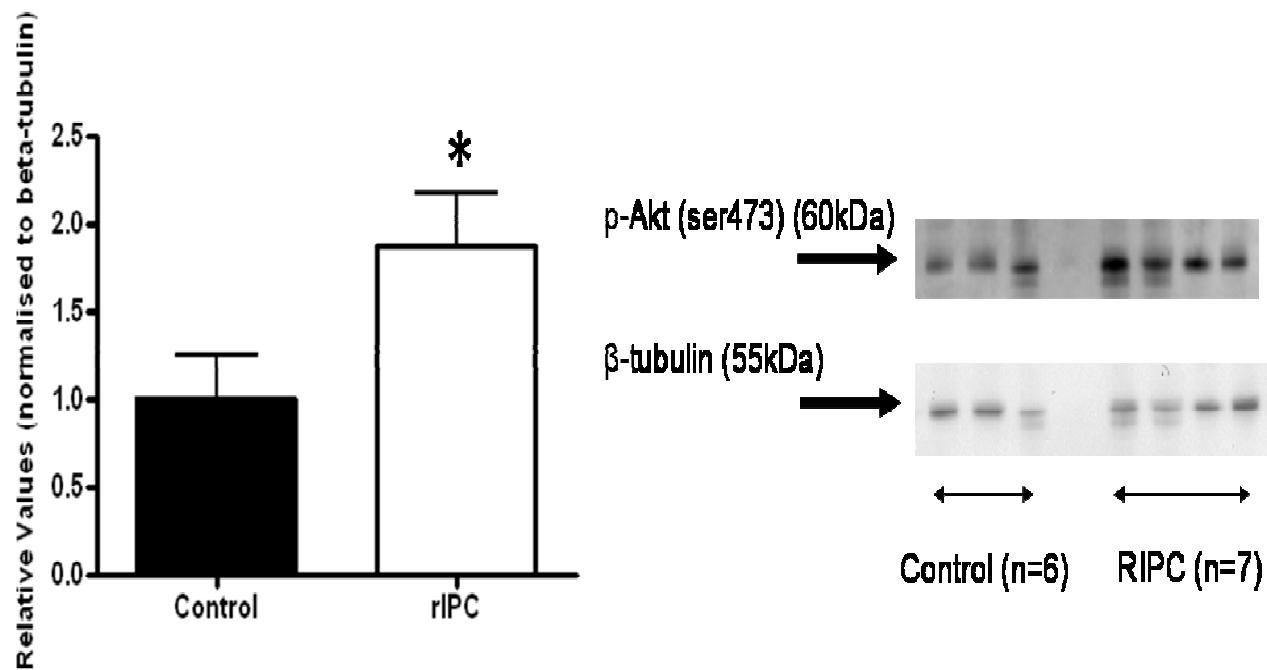


Figure 9.3: Relative expression of PAN-Akt between groups (p<0.01).

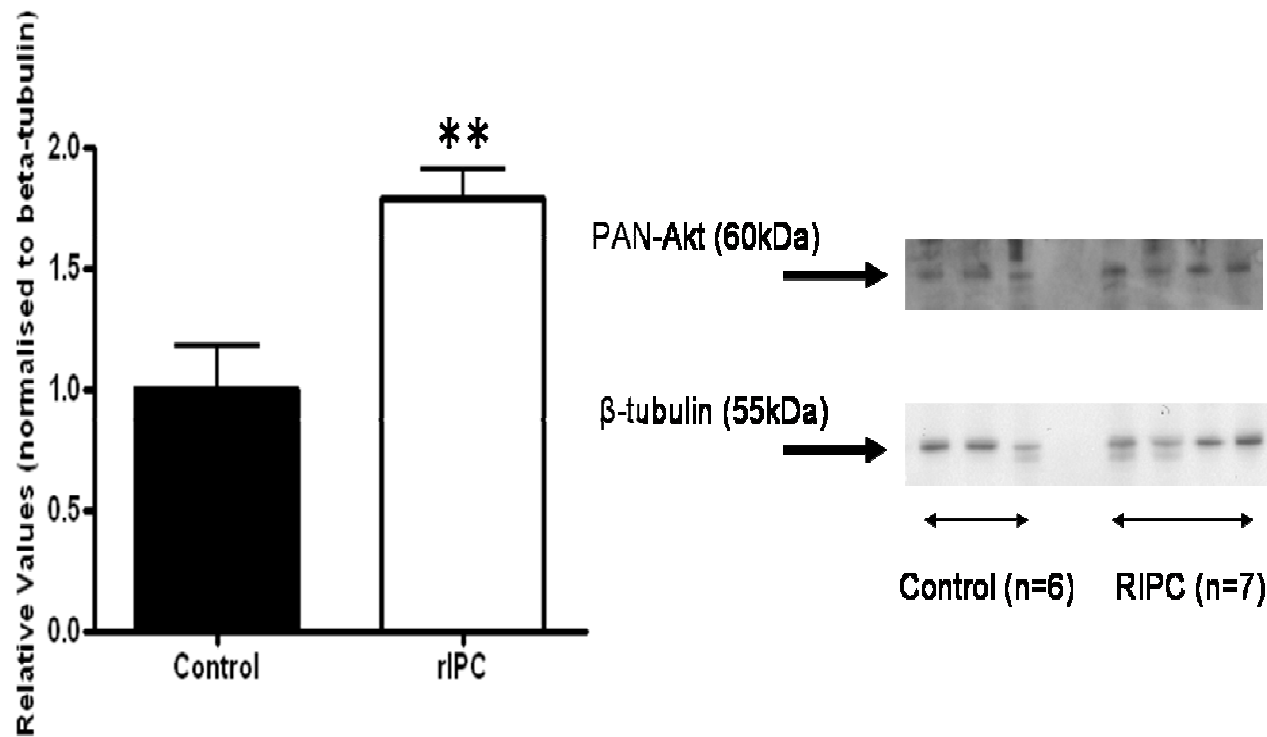


Figure 9.4: Relative expression of GSK-3 $\beta$  between groups ( $p<0.01$ ).

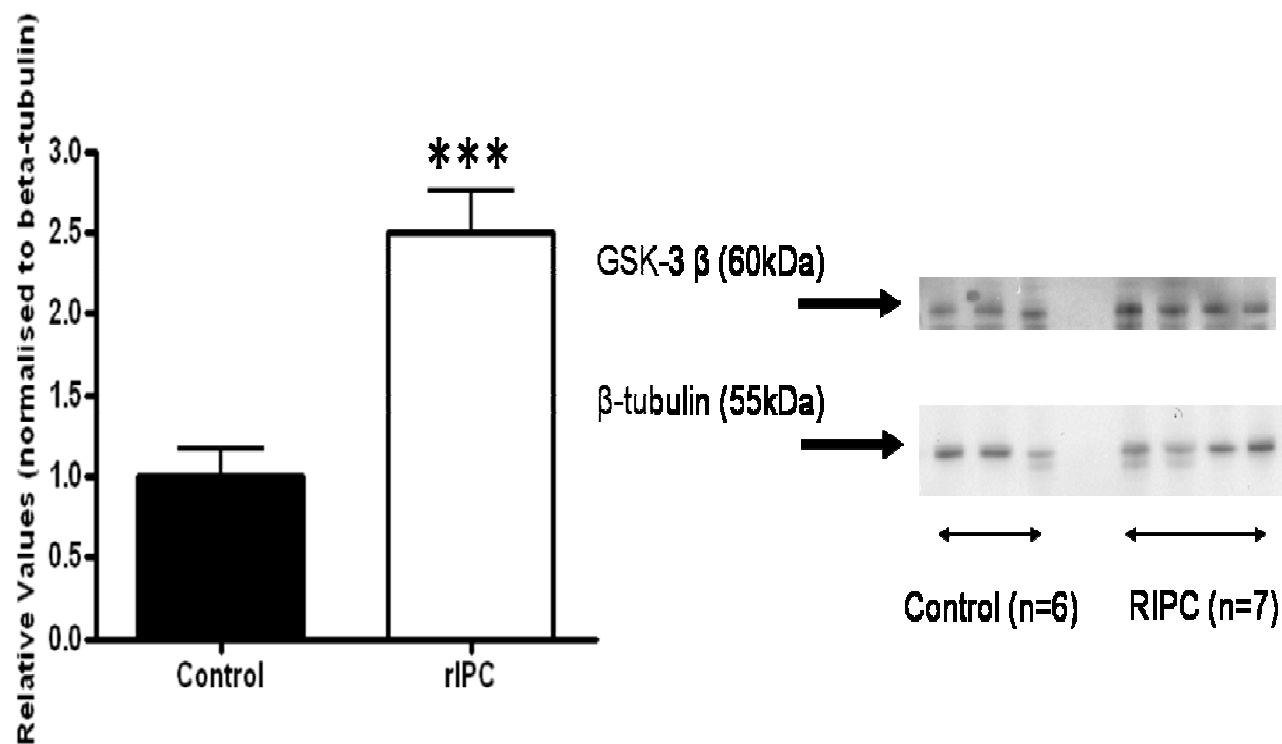
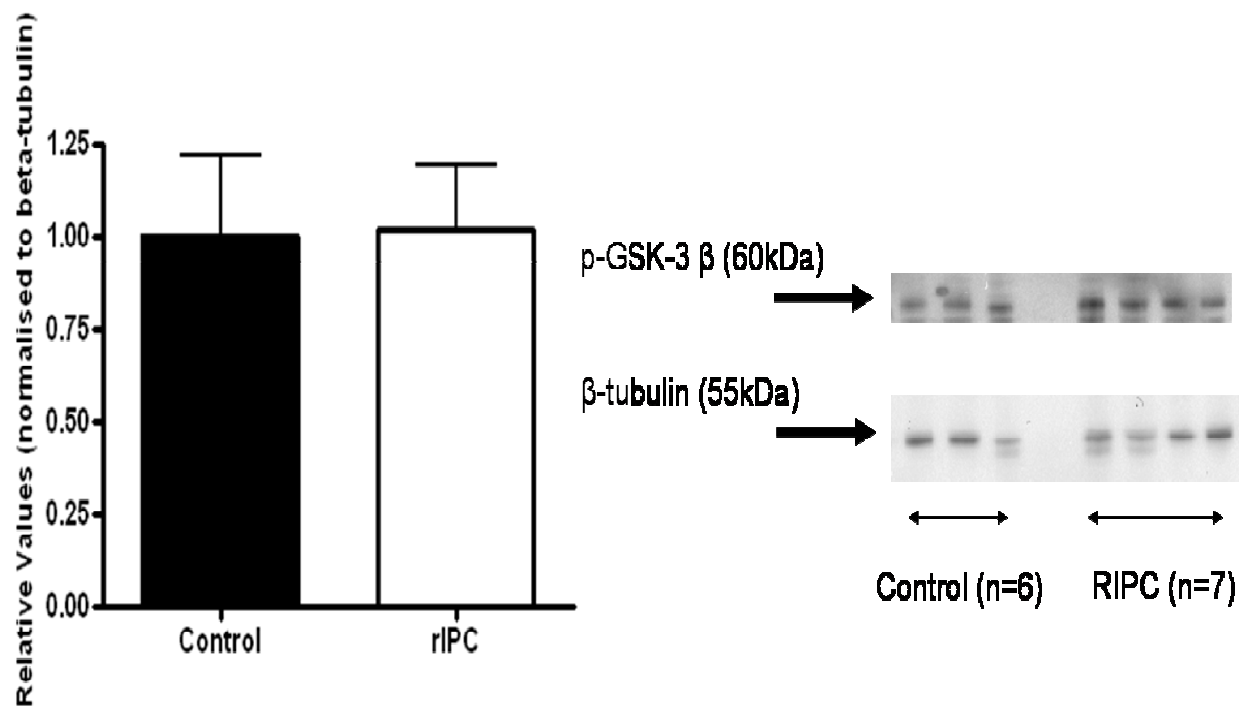
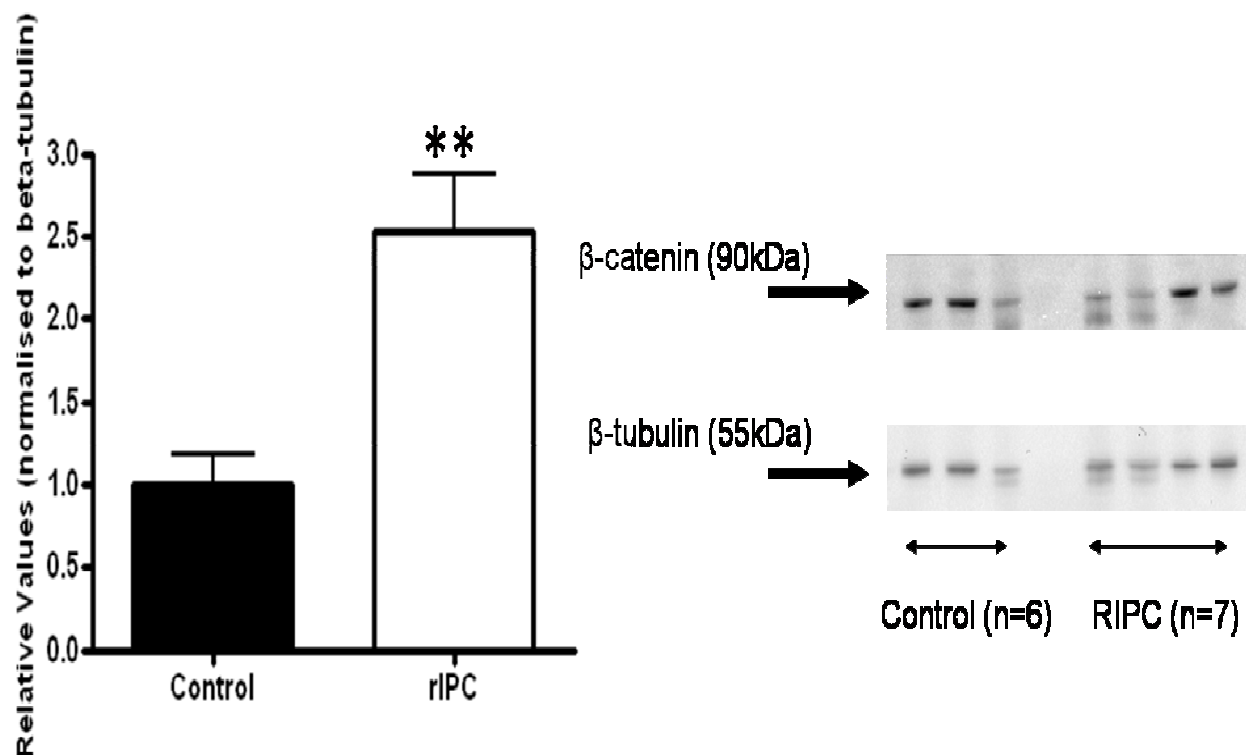


Figure 9.5: Relative expression of p-GSK-3 $\beta$  between groups ( $p=0.95$ ).



**Figure 9.6: Relative expression of  $\beta$ -catenin between groups ( $p<0.01$ ).**



#### 4.0 Discussion

The data presented from this small mechanistic sub-study is the first to demonstrate that RIPC increases myocardial expression of the  $\beta$ -catenin pathway in humans undergoing CABG.

The identification of a significant increase in the expression of p-Akt, pan-Akt, total GSK-3 $\beta$  and  $\beta$ -catenin represents an important step in understanding the cell biology of cardioprotection and the development of clinically viable therapeutic strategies for reducing IR injury.

The Yellon group observed an increase in the activation of the RISK pathway in atrium, harvested from patients undergoing cardiac surgery (CABG and/or valve replacement), subjected to postconditioning protocols. The results in the

present study add support to these findings. It appears that the heart globally (atrium and left ventricle) responds to ischaemic stimuli. As pre- and post-ischaemic protocols have been successful the timing of stimulus delivery in a clinical setting offers flexibility and thus could be selected on practicality. A larger scale study to compare the effects of timing of conditioning stimulus on magnitude of effect is warranted.

Haunseloy et al, Solenkova et al and Lecour et al observed increased activation of the prosurvival kinases in experimental IP models and correlated this with reductions in histological infarct risk-to-volume ratio. Although the present study can conclude that a remote ischaemic stimulus has been administered unlike other studies it has not correlated this with a functional change. In this study cardiac troponin release, a marker of myocardial necrosis, was unaltered between groups but with the arrival of advanced imaging techniques whether other endpoints such as infarct risk-to-volume ratio may show change should be investigated.

The results are in general concordance with previous work showing up-regulation and phosphorylation of constituents in the pathway by a cytoprotective stimulus. However, it is rather puzzling that there was no change in the phosphorylation status of GSK-3 $\beta$  and the reasons for this are unclear. Indeed this would appear to be a key step in animal models of preconditioning with GSK-3 $\beta$  inactivation allowing the preservation of  $\beta$ -catenin. Our data would suggest therefore that the human pathway differs from that in other mammalian models in this step.

Nevertheless this small scale study has shown three 5 min cycles of intermittent upper limb ischaemia to activate known cardioprotective pathways in humans following coronary bypass surgery.

## **5.0 Conclusion**

In this small mechanistic study in patients randomised, RIPC increases activation of cardioprotective pathways in the myocardium of non-diabetic adult humans undergoing first time coronary artery bypass surgery.

## **CHAPTER 10                      SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH**

### **1.0    Introduction**

Ischaemic preconditioning has been recognised as a major cardioprotective phenomenon for many years [102][326]. Cycles of non-lethal ischaemia and reperfusion applied to the heart prior to a potentially lethal ischaemic insult have the capacity to reduce infarct size by >50%. More recently, it became apparent that the protection generated by this classical form of direct ischaemic preconditioning could be replicated when the non-lethal ischaemia was applied to one segment of the heart and the lethal ischaemia applied to a separate segment [108]. Thereafter, it became established that the same protection could also occur even if the pre-conditioning ischaemic stimulus was applied completely distant to the target organ requiring protection, i.e. transient ischaemia of a remote organ or limb could still generate protection for the organ being subsequently challenged by lethal ischaemia [327]. There is now clinical evidence, suggesting that this remarkable remote ischaemic preconditioning (RIPC) phenomenon may represent a simple, inexpensive, easily applied method of increasing cardioprotection during an array of interventional procedures that require a period of cardiac ischaemia to allow repair or intervention. Moreover, as it is now recognised that such protection may be achieved by starting the cyclical remote ischaemia and reperfusion after the period of injurious cardiac ischaemia has commenced; so called remote post- or peri-conditioning, the potential arises of enhancing protection in other scenarios, including transplantation [328].

Several clinical reports of RIPC in cardiovascular surgery have now been published. In abdominal aortic aneurysm surgery, RIPC, induced by unilateral iliac artery clamping has reduced troponin release and renal injury [143]. In children undergoing congenital heart defect repairs utilising cardiopulmonary bypass, lower limb RIPC has been demonstrated to reduce troponin release and inotrope requirements [118]. In adults undergoing coronary artery bypass surgery (CABG), intermittent upper limb ischaemia has been followed by reductions in post-operative release of lactate dehydrogenase [144] and troponin T. In 57 patients, Haunseloy et al observed a >40% reduction in AUC serum cTnT where each anastomosis was constructed with either intermittent cross clamp fibrillation or cardioplegia [107]. Venugopal et al went on to observe 45 patients undergoing CABG ( $\pm$ concomitant aortic valve replacement) and antegrade $\pm$ retrograde cold blood cardioplegia produce a similar magnitude of effect [145].

## **2.0 Summary of effects of RIPC in patients undergoing CABG**

### *2.1 Cardiac outcomes*

Postoperative cardiac troponin is an independent predictor of in-hospital death and high concentrations are associated with a cardiac cause of death and major postoperative outcomes. Which troponin measurement; isolated values at specific time points or area under the curve (AUC) release provides the most prognostically important information is not yet known. Additionally, whether troponin release in the first few hours after surgery reflects true infarction or a change in sarcolemmal integrity or permeability has been questioned. Previous studies suggested a large treatment effect; a

standardised difference of 0.8. The current study hypothesised that RIPC would reduce 48 h AUC cTnT release by a standardised difference of 0.6 at 90% power and 5% alpha. This required a sample size of 120 subjects which was increased by 33% to accommodate withdrawal or missing data points. Sufficient patients with full data sets were recruited.

This double-blind study using an identical RIPC stimulus and cTnT assay has not corroborated previous smaller single blind studies that found RIPC to reduce troponin release following on-pump CABG. This absence of effect on measurable myocardial injury (48h AUC cTnT; peak cTnT; 6h cTnT release) is matched by a failure to demonstrate any advantage of RIPC in terms of cardiac performance (serial cardiac indices; IABP usage) and inotrope and vasoconstrictor requirement. To accommodate for changes in systemic vascular resistance left ventricular stroke work indices was analysed and was comparable between groups. Thus, there is no evidence to suggest the benefit of a 'first window' of RIPC protection.

Post-CABG myocardial injury often manifests as a transient period of reversible dysfunction, treatable by hemodynamic optimisation and temporary inotropic support. The data has provided no evidence of protection afforded by RIPC against this cardiac stunning phenomenon.

No subtle differences between the groups exist for known preconditioning volatile anaesthetic agents (Enflurane or Sevoflurane) usage or AUC cTnT when adjusted for calculated CrCl or elective/urgent cases which could bias results.

In conclusion, RIPC in this study has failed to augment myocardial protection in patients undergoing CABG.

## 2.2 *Functional impact*

During and following cardiac surgery there is considerable variability in preload and afterload. Isovolumic acceleration (IVA) was selected as the primary endpoint of LV contractile function due to its load-independent qualities. Due to logistical reasons only 72 of the planned 146 patients were recruited to this sub-study. With the achieved sample size this study would still have the power to detect a difference in IVA  $\geq 0.675SD$  ( $\alpha$  0.05,  $1-\beta$  0.80). The observed difference in  $\Delta IVA$  between the two groups was non-significant and the 95% confidence interval of the difference was small (-0.4 to 0.3). From this, it is suggested that should a difference between the two groups exist as a result of RIPC, this is likely to be small and offer minimal clinical benefit.

This double-blind study is the first to demonstrate that RIPC offers no long term functional benefit on LV or RV function assessed by transthoracic echocardiography during on-pump CABG.

Following surgery RIPC afforded no functional improvement in load-independent (IVA) and load dependent (LVEF with contrast; LV Tei) markers of systolic left heart function. Diastolic function (LV Tei; E/A; deceleration time; E/e') and RV contractile function (TAPSE; RV Tei) was not improved either.

Within groups, markers of LV function were unchanged following surgery. Conversely, reduced TAPSE but unchanged RV Tei in both groups would suggest a reduction in RV longitudinal function that is compensated for circumferentially as global function remains unchanged. This reduced RV function was not related to peri-operative myocardial necrosis or inotrope use.

Indeed deleterious echocardiographic change post-CABG is not predicted by cTnT release or inotrope requirement up to 12 hours postoperatively.

In conclusion, following surgery a reduction in RV longitudinal function occurs although global RV function is preserved. This is the first study to show that RIPC fails to preserve late LV or RV contractile function.

### *2.3 Arrhythmias*

This double-blind study is the first to demonstrate that RIPC has no role in reducing arrhythmia rates during on-pump CABG in non-diabetic adults.

All markers of arrhythmia: perioperative MI by 12 lead ECG (new onset LBBB; Q waves); ventricular tachyarrhythmia incidence by continuous Holter monitoring; treated AF incidence have found no anti- or pro-arrhythmic potential in humans.

Wu et al observed a benefit of >30% in postoperative ventricular tachyarrhythmia's with IP following CABG. The present study however has been unable to replicate these positive findings. A much lower overall incidence of reperfusion fibrillation and a lower overall rate in the post-operative 48 hour period was found. The lower arrhythmia incidence in the current study makes it under-powered to exclude a lack of protection by RIPC for this end-point.

### *2.4 Renal outcomes*

This double-blind study is the first to demonstrate that RIPC has no role in reducing rates of acute renal injury following on-pump CABG in non-diabetic adults.

No previous studies have investigated the renal protective effects of RIPC following cardiac surgery. However three relatively small experimental and human studies have shown a beneficial effect of RIPC on aortic aneurysmal surgery (including endovascular).

Intermittent upper limb ischaemia failed to offer any detectable benefit in urinary 24h AUC ACR and so at the glomerular level no protection effect from the insult of cardiac surgery was found. As urinary 24 h  $\alpha$ 1-microglobulin level was comparable there is no suggestion of protection by RIPC against damage to tubular reabsorptive function.

Other surrogate markers of renal injury: peak serum creatinine concentration, change in serum creatinine, incidence of intravenous frusemide requirement; incidence of dialysis requirement also failed to support the improvement of renal function by RIPC.

A sub-analysis of the incidence of those patients who suffered a more severe acute kidney injury as defined by  $>44 \mu\text{mol.L}^{-1}$  increase in creatinine once again demonstrated no benefit afforded in this group by RIPC.

In spite of early suggestions that RIPC may offer benefit against IR injury of the kidneys during on-pump coronary surgery this study has failed to offer evidence.

## *2.5 Lung outcomes*

This double-blind study is the first to demonstrate that RIPC has no role in reducing rates of acute lung injury following on-pump CABG in non-diabetic adults.

No previous studies have investigated the pulmonary protective effects of RIPC following CABG in humans. Small human studies have presented assuring evidence for the beneficial effect of RIPC in cardiac surgical procedures in younger patients. However in this study, RIPC did not improve lung preservation as assessed by 6 and 12 hour PaO<sub>2</sub>:FiO<sub>2</sub> ratio and time to extubation on the Intensive Care Unit following surgery.

RIPC does not offer benefit against IR injury of the lungs during on-pump coronary surgery in non-diabetic adults.

## *2.6 Genes and Pathways*

The data presented from this small mechanistic sub-study is the first to demonstrate that RIPC increases myocardial expression of the  $\beta$ -catenin pathway in humans undergoing CABG.

The identification of a significant increase in the expression of p-Akt, pan-Akt, total GSK-3 $\beta$  and  $\beta$ -catenin represents an important step in understanding the cell biology of cardioprotection and the development of clinically viable therapeutic strategies for reducing IR injury.

Although studies have observed an increase in the activation of the RISK pathway in atrium, harvested from patients undergoing cardiac surgery, subjected to postconditioning protocols this study shows that the heart is globally affected (left ventricular biopsy) 10 minutes after reperfusion from a prolonged period of ischaemia when a preconditioning stimulus is used also.

The results are in general concordance with previous work showing up-regulation and phosphorylation of constituents in the pathway by a cytoprotective stimulus. However, it is rather puzzling that there was no

change in the phosphorylation status of GSK-3 $\beta$  and the reasons for this are unclear. Indeed this would appear to be a key step in animal models of preconditioning with GSK-3 $\beta$  inactivation allowing the preservation of  $\beta$ -catenin. Our data would suggest therefore that the human pathway differs from that in other mammalian models in this step.

This small scale mechanistic study has shown three 5 min cycles of intermittent upper limb ischaemia activate known cardioprotective pathways in humans following on-pump CABG in non-diabetic adults.

### **3.0 RIPC from promise to disappointment**

#### *3.1 Cardiac outcomes*

This double-blind study using an identical RIPC stimulus and cTnT assay has not corroborated previous smaller single blind studies that found RIPC to reduce troponin release following on-pump CABG.

On initial review the design of Venugopal et al's investigation into RIPC effects on patients undergoing CABG using cold blood cardioplegic arrest is identical to the present study. Closer examination however reveals fewer similarities which may help explain the difference in results.

Firstly, blinding of treatment allocation was applied to patients and surgeons only; anaesthetists (who administer agents capable of preconditioning or affecting myocardial protection) and investigators were not blinded. Similar proportions of patients received isoflurane or sevoflurane for anaesthetic maintenance but dosages are not reported. As such volatile anaesthetic agents may induce a dose dependent conditioning effect [146], a potential for inadvertent bias arises. In the present study a scrupulous technique to blind

the surgeon, anaesthetist and investigators from the group allocation was developed. The stimulus was administered within an appropriate time-frame and delivery verified by observing disappearance of the oximetry pulse signal. Isoflurane, a known pre-conditioning agent was specifically avoided. Secondly, the Venugopal et al study was small (n=45) compared to the current study (n=162) and contained only half of the estimated number of patients to detect the initially anticipated difference in AUC cTnT of  $15 \mu\text{g.L}^{-1} \cdot 72\text{hrs}$  (standard deviation  $25 \mu\text{g.L}^{-1} \cdot 72\text{hrs}$ ) quoted in the statistical methodology. Statistical significance was actually attained with a smaller mean difference and sample size and this is attributable to the lower than anticipated variance observed in the RIPC group. Thirdly, the study also included patients requiring AVR; whether RIPC was effective in the CABG alone patients is not reported. The larger number of combined AVR/CABG cases contributed to a longer mean bypass time in the control group and bypass time was an independent predictor of greater troponin release. Despite this potentially confounding effect, an inter-group statistically significant difference was maintained after correction for bypass time using a generalised linear model. Fourthly, many of the important variables in the study e.g. bypass time, cross-clamp time and AUC cTnT had unequal variances yet were analysed parametrically. Lastly, although the drug history is reported, whether potentially relevant medications e.g. atorvastatin, potassium channel blockers [147][148] administered in the 24 hours pre-operatively is not clear. Nevertheless, the effect of RIPC on troponin release was large and the data encouraging.

Differences in other preoperative and intraoperative variables may offer clues as to why no cardioprotection was observed in the present study. Preoperatively, non-elective patients who had angina or an acute coronary syndrome within 30-days of surgery were recruited. However, no patients had experienced angina within 48 hours of surgery and no differences between those admitted electively and more urgent cases was observed. A higher usage of beta-blockers was continued until the morning of surgery. Intraoperatively bypass and cross-clamp times were slightly longer due to a single clamp technique. The incidence of volatile anaesthetic (Enflurane or Sevoflurane) use and AUC cTnT adjusted for calculated creatinine clearance (CrCl) was similar between treatment and control groups.

### *3.2 Functional outcomes*

Early evidence for a benefit from RIPC on LV function had been promising. Kharbanda et al in a porcine model undergoing intermittent hindlimb ischaemia observed a greater fall in LV function during ischaemia in controls, late effects were not studied. This improvement in contractile function was not replicated in the present study five to seven days following CABG. Differences in the designs of the studies: lower limb vs., upper limb ischaemia; invasive vs. non-invasive measurement of LV function; four vs. three 5 min cycles, in combination may have increased the likelihood of realisation of cardioprotection in the Kharbanda et al study.

The lack of effect observed in the current study could have been due to a lack of statistical power arising from the failure to perform echocardiography in all subjects recruited. As mentioned above, with the achieved sample size of 72

patients this study would still have the power to detect a difference in IVA  $\geq 0.675SD$  ( $\alpha$  0.05,  $1-\beta$  0.80). The observed difference in  $\Delta IVA$  between the two groups was non-significant and the 95% confidence interval of the difference was small (-0.4 to 0.3). From this, it is suggested that should a difference between the two groups exist as a result of RIPC, this is likely to be small and offer minimal clinical benefit.

Another potential reason for the failure to demonstrate an effect could be the degree of variability between pre-operative and post-operative measurements on echocardiography. Image acquisition is dependent not only on observer seniority but also patient morphological variation. Intra-patient variability exists particularly in the post-operative setting where “poor acoustic windows” are commonplace. Poor pain control, less patient compliance, post-operative fluid and ventilation requirements result in a difficulty in obtaining a “good acoustic window” postoperatively. This variability may be reflected in the wide standard deviations for the LVEF values recorded, albeit similar in both groups. The inter-study variability in our population was minimised as far as possible by using a single blinded observer to analyse all studies.

### 3.3 *Arrhythmias*

No previous studies have investigated the anti-arrhythmic potential of RIPC in humans but reports from experimental models had been encouraging. Again whether the use of the lower limb and an extra cycle of stimulus confer extra cardioprotective benefit and/or whether RIPC is species-dependent is difficult to ascertain but may offer reasoning as to why the purported benefit was not observed.

The present study did not replicate the >30% reduction in postoperative ventricular tachyarrhythmia's observed by Wu et al, in those receiving IP, following CABG. A much lower overall incidence of reperfusion fibrillation and a lower overall rate in the post-operative 48 hour period was found suggesting important differences in protection efficacy between studies but the lower arrhythmia incidence in the current study makes it under-powered to exclude a lack of protection by RIPC for this end-point. Other differences between studies: central, invasive vs. remote, non-invasive stimulus; antegrade+retrograde vs. antegrade only cardioplegia; hot shot vs. no hot shot may have advantaged the study by Wu et al to demonstrate a change.

### *3.4 Renal outcomes*

Although no studies into the renal protective effects of RIPC following cardiac surgery have been conducted, three small experimental and human studies have presented evidence for a beneficial effect on aortic aneurysmal surgery (including endovascular). Lazaris et al and Ali et al utilised invasive clamping of large blood vessels to deliver remote stimuli and Walsh et al although using a non-invasive protocol did so to both lower limbs and for a longer duration than the current study. Thus a difference in the 'strength' of remote stimulus may have been able to produce change in these studies.

### *3.5 Lung outcomes*

The current study has not replicated the benefit in lung preservation seen in three small human studies receiving RIPC in cardiac surgical procedures.

Small sample size, age of population, differences in remote stimulus delivery and cardioplegia protocols may help explain why RIPC did not produce effect. An age-dependent response to RIPC cannot be ruled out as all three human studies investigated a considerably younger population group than the present study. The three studies used either non-invasive protocols that were longer in duration or an invasive protocol for delivering remote stimuli possibly providing extra stimulus to cause effect. Although a positive response was seen with upper limb ischaemia in Wenwu et al's study, the stimulus was delivered twice at 24h and 1h before surgery again possibly offering a synergistic benefit.

In this study PEEP was set to a standardised level of 5cm H<sub>2</sub>O. Although PEEP improves arterial oxygenation, it can adversely affect systemic haemodynamics, reducing venous return and cardiac output. These effects are proportional to the PEEP level.[175][329] Any future work should address this potential methodological limitation to ensure unbiased results.

Analysis of bronchoalveolar lavage has been shown to be a sensitive technique for the diagnosis of acute lung injury in a wide spectrum of clinical scenarios. [310][330][331][332] Although due to resource availability it was not employed in this study it may offer useful information in future studies investigating the efficacy of RIPC on lung preservation post cardiac surgery.

### *3.6 Genes and Pathways*

The data presented from this small mechanistic sub-study is the first to demonstrate that RIPC increases myocardial expression of the  $\beta$ -catenin pathway in humans undergoing CABG. The remote stimulus of three 5 min

cycles to the upper limb has replicated findings from the Yellon group which showed activation of the RISK pathway in myocardium subjected to postconditioning following CABG. IP protocols in three studies have also demonstrated activation of prosurvival kinases.

In conclusion, the remote stimulus delivered in the current study has been confirmed to have occurred due to positive changes in the cardioprotective pathways within the myocardium.

#### **4.0 Future Direction**

The activation of cardioprotective pathways within the myocardium by three 5 min cycles of upper limb ischaemia has concurred with previous studies for the existence of the RIPC phenomenon. However, it is clear that the stimulus was unable to produce functional beneficial change to the heart, kidneys or lungs.

A closer look at the body of literature suggests that use of the lower limb and extra cycles of stimulus may be able to increase the 'strength' of the stimulus itself. Whether this would be able to effect a change in patients undergoing CABG where other confounding variables such as CPB, cardioplegia and volatile anaesthesia exist is difficult to predict. Invasive remote stimuli have also delivered improvement in cardiac, renal and lung preservation but it is unlikely this method will ever offer a practical solution.

In order to corroborate the findings of this study a further study is recommended. A more definitive conditioning protocol should be incorporated. Biochemical markers of preconditioning should be developed to differentiate those that are preconditioned from those that are not. Population investigated

may be those that are higher risk of myocardial injury e.g. left ventricular hypertrophy. The role of RIPC in transplantation surgery should also be explored further.

## **5.0 Recommendation I: Trial**

### *5.1 Aims*

To assess whether four 5 min cycles of lower limb ischaemia at two time points (24h and 1 h before surgery) improves myocardial, renal and lung outcomes in non-diabetic adult patients undergoing on-pump multi-vessel coronary artery surgery.

### *5.2 Design*

A large scale single centre, prospective randomised, placebo intervention-controlled trial. Patients, investigators, anaesthetists, surgeons and critical care teams will all be blinded to group allocation. Subjects will be randomized (1:1) to RIPC (or placebo) stimuli (4x lower limb (or dummy leg) 5 minute cycles of cuff inflation/deflation) 24h and 1h prior to surgery. Anaesthesia, perfusion, cardioplegia and surgical techniques will be standardized. Patients will be stratified to Urgent and Elective CABG.

Primary endpoint:	48h AUC cTnT release
Cardiac secondary endpoints:	MRI assessed infarct risk-to-volume ratio
	6h and peak cTnT release
	Perioperative MI (12 lead ECG)
	Ventricular tachyarrhythmia's (Holter)

	Treated atrial fibrillation incidence
	Serial cardiac indices
	Serial left ventricular stroke work indices
	Low cardiac output episodes
	IABP usage
	Inotrope and vasoconstrictor usage
	Echocardiographic parameters
Renal secondary endpoints:	Peak serum creatinine
	>44 $\mu\text{mol.L}^{-1}$ increase in creatinine
	$\Delta$ - creatinine (day 0-4)
	Urinary 24h AUC ACR
	Urinary 24 h $\alpha_1$ - $\mu\text{globulin}$
	Temporary dialysis incidence
	Intravenous frusemide infusion incidence
Lung secondary endpoints:	6h $\text{PaO}_2\text{:FiO}_2$ ratio
	12h $\text{PaO}_2\text{:FiO}_2$ ratio
	Extubation time
Other endpoints:	Infection incidence
	ITU length of stay
	In-hospital length of stay

## **6.0 Recommendation II: Other future work**

There is a need :

- (i) to determine the efficacy of RIPC in promoting protection in other forms of cardiac surgery
- (ii) to ascertain whether changes in troponin release reflects true infarction or a change in sarcolemmal integrity or permeability
- (iii) to establish if changes are reflected in improved clinical outcomes and that RIPC independently reduces risk
- (iv) to test if urgent CABG or elective CABG patients are already preconditioned

## **7.0 RIPC in Transplantation**

The national early mortality for heart transplantation is 12.5% and >60% of deaths are secondary to dysfunction of the donor heart and so transplantation represents a specific challenge to a therapeutic role of RIPC. Theoretically, RIPC could be used in the brainstem dead donor to generate cardioprotection. However, although the pre-conditioning site would remain innervated, central neural connection is lost. Moreover, in this circumstance, reperfusion does not occur in the pre-conditioned environment unless the stimulus is repeated in the recipient. This is indeed the case, a donor heart, denervated at transplantation may still be protected from a post-implantation ischaemic insult by a RIPC stimulus in the recipient [333]. However, whether such conditioning could protect during the retrieval, transport and implant period of transplantation is not clear.

## **8.0 Limitations**

Despite its true double-blind status, the confirmation of stimulus delivery and its objective clinical and biochemical end-points our study remains undersized to detect smaller differences in a protective effect of RIPC and does not explore effect in a higher risk population. Subtle differences in conditioning status may occur in patients presenting with urgent coronary syndromes status that preventing further conditioning in this group.

This study relies heavily on the use of troponin release as a marker of true infarction but further research is required to confirm changes postoperatively are not due to change in sarcolemmal integrity or permeability.

## **9.0 Conclusion**

The prospective randomised placebo intervention-controlled trial of RIPC described in this thesis has, like its predecessor cardiac same-organ IP, not fulfilled the promise of a practically useful form of improved myocardial protection. An identical stimulus to smaller positive studies was used with a robust technique designed to ensure blinding of all team-members. The stimulus of three 5 min cycles of upper limb ischaemia, administered within an appropriate time-frame with verified delivery, has been shown to activate cardioprotective mechanisms within the myocardium but has not been able to mount a beneficial functional response. An absence of effect on measurable myocardial injury is matched by a failure to demonstrate any advantage of RIPC in terms of cardiac performance, inotrope requirement, echocardiographic function, arrhythmia protection or renal and lung outcomes.

As the optimal RIPC stimulus remains elusive further lines of research has been generated by this study: the significance of troponin release during on-pump surgery, the conditioning status of those presenting with urgent coronary syndromes, the efficacy of more cycles of lower limb ischaemia, the role of RIPC in higher risk and transplantation surgery. The hope is to establish a novel practical myocardial protective technique with proven benefit in a range of clinical end-points.

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# **DATA COLLECTION SHEET**

## **REMOTE TRIAL**

**Case .....**

If found please contact  
Mr I A Rahman

Mobile : 

**Demographics**

REMOTE Code (IR151106)	Hospital Reg No.	Study No.	D.o.B.	D.o.S.

Surgeon	Gender	Height pre-op	Weight pre-op	Weight POD 4	BSA pre-op
A - RSB B - JM	M      F				

**Cardiac History**

CCS	NYHA	MI	Intervention
		None / Q-wave / Non-Q	None / PCI / Stent / PPM

Cardiac Catheterisation	Vessels diseased:
Date:	Angio.LVF: Good / Moderate / Poor EF (echo):

**Holter monitoring**

Time monitor ON (12hrs pre-op)	Time Monitor OFF (48 hrs post-op)

**Pre-op Rhythm**

Sinus	AF/Flutter	PPM

**Admission from Home    /    In-Patient Transfer**

**Risk Factors**

<b>Cholesterol / TG</b>	<b>Hypertension</b>	<b>Family Hx</b>	<b>TIA (date)</b>	<b>CVA (date)</b>

<b>Peripheral Vascular disease</b>	Carotid Date:  Other surgery:                      Date:  Endarterectomy
<b>Smoking</b>	Never Current                      No. of pack years..... (20 cig / day / yr= 1 pack yr) Ex                                      Date:
<b>Respiratory disease</b>	None Asthma COPD Bronchodilators Steroids   PO

**Spirometry**

<b>Pre-op SaO<sub>2</sub> &gt;95% on RA</b>		
<b>FEV<sub>1</sub></b>		
<b>FVC</b>		
<b>FEV<sub>1</sub> / FVC</b>		
<b>Pre-op FEV<sub>1</sub> / FVC &gt;70%</b>		

**Medications**

**Pre op medication**

Premed given        yes    no

Aspirin (dose        mg)                    days stopped prior to surgery

Clopidrogel (dose   mg)                    days stopped prior to surgery

Clexane (dose        mg)                    days stopped prior to surgery

IV Nitrates    Yes    No

IV Heparin    Yes    No

IABP            Yes    No

**Echocardiography**

PRE\_OP

POD 6

**Operative Details**

<b>Date and time fasted from</b>	
<b>Date and time knife to skin</b>	

	<b>Time on / applied</b>	<b>Time off / released</b>	<b>Reason Re- instituted</b>
<b>1st CPB</b>			
<b>2<sup>nd</sup> CPB</b>			
<b>3<sup>rd</sup> CPB</b>			
<b>4<sup>th</sup> CPB</b>			
<b>1<sup>st</sup> AXC</b>			
<b>2<sup>nd</sup> AXC</b>			
<b>3<sup>rd</sup> AXC</b>			

**Trucut Biopsies**

		<b>Time taken</b>
Atrial		
LV1 Pre Ischaemia		
LV2 Ischaemia		
AXC removed		
LV3 Reperfusion		

**Conduit**

LAD	
Diagonal	
Circumflex	
OM1	
OM2	
RCA	
PDA	
Marginal	

**Cold Blood Cardioplegia**

<b>Induction:</b> 12mls/kg volume		<b>Maintenance:</b> 300 ml extra Timing	
<b>Timing</b>			

Enflurane:                      pre CPB              intra CPB

**Additional protamine**

Yes

No

Time heparin given	
Mannitol 20% volume given	

**CARDIAC OUTPUT & OXYGEN EXTRACTION DATA**

	<b>Pre-Sternotomy</b>	<b>Pre-Protamine</b>	<b>Post - Protamine</b>	<b>Post-sternal closure</b>	<b>ITU 0 hrs</b>	<b>2 hrs</b>	<b>4 hrs</b>	<b>6 hrs</b>	<b>9 hrs</b>	<b>12 hrs</b>
<b>Time</b>										
<b>HR</b>										
<b>Rhythm</b>										
<b>CO 1</b>										
<b>CO 2</b>										
<b>CO 3</b>										
<b>Average CO</b>										

Average CI										
BP										
MAP										
PAP										
MPAP										
PAWP										
CVP										
Hb										
PaO2										
SaO2										
Temp 1										
Temp 2										
Mixed Venous SaO2										
SV										
SVR										
SVRI										

### **LCOS**

**e.g. Dopamine 5ml/h started at AXC+2, 5,5,6,7,10**

### Inotropes, Insulin and Electrolytes

	KCL	Ca	Insulin
Pre-CPB			
CPB			
Post CPB – ITU			
ITU - 6hrs			
6 – 12hrs			
12 – 24 hrs			
24 – 48 hrs			
48 – 72 hrs			

### Inotrope & Drug Requirements

Time	Drug Name	Dose	Diluent	Rate	Time	Drug Name	Dose	Diluent	Rate
Pre-CPB	Dopamine				1	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
CPB	Dopamine				2	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
Post-CPB	Dopamine				3	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
0	Dopamine				4	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			

	Enoximone					Enoximone			
Time	Drug Name	Dose	Diluent	Rate	Time	Drug Name	Dose	Diluent	Rate
5	Dopamine				11	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
6	Dopamine				12	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
7	Dopamine				13	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
8	Dopamine				14	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
9	Dopamine				15	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
10	Dopamine				16	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			

	GTN					GTN			
<b>Time</b>	<b>Drug Name</b>	<b>Dose</b>	<b>Diluent</b>	<b>Rate</b>	<b>Time</b>	<b>Drug Name</b>	<b>Dose</b>	<b>Diluent</b>	<b>Rate</b>
17	Dopamine				22	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
18	Dopamine				23	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
19	Dopamine				24-48	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
20	Dopamine				48 - 72	Dopamine			
	Dobutamine					Dobutamine			
	Noradrenaline					Noradrenaline			
	Adrenaline					Adrenaline			
	Phenylephrine					Phenylephrine			
	Vasopressin					Vasopressin			
	GTN					GTN			
	Enoximone					Enoximone			
21	Dopamine								
	Dobutamine								
	Noradrenaline								
	Adrenaline								
	Phenylephrine								
	Vasopressin								
	GTN								
	Enoximone								

Time	Gelofusine	HAS	Platelets	FFP	Cryo	Pump	PRBC
Pre-CPB							
CPB							
Post-CPB							
0							
1							
2							
3							
4							
5							
6							
7							
8							

9							
Time	Gelofusine	HAS	Platelets	FFP	Cryo	Pump	PRBC
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

21							
22							
<b>Time</b>	<b>Gelofusine</b>	<b>HAS</b>	<b>Platelets</b>	<b>FFP</b>	<b>Cryo</b>	<b>Pump</b>	<b>PRBC</b>
23							
24-48							
48-72							

### **Blood Results**

	<b>Pre-op</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 4</b>
Na				
K				
Urea				
Creatinine				
PTTR				
Hb				
Fasting glucose				

### **Assays - Blood**

	<b>Pre-sternotomy</b>	<b>6 hours post-XC</b>	<b>12hours post-XC</b>	<b>24 hours post-XC</b>	<b>48 hours post-XC</b>
<b>cTnT</b>					

	<b>Pre-op</b>	<b>4 hours post op</b>	<b>8 hours post op</b>	<b>12 hrs post op</b>	<b>24 hours post op</b>
<b>Urine ACR</b>					
<b>Urine microglobulin</b>					

**IABP Data**

Pre-op IABP	Y / N	Date: Reason:
IABP post bypass	Y / N	Date: Reason:
Post-op IABP	Y / N	Date: Reason:
Date and time IABP Removed:		

**ITU Data**

Date and Time Arrived ITU	
Date and Time Reached 36 C	
Bair Hugger (all patients should have a bair hugger)	Y / N
Total Blood drained at 12hrs or reopening	
Total Blood drained at 24 hours or reopening	
Date and Time Actual Extubation	
Date and Time Actual Discharge	

**Kidneys**

	Start (date and time)	End (date and time)
CVVH		
Frusemide infusion		

**ECG**

ECG Preop (date )

Immediate POD 1

POD 4

	<b>Preop</b>	<b>POD 0</b>	<b>POD 1</b>	<b>POD 4</b>
Comment				
Nil				
SVT				
AF/Flutter				
VT/VF				
Asystole				
EMD				
Amiod				
Dig				
Adenosine				
Lignocaine				
Shock no.				
Shock tot J				

### **New Arrhythmias**

	Pre-CPB	Post AXC	0-6hrs	6-12hrs	12-24hrs	24-48hrs	48-72hrs
Nil							
SVT							
AF/Flutter							
VT/VF							
Asystole							
EMD							
Amiod							
Dig							
Adenosine							
Lignocaine							
Magnesium							
Shock no.							
Shock tot J							

### **pO<sub>2</sub> / FiO<sub>2</sub> Ratio**

	preop	1 hr postop	6 hrs postop	12 hrs postop
<b>pO<sub>2</sub></b>				
<b>FiO<sub>2</sub></b>				
<b>pO<sub>2</sub> / FiO<sub>2</sub> ratio</b>				

<b>Reoperation</b>	No LCOS	Cardiac Arrest	Bleeding/Tamponade
<b>Bypass used</b>	yes / no		
<b>Pulmonary Complication</b>	yes / no		
Chest infection	Re-intubation	Tracheostomy	Other
<b>Renal Complication</b>	yes / no		
Mild/Moderate (Cr>200)	Haemofiltration	Haemodialysis	
<b>GI Complication</b>	yes/no		
Pancreatitis	GI bleed	Ischemic bowel	Other:
<b>Leg wound complication</b>	yes / no / N/A		
Infection	Dehiscence		
<b>Sternal wound complication</b>	yes / no		
Infection	Dehiscence	VAC/Surgery	
<b>Endocarditis</b>	yes / no		
Root abscess	Re-operation	Organism:	
<b>MRSA status</b>	Pre-op	yes / no	
	Post-op	yes / no	
<b>Neurological complications</b>			
<b>Type I neurological complications</b>	yes / no		
Fatal injury	Stroke		Coma
<b>Type II neurological complications</b>	yes / no		
<b>Discharge Rhythm</b>			
SR	AF	PPM	
<b>Discharge details</b>			
Home	Transferred	Died	
Date:			

## **PATIENT INFORMATION SHEETS**

**REC Ref. No. 06/Q2702/7**

### **Application of remote ischaemic pre-conditioning to human coronary artery bypass surgery (CABG)**

#### **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 coronary artery surgery safer. We include a simple and non-technical summary of the reasons for the trial and what it involves for patients over and above their routine treatment if they take part. If you are being approached in the pre-admission clinic we would like you to take these information sheets home with you to read and consider participating in this trial between now and your admission date at which time a research doctor will be available to discuss the trial further and answer any questions. You can only be included in this clinical trial if you give your express permission in the form of signed consent. If you are already an inpatient on the ward then a member of the heart surgical team will be happy to discuss the trial with you once you have had at least 24 hours to read through these information sheets.

## **PATIENT INFORMATION SHEETS**

REC Ref. No. 06/Q2702/7

### **Application of remote ischaemic pre-conditioning to human coronary artery bypass surgery (CABG)**

#### **WHAT IS THE STUDY ABOUT?**

This study is about trying to improve the protection of the heart in patients undergoing coronary artery bypass surgery (CABG). During CABG the blood flow to the heart is reduced markedly for 30-90 minutes so that bypass grafts can be constructed and during this period 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 operation. We are seeking ways to improve on standard methods to protect the heart by using a technique called remote ischaemic pre-conditioning (RIPC).

Experimentally, we know that short periods (minutes) of reduced blood flow (ischaemia) to the heart can substantially reduce the injury caused by a subsequent more prolonged episode of reduced blood flow. This phenomenon is called ischaemic pre-conditioning. This protective pre-conditioning effect has been observed in cardiac surgery but there are concerns that even the short period of reduced blood flow may itself cause injury and therefore, this technique is not in general usage. Recently, it has been demonstrated that the same degree of protection against injury occurs when a remote organ or limb is pre-conditioned without any reduction in blood flow for the heart. Thus, three 5 minute periods of reduced blood flow of a limb performed immediately before a more prolonged period may improve protection of the heart. This study assesses whether remote pre-conditioning of the arm improves heart protection during coronary artery surgery. Thus, remote ischaemic pre-conditioning means that brief episodes of reduced blood supply to a limb can pre-condition or prime the heart to better cope with any period during which its own blood flow is reduced.

#### **HOW CAN WE DO THIS?**

We seek to investigate whether remote pre-conditioning (RIPC) can improve heart protection during coronary artery bypass surgery. To do this, we have designed a randomised control trial in which some patients will be allocated by chance to receive RIPC or not. Randomized controlled trials are recognized as the very best way of answering such a question. This study will compare whether RIPC improves heart protection. The injury that a heart sustains during a heart operation can be detected in a number of ways. Blood tests may show biochemical abnormalities, electrocardiograms (ECGs) may show changes indicative of injury, echocardiograms (ultrasound scans of the heart similar to those used in pregnant women) can show changes in muscular function of the heart, and monitoring lines can show changes in the pressures and blood flows generated within the heart. Some patients undergoing heart surgery require drug therapy to support the heart afterwards. A requirement for such drugs and the quantity needed is a further index of heart injury. In addition, analysis of tiny samples of heart muscle can show changes in the heart's energy stores. Thus, by performing these investigations in a standard way we can compare whether RIPC is beneficial or not.

### **HOW HAS THIS CLINICAL TRIAL BEEN DESIGNED?**

This study is what is called a double-blind randomized placebo-controlled trial. This means that if you agree to take part you may be allocated by chance to receive RIPC. The RIPC is conducted while you are under anaesthetic in the early stages of your heart surgery. Prior to operation, standard blood pressure cuffs will be applied to your left arm and to a dummy cylinder. These will be connected by a switch and depending on your chance allocation, either cuff will be inflated for 3 periods of 5 minutes each, with 5 minutes of cuff deflation prior to the time when your heart's blood flow is reduced to perform the CABG operation. The cuffs will be obscured from view so that the researchers will not know if you received RIPC or not until such time that we open the allocation code. Your operation will then proceed in a standard way, you will be cared for on our Intensive Care Unit and you will be recovered from surgery in the normal manner. This is done to prevent any bias in the results. By comparing the results of biochemical, ECG, echocardiographic, muscle analyses and post-operative drug requirements between those patients receiving RIPC and those not, we will be able to answer whether RIPC is beneficial.

### **HOW WILL WE MEASURE THE EFFECTS OF RIPC?**

To detect changes in heart function, some measurements need to be taken before, during and after surgery. Prior to your operation an ECG and echocardiogram will be performed to compare with those taken after the operation. On the evening before surgery a portable monitoring ECG will be connected to carefully monitor and record your heart rhythm before, during and for 48 hours after operation. All these tests are harmless and cause minimal discomfort if any. When you are placed under anaesthetic, small monitoring catheters (plastic tubes) are routinely inserted in blood vessels and are usually removed on the first or second post operative day. We will record measurements from these catheters throughout this period at specific time points. These catheters are all a standard part of your heart operation and are removed in the first few days after operation, usually within 48 hours. From these catheters, we will draw some additional blood samples for biochemical analysis. During your operation, your surgeon will take 3 small samples of heart muscle about the size of short length of pencil lead (actual size =====). Analysis of these samples will enable us to examine the possible effects of RIPC on the heart's energy stores or other chemicals. Also during your heart operation, a small catheter is routinely placed in your bladder so that your urine output can be measured. The urine is usually discarded but we will take some timed samples to measure any possible additional protective effect of RIPC on the kidney.

### **MEASURING THE POSSIBLE BENEFICIAL EFFECTS OF RIPC**

**The sampling of additional blood and urine tests:** the blood tests performed on these samples can tell us how well the heart has tolerated 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 half a cupful (50ml). Samples of urine will be collected for analysis from the catheter routinely placed under anaesthesia.

**Imaging the heart using a trans-thoracic echocardiogram:** An echocardiogram is a scan that uses ultrasound (sound waves) to produce pictures of the heart. The test is painless and without side effects. An ultrasound probe and a small amount of gel are gently placed on your chest and will be moved to different positions. The scan will

take approximately 20 –40 minutes to complete and will be performed once before surgery and once after. The scan will be performed in a special Echo room. Following all our heart surgery, small wires are left on the surface of the heart so that we can ensure that your heart rate is satisfactory after the operation. During the second scan, we will adjust your heart rate between 70 and 120 beats per minute (a normal range of heart rate) using these wires. This will give us extra information about heart function. You may be able to feel a change in heart rate. This is harmless, but if uncomfortable we would stop and complete the scan without varying the heart rate.

**ECG and heart monitoring:** ECGs are routinely performed before and several times after surgery. We will analyse these ECGs carefully as part of the study. Heart injury may cause some rhythm changes of the heart that are not detectable by standard ECGs. We will, therefore, connect you to a continuous portable (Holter) monitor on the evening before your operation which will record your ECG for the pre-operative period and for 48 hours afterwards. This will then be removed and analysed by computer. This will be able to tell us how many and what duration of any abnormal rhythms you had during that period. This test is painless and silent.

#### **Monitoring your heart output**

This will be done at specified time points from the lines inserted during your anaesthetic. The lines themselves are a necessary and standard part of the operation. We will gather the information from these lines as part of our research.

**The removal of tiny samples of the heart muscle, skeletal muscle and fat known as biopsies:** This will tell us whether RIPC protects the heart by conserving its energy stores. We would aim to obtain 3 heart muscle biopsies all whilst you are asleep under the anaesthesia, one at the beginning, middle and end of the CABG procedure. A stitch is placed in the tiny defect left behind and their removal incurs no increased risk during the surgery nor has any effect on the strength of the heartbeat. We have performed more than 1500 such biopsies in patients at the time of writing of this information leaflet with no complications.

#### **The RIPC stimulus**

If you have been allocated to the RIPC group, a blood pressure cuff will be inflated on your left arm for three 5 minute periods at the start of your operation while you are under anaesthetic. This is exactly the same as having your blood pressure taken but for a longer period. Previous studies have shown this to be quite safe and harmless and only modestly uncomfortable even in conscious patients. You should not be aware of it whatsoever and we would expect that patients will not be able to tell if they underwent RIPC or not. To be quite sure that this has not caused any problems whatsoever we will ask you to fill in a questionnaire about any symptoms that may have arisen in your arm.

#### **WHAT WILL I HAVE TO DO?**

If you decide to participate then 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 will by now have received the written relevant information and asked to read this at your leisure at home or on the ward if you are already an inpatient. On the day prior to your surgery a member of the research team will visit you so you can ask any questions. If you give signed consent to participate then we will take the opportunity to perform an echocardiogram (20 minutes) on the day before surgery and also connect the ECG monitor.

In the anaesthetic room of the operating theatre the anaesthetist will give you a general anaesthetic. The anaesthetists will insert the pressure measuring catheters normally inserted at this stage. Once you are asleep, an extra blood pressure cuff will be applied to your left arm and inflated for three 5 minute periods as above depending upon your allocation code. The operation will be conducted in a normal and standard 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.

When you awake you will be on the intensive care unit as routine. Blood and urine tests will be drawn from the lines and catheters already in place. The last blood test will be performed at 72 hours after the operation. Forty-eight hours after your operation, the continuous ECG monitor will be removed. On about the fourth day after your operation we will perform the second echocardiogram. Standard tests will be performed in the recovery period as for all patients.

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 50 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 RIPC will actually be of benefit. There is sufficient evidence to believe that it may be but this study is being performed to prove or disprove this. Therefore, we cannot say if you will benefit as an individual. It is hoped that this study will provide information that will benefit future populations of patients undergoing cardiac surgical procedures.

#### **WHAT ARE THE RISKS?**

Previous studies of RIPC have shown that it is a safe. There is no reason to suspect that it would be harmful. Nevertheless the extensive monitoring that is performed as a matter of routine in a patient undergoing cardiac surgery would be able to detect any side effects at their earliest stages before a problem developed. In addition, the questionnaire will help us to establish that it is harmless. All heart operations carry some risk and these will have already been discussed with you by your surgical team. For this study we insert routinely used monitoring lines to measure heart function. These are used in 60% of patients undergoing coronary artery surgery. In the study we would use them in all patients to more closely monitor heart function. The risks of insertion of these monitoring lines are minimal and the possibility of a severe complication is in the region of 1 in 15000. The only additional invasive procedure performed on you is the biopsy which as stated above has been performed in over 500 patients without ill-effect.

**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?**

The information from the study will be collected confidentially and coded for anonymity. At the end of the study, the information will be analysed. The information will be presented at scientific meetings and published in scientific journals to inform other doctors and health professionals of our findings. All data is confidential and stored by code on secure computers. This ensures that your identity will not be revealed at any time.

**WHO ELSE IS TAKING PART?**

We hope that 162 patients will participate in this study. This is the number required to establish that we are able to detect a true effect of RIPC.

**WHAT IF SOMETHING GOES WRONG?**

Throughout your surgery, your safety is of paramount concern. If for any reason, your surgeon and anaesthetist feel that the study should not proceed because of other safety concerns, the research will not take place. 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 all the measurements you will be in the theatre or I.T.U. where trained staff is at hand at all times. Should you become ill for any reason related to the study, the study would be stopped immediately. Your safety during and after surgery is paramount, and takes precedence over any research.

**WHAT HAPPENS AT THE END OF THE STUDY?**

The main study is undertaken during the surgery and our collection of additional information ends on the 4<sup>th</sup> or 5<sup>th</sup> day after your operation. At the end of the complete study, the codes of allocation will be broken and the overall results analysed. Your direct involvement is complete within your stay in hospital. If you would like to receive summary information about the study results, this will be sent to you once the study is complete (in approximately 2 ½ years time).

**WHAT IF I HAVE MORE QUESTIONS OR DO NOT UNDERSTAND SOMETHING?**

Please feel free to 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 you like. If you would like more information this can be provided by the Research Fellow coordinating the study (see below) or from Professor Bonser's office extension 2543.

**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. You will be listed for surgery as normal. When your surgery is scheduled we will ask your permission to start the study on the evening prior to operation and undertake the echocardiogram and ECG monitoring. You are free to withdraw from this study after initially consenting without prejudice to your continuing care.

**CONSENT FORM**  
**Application of remote ischaemic pre-conditioning to human coronary artery bypass surgery (CABG)**

This is a consent form that documents your agreement to participate in this particular research. Before signing this form please tick the boxes below.

- |  |                          |
|--|--------------------------|
| I have read the invitation form  | <input type="checkbox"/> |
| I have read the information sheet  | <input type="checkbox"/> |
| I have understood the nature of the research   | <input type="checkbox"/> |
| I have had time to consider my response/reply  | <input type="checkbox"/> |
| I have been able to ask any questions and am satisfied with the answers                      | <input type="checkbox"/> |
| I understand that I can withdraw at any time without giving explanation                      | <input type="checkbox"/> |
| The participation or lack of it in this research will not affect my clinical care in any way | <input type="checkbox"/> |
| I would like to receive a summary of the research when it is complete.                       | <input type="checkbox"/> |

Please remember that there is no obligation to participate in this study and that even if you do you can withdraw at any time. If you do not consent to this study your operation and care will proceed as planned. There are no rewards or penalties for participating in research although we are grateful for your efforts and inconvenience. If after reading this consent form and the patient information sheet you agree to participate please sign the form below and return to Mr I Rahman. If you have any further questions about any aspect of the research feel free to ask Mr I Rahman.

The study has been explained by:

Print Name:

Signature:

Date:

Please sign below if you agree to participate in this study

I have read and understand the above, and I am happy to take part in the above study.

Signed

Name (Print)

Date

## **APPENDIX B - STANDARDISED PROTOCOLS**

Procedures that have been standardised across a number of trials including protocols for departmental postoperative management of patients are referenced below:

### **1.0 Preparing the operating schedule**

Operating list for trial patients was annotated thus:

*SMITH John DOB 12/12/45 G236819/A CABG (REMOTE TRIAL) E2B*

This practice was to ensure ward staff, anaesthetists, ODP, theatre staff, perfusionists, ITU staff and surgical team were aware of the trial patient and that all protocols were adhered to.

### **2.0 Protamine dose**

Protamine was administered intraoperatively at a dose of 1mg per 100 units of heparin once the patient is off CPB.

Additional protamine to cover the pump blood returned to the patient was given to the following regimen:

- 0-499 ml of pump blood → no additional protamine
- 500-999 ml of pump blood returned → 50 mg protamine
- $\geq 1$ l of pump blood returned → 100 mg protamine

### **3.0 Placement of epicardial pacing wires**

The placement of atrial and ventricular pacing wires is mandatory. Two epicardial atrial pacing wires were sited in the region of the atrial cannulation

site and two epicardial ventricular wires were placed in the right ventricular outflow tract.

#### **4.0 Postoperative medication**

##### *4.1 Antiplatelet therapy*

Patients received on return to the ITU received a dose of aspirin 300mg rectally and thereafter 150mg orally once a day. In the case of aspirin allergy or other contra-indications, anti-platelet therapy was at the discretion of the attending team.

##### *4.2 Angiotensin converting enzyme (ACE) inhibitors*

All patients received an ACE inhibitor in the postoperative period (unless contraindicated). Reinstitution and new prescribing of ACE inhibitor therapy was not permitted until the fourth postoperative day.

##### *4.3 Other cardiovascular medication*

All patients received initial diuretic therapy with oral frusemide 40mg daily. Long-acting nitrates or nitrate patches were not permitted postoperatively. Preoperative antihypertensive medications were recommended as clinically indicated.

$\beta$ -receptor antagonists, statins and other lipid lowering agents were started as soon as possible in the postoperative period.

#### 4.4 *Non cardiovascular medication*

Treatment was restarted on as soon as possible after surgery at the discretion of the caring team.

### 5.0 **Protocols for the management of post-operative complications**

#### 5.1 *Protocol for management of pacing and arrhythmias*

##### 5.1.1 *Management of ventricular fibrillation post AXC removal*

The management was as follows until the restoration of sinus rhythm:

- 2 x 10 J shock
- Administration lignocaine 100mg IV

All reperfusion VF was recorded, together with shock strengths. Similarly, the use of induced VF and subsequent defibrillation to facilitate biopsy or other repairs was recorded.

Treatment beyond this is at the discretion of the patient's clinical team but was recorded as part of the results

##### 5.1.2 *Post-operative pacing management*

At the end of cardiopulmonary bypass all patients had ventricular and atrial pacing wires in situ.

During weaning from CPB, the target synchronised heart rate was 90bts/min.

This was achieved by:

- native sinus rhythm ( $\pm$  atropine / glycopyrrolate)
- AAI pacing (90 bts.min<sup>-1</sup>)
- DDD (or DVI) pacing (90bts.min<sup>-1</sup>) with a default AV interval of 160ms.

Initial haemodynamic studies were performed in the rhythm or pacing mode selected by the Consultant Surgeon/Anaesthetist to achieve best overall haemodynamic performance. These settings were maintained through transfer to ITU and reviewed by the Research Fellow 2 hours following release of the aortic cross-clamp.

At this time, prior to the 2 hour haemodynamic assessment:

- in patients in SR with a heart rate of  $\geq 90 \text{bts.min}^{-1}$  and normal AV conduction no action was required
- In patients in SR with a heart rate  $< 90 \text{bts/min}$  AAI pacing at  $90 \text{bts.min}^{-1}$  was instituted
- In patients DDD or DVI paced ( $90 \text{bts.min}^{-1}$ ) the PAUSE button on the pacing box was depressed briefly:

If this demonstrated persistent AV block, DDD or DVD pacing (default AV interval 160ms) was continued throughout the study period at  $90 \text{bts.m}^{-1}$ .

If the PAUSE query demonstrated a rhythm which had AV conduction, the pacing mode was adjusted. The AV interval setting was increased to a maximum of 200ms and the rhythm reviewed.

If the rhythm was now atrially paced with native AV conduction, this means that the native AV interval is  $< 200 \text{ms}$ . This rhythm is haemodynamically preferable and provided there was not a variable native AV interval and thus intermittent pacing, these pacing settings (DDD AV interval 200ms) were maintained.

In circumstances in which the AV interval was  $\geq 200 \text{ms}$  or in which the native AV interval was variable with intermittent ventricular pacing, the previous DDD settings (AV interval 160ms) was resumed.

The trial staff would document in the patient notes any pacing changes, record the data on the study datasheet and proceed with the haemodynamic studies.

This pacing assessment is the only time when trial staff had a direct clinical responsibility regarding a patient's management.

#### *5.1.3 Protocol for the management of suspected atrial fibrillation and other supraventricular tachyarrhythmias*

- Check hypoxia and hypokalaemia ( $K^+ < 4.5 \text{ mmol.L}^{-1}$ ), record the result and correct appropriately
- Confirm diagnosis with a12 lead ECG (2 copies named, dated and timed)
- If hypoxia is present a chest radiogram was obtained

#### *5.1.4 Management of AF or atrial flutter: Scenario 1*

If the PR interval  $< 180$  millisecs, no new LBBB or no new bifasicular block and ventricular rate  $> 80.\text{min}^{-1}$  the treatment plan was:

##### *5.1.4.1 Patient unable to take oral medication*

If the patient was intubated and ventilated we would strive to ensure oxygen saturations were greater than 95%, using supplemental oxygen as required. We aimed to maintain serum  $K^+$  greater than  $4.0 \text{ mmol.L}^{-1}$

Intravenous Amiodarone 1.2g over 24 hours was administered. The initial bolus dose of 300mg was administered intravenously over 1 hour followed by 900mg for the remaining part of 24 hours.

Intravenous Amiodarone 1.2g was repeated in the second 24 hours, and reduced to 600 mg over 24 hours for the next 5 days, and 300mg over 24 hours thereafter.

Oral Amiodarone was commenced when oral or nasogastric medications were tolerated.

Prophylactic anti-coagulation was administered using s/c Enoxaparin 40mg daily for the first 48 hours after the commencement of atrial fibrillation, unless other anti-coagulation was already ongoing, or unless anti-coagulation was contra-indicated. If atrial fibrillation persisted for more than 48 hours, Enoxaparin was increased to 1mg/kg s/c BD, unless contraindicated, until 24 hours after sinus rhythm was restored.

DC cardioversion was considered if sinus rhythm was not restored after 72 hours atrial fibrillation.

If the ventricular capture rate remained high loading with Digoxin 500µg 6 hourly up to 3 doses according to response was considered. Maintenance dose was then continued at half the recommended dose for age, size and renal function.

DC cardioversion was also considered, at any stage, if haemodynamic instability ensued. Patients who failed to cardiovert to sinus rhythm were warfarinised. Target INR 2.5.

#### 5.1.4.2 *Patient able to take oral medication*

For awake, extubated patients on the ward or ITU we would strive to ensure oxygen saturation's were greater than 95%, using supplemental oxygen as required and aim to maintain serum K<sup>+</sup> greater than 4.0 mmol.L<sup>-1</sup>.

The regimen of oral Amiodarone 400 mg TDS for 48hrs, followed by 200mg TDS for one week, then 200mg BD for one week and finally 200mg OD for one week was followed, or until the day of discharge, whichever is the shorter. Doses were reviewed if patient weight was less than 50Kg.

Prophylactic anti-coagulation was administered using s/c Enoxaparin 40mg daily for the first 48 hours after the commencement of atrial fibrillation, unless other anti-coagulation was already ongoing, or unless anti-coagulation was contra-indicated. If atrial fibrillation persisted for more than 48hours Enoxaparin was increased to 1mg.kg<sup>-1</sup> s/c BD, unless contraindicated, until 24 hours after sinus rhythm was restored.

DC cardioversion was considered if sinus rhythm was not restored after 72 hours of atrial fibrillation or if at any stage haemodynamic instability ensued.

If the ventricular capture rate remained high loading with Digoxin 500µg 6 hourly up to 3 doses according to response was considered. Maintenance dose was continued at half the recommended dose for age, size and renal function.

If atrial fibrillation/atrial flutter was intermittent but persistent despite Amiodarone loading anti-coagulation with Warfarin was commenced prior to discharge home and continued until review in the outpatient clinic (target INR 2.5). If sinus rhythm was restored when reviewed in the outpatient clinic Warfarin and Amiodarone was discontinued. If atrial fibrillation/atrial flutter persisted DC cardioversion was considered.

Amiodarone was discontinued on review in the outpatient clinic at 6 weeks if sinus rhythm persisted.

#### *5.1.5 Management of AF or atrial flutter: Scenario 2*

If the PR interval >180 millisecs, or new LBBB or new bifasicular block and ventricular rate 80.min<sup>-1</sup> the treatment plan was:

##### *5.1.5.1 Patient unable to take oral medication*

If the patient was unable to take oral medication intravenous Amiodarone 600 mg over 24 hours for 2 days and 300 mg daily thereafter until able to tolerate oral/NG medication was commenced. We would strive to ensure oxygen saturations were greater than 95%, using supplemental oxygen as required and aim to maintain serum K<sup>+</sup> greater than 4.0 mmol.L<sup>-1</sup>.

Prophylactic anti-coagulation was administered using s/c Enoxaparin 40mg daily for the first 48 hours after the commencement of atrial fibrillation, unless other anti-coagulation was already ongoing, or unless anti-coagulation was contra-indicated. If atrial fibrillation persisted for more than 48 hours, Enoxaparin was increased to 1mg.kg<sup>-1</sup> s/c BD, unless contraindicated, until 24 hours after sinus rhythm was restored.

DC cardioversion was considered if sinus rhythm was not restored after 72 hours atrial fibrillation.

If ventricular capture rate remained high loading with Digoxin 500µg 6 hourly up to 3 doses according to response was considered. Maintenance dose was continued at half the recommended dose for age, size and renal function.

DC cardioversion was also considered, at any stage, if haemodynamic instability ensued.

Patients who failed to cardiovert to sinus rhythm were warfarinised. Target INR 2.5.

#### *5.1.5.2 Patient able to take oral medication*

If the patient was able to take oral medication oral Amiodarone 200 mg TDS for 8 days or until the time of discharge, whichever is the shorter was commenced. We would strive to ensure oxygen saturations were greater than 95%, using supplemental oxygen as required and aim to maintain serum K<sup>+</sup> greater than 4.0 mmol.L<sup>-1</sup>.

Prophylactic anti-coagulation was administered using s/c Enoxaparin 40mg daily for the first 48 hours after the commencement of atrial fibrillation, unless other anti-coagulation was already ongoing, or unless anti-coagulation was contra-indicated. If atrial fibrillation persisted for more than 48hours, Enoxaparin was increased to 1mg.kg<sup>-1</sup> s/c BD, unless contraindicated, until 24 hours after sinus rhythm was restored.

DC cardioversion was considered if sinus rhythm was not restored after 72 hours atrial fibrillation and also at any stage if haemodynamic instability ensued. If the ventricular capture rate remained high loading with Digoxin 500µg 6 hourly up to 3 doses according to response was considered. Maintenance dose was continued at half the recommended dose for age, size and renal function.

If atrial fibrillation/atrial flutter was intermittent but persistent despite Amiodarone loading anti-coagulation with Warfarin was commenced prior to discharge home and continued until review in the outpatient clinic (target INR

2.5). If sinus rhythm was restored when reviewed in the outpatient clinic Warfarin and Amiodarone was discontinued. If atrial fibrillation/atrial flutter persisted DC cardioversion was considered. Oral Amiodarone 200 mg daily thereafter. Amiodarone discontinued on review in the outpatient clinic at 6 weeks if sinus rhythm persists

#### *5.1.6 D.C. Cardioversion technique for atrial fibrillation/flutter*

Under anaesthesia the cardiovert protocol followed according to the incremental energies detailed below:

##### **Monophasic**

50J x2 attempts

100J x 2 attempts

150J x 2 attempts

360J x 2 attempts

##### **Biphasic**

150J x 3

Abandon if unsuccessful. Consider anticoagulation and further management at team discretion.

#### *5.1.7 Diagnosis and management of other supraventricular tachycardias*

In the presence of supraventricular arrhythmias not believed to be AF the aim of treatment was to restore normal sinus rhythm. Hypokalaemia and hypoxia were corrected. Carotid sinus massage was attempted and if this failed intravenous adenosine therapy was instituted as 3 mg in increasing doses of 3 mg (i.e. 3 mg, 6 mg, 9 mg) unless the patient had contraindications to adenosine (asthma, second or third degree heart block, sick sinus syndrome). Further treatment was at the discretion of the patient's consultant/clinical team.

### *5.1.8 Protocol for the management of ventricular dysrhythmias*

The following ventricular dysrhythmias require attention:

- Bigeminy or Trigeminy
- ‘Salvos’ of ventricular tachycardia
- ‘R on T’ phenomenon
- 15 ventricular ectopics/minute for > 5 minutes
- Sustained VT or VF

In all cases hypoxia, hypokalaemia and hypomagnesaemia were corrected, the diagnosis was confirmed by 12-lead ECG with a lead II rhythm strip unless patient was in theatres and a chest x-ray was performed (if no obvious cause for hypoxia).

#### *5.1.8.1 Treatment of sustained pulseless ventricular tachycardia or ventricular fibrillation*

The decision to cardiovert, defibrillate or proceed to resternotomy was at the discretion of the caring team. Defibrillation should be the immediate method of choice (The Advanced Life Support Guidelines 2000) up to three shocks given initially with energies of 200 J, 200 J, 360 J (or their equivalent when using defibrillators with alternative waveforms). Amiodarone or lignocaine could next be utilised:

- Amiodarone 300 mg (made up to 20 ml with 5% dextrose or from a prefilled syringe) may be administered. A further dose of 150 mg may be given for recurrent or refractory VT/VF, followed by an infusion of 1 mg min<sup>-1</sup> for 6 -12 hours then at 0.5 mg min<sup>-1</sup>.

- Lignocaine was given at  $1 \text{ mg min}^{-1}$ , with a further 1-2 doses of  $0.5 \text{ mg min}^{-1}$  10 minutes apart. A continuous infusion may be considered as 1000 mg of lignocaine to a total volume of 50 ml of 0.9%NaCl (20 mg/ml) and infused at a rate of  $12 \text{ ml.hr}^{-1}$  ( $4 \text{ mg.min}^{-1}$ ) for 3 hours.

#### *5.1.9 Protocol for the management of bradycardias (<60bpm for >5 minutes)*

In the instance of bradycardias we would check initially the ECG monitors scale. The haemodynamic targets of CVP, MAP and CI would be assessed. A 12lead ECG would be performed.

Sinus bradycardia, nodal bradycardia or first-degree heart block but with MAP 65-85 mmHg and cardiac index  $>2.2 \text{ L.min}^{-1} \text{ m}^{-2}$  required no treatment.

Sinus bradycardia, nodal bradycardia or first degree heart block but with MAP <65-85 mmHg or CI  $<2.2 \text{ L.min}^{-1} \text{ m}^{-2}$  either glycopyrolate boluses of 0.6 mg up to a maximum of 1.8 mg or IV atropine boluses of 0.6 mg up to a maximum of 1.8 mg were used. Pacing or isoprenaline infusion was considered.

Second or third degree heart block management was at the discretion of the managing team. All therapy was recorded.

### **6.0 The management of post-operative hypertension on ITU**

Hypertension is defined as MAP  $>85 \text{ mmHg}$ . If a sustained period of hypertension or repeat temporary episode of hypertension develop then the patient was managed as below:

- Ensure adequate analgesia
- GTN (i.v. 50 mg in 50 ml) titrating the dose to achieve MAP  $\leq$ 85 mmHg until the upper limit of infusion of 15 ml.h has been reached.
- If this failed to reduce the blood pressure then SNP (i.v. 50 mg in 50 ml dextrose 5% solution) at 0-15 ml.h<sup>-1</sup> was added as directed by the caring medical team.
- Once the patient has been extubated, the need for hypertension control should be re-assessed by the caring medical team. Where appropriate, intravenous medication should be substituted with either re-commencement of oral anti-hypertensive therapy or institution of oral agent is at the discretion of the managing team.

## **7.0 Assessment and management of post-operative hypotension (i.e. sustained MAP <60mmHg)**

Hypotension is defined as MAP<60mmHg. In the case of hypotension the following was assessed:

- Arterial line calibration and accuracy
- Cardiac rhythm (if abnormal see treatment of cardiac rhythm disturbances)
- Filling pressures (CVP and PAWP)

- If below the range set by the patient's consultant/clinical team or  $< 8$  cm H<sub>2</sub>O if no range has been set then volume was administered.
- If above the range set by the patient's consultant/clinical team or  $> 12$  cm H<sub>2</sub>O then cardiac output studies were performed.
- If cardiac index was  $\leq 2.2$  L.min.<sup>-1</sup>m<sup>-2</sup> the protocol for the assessment and management of low cardiac output state was followed
- If cardiac index was  $> 2.2$  L.min.<sup>-1</sup>m<sup>-2</sup> and a normal SVR (800-1200dyne.cm.sec<sup>-5</sup>) associated with hypotension management was directed by the attending clinical team.
- If cardiac index  $> 3$  L.min.<sup>-1</sup>m<sup>-2</sup> with a low SVR ( $< 800$ dyne.cm.sec<sup>-5</sup>) was the cause of hypotension phenylephrine was prescribed as an infusion of 10mg made to 50 ml with 5% dextrose. The infusion was to start at 2 ml.h<sup>-1</sup>

## **8.0 Assessment and management of suspected low cardiac output state**

For the purpose of this study a low cardiac output state is defined as a C.I. of  $\leq 2.2$  L.min.<sup>-1</sup>m<sup>-2</sup> refractory to appropriate intra-vascular volume expansion following correction or attempted correction of any dysrhythmias including sinus bradycardia, nodal rhythms, atrial and ventricular tachycardia or dysrhythmias.

### *8.1 Low cardiac output state in theatres and anticipatory treatment*

Whenever possible the surgeon/anaesthetist was to await the results of cardiac output studies performed and recorded after CPB had been discontinued. In some cases the surgeon/anaesthetist will anticipate a low cardiac output state

and the need for inotropes on subjective and objective features of cardiac performance before and during surgery. If possible confirmatory cardiac output studies should be awaited but if not inotropic therapy is started at the discretion of the managing team. The exact time of commencement and dosage was recorded.

Low cardiac output state in the presence of adequate 'filling pressures' is an endpoint for this study.

The charts of patients who have had anticipatory inotropic treatment underwent a review by the low cardiac output committee. The aim of the review was to confirm that in the absence of C.O.S. data we had a bonafide case of low cardiac output.

## *8.2 Post-operative low cardiac output state diagnosed in the ITU/HDU*

Any patient having a measured cardiac index of  $\leq 2.2 \text{ L.min.}^{-1}\text{m}^{-2}$  was assessed as follows:

- Assess heart rate and rhythm and correct as appropriate
- Measure filling pressures (CVP and PAWP) and correct as appropriate within targets set by the caring team. Consider overt or covert haemorrhage
- Consider the possibility of cardiac tamponade

Appropriate management of the above factors is at the discretion of the caring team. Once these factors were assessed and treated and if the patient still had a cardiac index of  $\leq 2.2 \text{ L.min.}^{-1}\text{m}^{-2}$  then the patient was considered to be suffering from a low cardiac output state and initial management was as follows:

- Dopamine (first line inotrope) 200mg in 50ml 5% dextrose commenced at a dose of  $1-10 \mu\text{g}.\text{kg}^{-1}.\text{min}^{-1}$  at the discretion of the caring team.
- All management considered necessary above and beyond dopamine  $10\mu\text{g}/\text{kg}/\text{min}$  is at the discretion of the caring team.

### 8.3 *Management of a 'Reopening' Procedure*

The decision to reopen a patient is purely a clinical one at the discretion of the patient's consultant/team. The intra-operative reopening 'findings' were recorded.

## 9.0 **Protocol for the administration of colloids and blood products**

Colloid is prescribed to replace drain losses and maintain adequate filling pressures to achieve the desired mean arterial pressure (MAP 65-85mmHg) and cardiac index. The colloid of first choice for this study was Gelofusin<sup>®</sup>.

The maximum volume of gelofusin<sup>®</sup> permissible over 12 hours is

- 2 L if the patient is  $\geq 70$  kg
- 1.5 L if  $< 70$  kg.

If additional filling was required beyond these volumes:

- H.A.S. 4.5% if  $\text{HB} \geq 8.5$
- Packed red cells if  $\text{HB} < 8.5$  or active bleeding through drains above specified rate

Clotting factors, platelets and cryoprecipitate administered to restore clot formation also served as volume expanders in addition to the Gelofusin<sup>®</sup>.

## **10.0 Protocol for the management of a low urine output**

Low urine output was defined as  $<0.5\text{ml.kg}^{-1}.\text{hr}^{-1}$ . If the urine output fell below this in any 1 hour the following was checked:

- adequate mean arterial pressure
- adequate CVP and PAWP
- a working urinary catheter. Consider a 50 ml flush

If this checklist is normal then intravenous frusemide 10mg was administered and response awaited.

If any of the above checklist were not in the target parameters appropriate colloid was given and cardiac indices repeated considering the use of inotropes as protocol.

Failure to maintain a satisfactory diuresis would prompt an enquiry regarding a possible low cardiac output state. If a satisfactory cardiac output is the case but urine output remains inadequate dopamine (200 mg in a 50ml of 5% dextrose) run at  $3\text{ }\mu\text{g/kg/hr}$  was used supplemented by a low dose frusemide infusion as necessary. Treatment beyond this was at the discretion of the patient's clinical team.

## **11.0 Protocol for the management of suspected wound infection**

### *11.1 Suspected sternal or leg wound Infection*

Bacteriological swabs were taken from sternal or leg wounds in cases of suspected infection. Treatment was at the discretion of the clinical team.

### 11.2 Other infections

Bacteriological specimens were taken as appropriate in all suspected infection episodes and treatment was at the discretion of the clinical team.

### 12.0 Protocol for management of blood glucose

The normal range for random blood glucose was defined as 4-10 mmol.L<sup>-1</sup>

Post-operative hyperglycaemia and insulin resistance was managed with standard Trust sliding scale insulin protocols.

3 examples of sliding scale protocols (A, B and C) are given according to insulin-resistance of the patients below.

Management would start from protocol A and escalate if necessary. The aim was to maintain blood sugars ≤10mmol/L.

A		B		C	
BM (mmol.L <sup>-1</sup> )	Units.h <sup>-1</sup>	BM (mmol.L <sup>-1</sup> )	Units.h <sup>-1</sup>	BM (mmol.L <sup>-1</sup> )	Units.h <sup>-1</sup>
0 - 7	0	0 - 7	0	0 - 7	0
7.1 – 11	1	7.1 – 11	2	7.1 – 11	4
11.1 – 15	2	11.1 – 15	4	11.1 – 15	8
15.1 – 20	4	15.1 – 20	6	15.1 – 20	10
>20	6	>20	8	>20	14

In more severe cases of insulin-resistance intermittent boluses of insulin of 5-10 units could be administered if serum potassium concentration was ≥4 mmol.L<sup>-1</sup>

### **13.0 Post-operative prescription for all ITU trial patients**

#### *13.1 Fluids*

5% Dextrose at  $1\text{ml.kg}^{-1}\text{h}^{-1}$  was prescribed

#### *13.2 Potassium*

Potassium chloride (KCl) 10-20mmol in 30ml 5% dextrose over 30 minutes was prescribed as required to maintain  $\text{K}^+$  4.5-5.5mmol.L<sup>-1</sup>.

#### *13.3 Insulin*

As per ITU sliding scales

#### *13.4 Sedation and continuous intravenous analgesia*

Propofol 10mg/ml at 0-15 ml.h<sup>-1</sup>

Alfentanil 25mg in 50ml 5% Dextrose at 0-2ml.hr<sup>-1</sup>

Followed by:

Paracetamol 1q p.o. QDS

Tramadol (PRN) i.v. 50-100 mg 4 hrly or Tramadol p.o. 50-100 mg qds 4hrly

And or Codeine Phosphate 30-60mg 4hrly (max 240mg.24h<sup>-1</sup>) or

Dihydrocodeine 30-60mg qds (max 240mg.24h<sup>-1</sup>).

#### *13.5 Antihypertensive medication*

Glyceryl trinitrate solution (GTN) 50 mg in 50 ml at 1-15 ml.hr<sup>-1</sup> ±

Sodium nitroprusside (SNP) 50mg in 50ml. D5%W at 0-20ml.h<sup>-1</sup> or

Nifedipine 10mg sub-lingual PRN for sustained hypertension (>160/90)

### *13.6 Anti-emetic medications*

Maxalon 10 mg 8 hrly i.v./i.m./p.o. ±

Cyclizine 50 mg i.v. 8 hrly ±

Ondansetron 4-8 mg IV/IV/PO PRN 6-8 hourly

### *13.7 Antibiotics*

As per the standard unit policy.

### *13.8 Heparin DVT prophylaxis*

Enoxaparin 40mg s/c o.d.

### *13.9 Intravenous diuretic therapy*

Frusemide 10mg i.v. PRN

### *13.10 Inotropes and Vasoconstrictors*

The trial staff would always aim to perform a full series of cardiac output studies prior to the commencement or escalation of inotropes.

Inotropes would always be prescribed and used in the 50ml 5% dextrose as per unit policy. The dose of drug required to constitute a single strength solution for each inotrope is detailed below:

Dopamine 200mg

Dobutamine 250mg

Adrenaline 2mg

Noradrenaline 2mg

Phenylephrine should be made up as 10mg / 50mls 5% dextrose.

Enoximone should be made up as 100mg / 40mls N/Saline

The loading dose should be run at  $1\text{mg.kg}^{-1}$  for 1 hour

Enoximone in the pump was not allowed.

## **APPENDIX C - PRESENTATIONS AND PUBLICATIONS ARISING FROM THIS THESIS AND THE RESEARCH FELLOWSHIP**

### **1.0 PRESENTATIONS**

**[1] Does remote ischaemic preconditioning during on-pump coronary artery bypass grafting enhance lung preservation?** Rahman IA, Mascaro JG, Green D, Townsend P, Bonser R. *ESCVS International Congress*, 2010 (oral presentation).

**[2] Does remote ischaemic preconditioning protect the heart and kidneys in human coronary artery bypass surgery – a randomised controlled trial.** Rahman IA, Mascaro JG, Steeds RP, Frenneaux MP, Nightingale P, Gosling P, Townsend P, Townend J, Green D, Bonser RS. *SCTS 2010* (oral presentation).

**[3] Remote ischaemic pre-conditioning in human coronary artery bypass surgery, from promise to disappointment.** Rahman IA, Mascaro CJ, Steeds RP, Frenneaux M, Nightingale P, Gosling P, Townsend P, Townend J, Green D, Bonser RS. *American Heart Association Scientific Session*, November 2009 (oral presentation)

**[4] Increased beta-catenin pathway expression by a remote ischaemic preconditioning stimulus: first evidence in humans.**

Contractor H, Ashrafian H, Rahman IA, Redington A, Frenneaux M, Bonser RS, Kharbanda R. *American College of Cardiology*, 2009 (oral presentation)

**[5] The functional impact of remote ischaemic preconditioning on patients undergoing first time coronary artery bypass surgery.** Rahman IA, Nightingale P, Marsh AM, Williams L, Mascaro CJ, Bonser RS, Steeds R. *British Cardiovascular Society*, 2009 (poster presentation)

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## **2.0 PUBLICATIONS**

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