FACTORS AFFECTING THE CARDIOPROTECTIVE RESPONSE TO REMOTE ISCHAEMIC PRECONDITIONING IN PATIENTS UNDERGOING CARDIAC SURGERY

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Abstract

Death from ischaemic heart disease (IHD) remains the most common cause of death worldwide, and is also a significant cause of morbidity. Coronary artery bypass graft (CABG) surgery is performed in a significant number of IHD patients that meet certain clinical and angiographic criteria or are unsuitable for percutaneous coronary intervention. Increasingly higher risk surgeries are taking place, as the population survives to become more aged with well-managed co-morbidities. As such, the myocardium is at risk of peri-operative myocardial injury (PMI) during CABG surgery, the presence of which impacts on clinical outcomes. There are a number of strategies already in place to protect the myocardium during CABG surgery including therapeutic hypothermia, and the use of cardioplegic solutions, but there is a need to increase cardioprotection especially in higher-risk patients.

In this regard, remote ischaemic conditioning (RIPC) is a promising, yet simple and cheap, non-invasive intervention, which shows huge promise in reducing PMI during CABG surgery. RIPC involves the serial inflations and deflations of a blood pressure cuff to the upper and/or lower limbs to induce brief cycles of ischaemia and reperfusion to the skeletal muscle. Despite the promise, clinical studies have produced variable results RIPC in the setting of CABG surgery, with confounding factors such as co-morbidities (age and diabetes) and comedications (propofol and glyceryl trinitrate) being proposed to interfere with the cardioprotective effect.

In two separate clinical studies of adult patients undergoing CABG surgery, we investigated the effect of diabetes and glyceryl trinitrate (GTN) on the cardioprotective effect elicited by RIPC. We used an intensified RIPC

protocol comprising 3 cycles of simultaneous inflation and deflation of two cuffs – one placed on the upper arm and the other on the thigh. The primary outcome measures of both clinical studies, was the extent of peri-operative myocardial injury (PMI), as evidenced by the 72 hours serum Troponin T area-under-the-curve. Secondary outcomes measures included the incidence of post-operative atrial fibrillation, acute kidney injury, inotrope score and length of intensive care and hospital stay. In the diabetic study, we found that RIPC significantly reduced the extent of PMI, whereas in the GTN study, RIPC resulted in only a non-significant reduction in PMI, when compared to control. There were no differences in the secondary outcome measurements with RIPC versis control in either study. In conclusion, we have demonstrated that intensifying the RIPC stimulus can overcome the confounding effects of diabetes and GTN on RIPC cardioprotection in patients undergoing CABG surgery.

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Abbreviations

ACC - American College of Cardiology

AHA – American Heart Association

ACE - angiotensin converting enzyme

ATP - adenosine triphosphate

Ca2+ - calcium

CABG – coronary artery bypass surgery

CIN - contrast-induced nephropathy

CK-MB creatinine kinase MB

CRISP - Cardiac Remote IPC in coronary stenting

cTn - Cardiac troponin

DAG – diacylglycerol

DPP-4 - dipeptidyl peptidase-4

DM - diabetes

ERK - extracellular-signal kinases

GLP-1 - glucagon-like peptide

G-PCR - G-protein coupled receptors

GSK3-β - glycogen synthase kinase 3 β

GTN – glyceryl trinitrate

H_2S - Hydrogen sulphide

iNOS - inducible nitric oxide synthase

IPost - Ischaemic postconditioning

IPC – IPC

JAK/STAT-3 - Janus Kinase/Signal Transducer and activator of transcription

JNK - c-Jun N-terminal

K_{ATP} - K+ adenosine triphosphate (ATP)

MACCE - all-cause mortality, major adverse cardiac and cerebrovascular events

LDH - lactate dehydrogenase

LBBB – left bundle branch block

LMS – left main stem

LV – left ventricular

MAPK - Mitogen activated kinases

MACCE – Major Adverse Cardiovascular and Cerebrovasular events

mitoK$_{ATP}$ - mitochondrial ATP-sensitive potassium channel

mPTP - mitochondrial permeability transition pore

MVO - microvascular obstruction

Na+ - sodium

NO – nitric oxide

NF-κB - nuclear factor-κB

PI3k/Akt - Phosphatidylinositol-3-Kinase and Protein Kinase B

PCI – percutaneous coronary intervention

PPAR - peroxisome proliferator-activated receptor

PPCI - primary percutaneous coronary intervention

PKC – Protein Kinase C

RACK - receptor for activated C-Kinase

RI - reperfusion injury

RIC - Remote ischaemic conditioning

RIPC - Remote IPC

RIPerC – Remote ischaemic per-conditioning

RIPost - Remote ischaemic postconditioning

RISK - Reperfusion injury salvage kinase

ROS – reactive oxygen species

SIP - sphingosine-1-phosphate

SPECT - single proton emission computed tomography

STEMI – ST-elevation myocardial infarction

STZ - streptozotocin

SWOP - second window of preconditioning

TIMI – Thrombolysis in myocardial infarction

TNF–α - tumour necrosis factor α

UK - United Kingdom

URL – upper reference limit

Chapter 1: Introduction

1.1 An overview of coronary artery disease and valvular heart disease and cardiac surgery

1.1.1 Coronary artery disease – a perspective.

Coronary artery disease mortality has declined significantly over the last four decades in developed countries, although it remains the most common cause of death worldwide (1). In the United Kingdom, there are 82,000 deaths a year from coronary artery disease. In addition, there is a significant impact on morbidity from coronary artery disease with 2 million sufferers of angina in the UK, and some 750,000 people with heart failure. The economic burden from this is vast, with £3.2 billion spent per year in the UK on ischaemic heart disease (2).

1.2 Revascularisation in coronary artery disease

1.2.1 Coronary artery bypass graft surgery

The main indications for revascularisation in coronary artery disease are to improve symptoms in patients with angina on maximal medical therapy, and to improve prognosis in patients with a significant burden of ischaemia (3). The Class 1 indications of the AHA/ACC guidelines for coronary artery bypass graft (CABG) surgery include the following (4, 5):

- Left main coronary artery stenosis >50%

- Proximal left anterior descending artery (LAD) and proximal circumflex stenosis >70%

- 3-vessel disease in those with stable or mild angina or even asymptomatic disease

- 3-vessel disease with proximal LAD stenosis in patients with poor left ventricular (LV) function
- A large area of viable myocardium in a high-risk area in patients with stable angina and 1 to 2 vessel coronary artery disease
- Proximal LAD stenosis >70% with either LV ejection fraction <50% or demonstrable ischaemia on non-invasive testing

Rarely CABG surgery, may be performed as an emergency in the context of an ST-segment elevation myocardial infarction (STEMI), in cases where it has not been possible to revascularise with primary percutaneous coronary intervention (PCI), and despite medical therapy there is ongoing pain and ischaemia threatening a significant area of myocardium. Surgery should be performed in the initial 4 hours from presentation before myocardium becomes necrotic. After this window, there is an inverse relationship between the time elapsed from STEMI and surgical mortality. A waiting period of 3 to 7 days appears to be the optimal time for outcome in the absence of symptoms and haemodynamic instability (6).

Initially CABG was the treatment of choice for coronary artery disease but over the last two decades, PCI has challenged CABG especially with the advent of drug eluting stents. The relative efficacies of both treatment strategies have been hotly debated but in-roads have been made providing guidance for clinicians with the publication of the SYNTAX (7) and FREEDOM (8) trials, and analysis of several large registries. The SYNTAX trial randomised 1800 patients with 3 vessel coronary artery disease and left main stenosis (LMS) stenosis to PCI or CABG. A SYNTAX score was calculated

using angiographic appearances to the effect that the higher the score the more severe and complex the disease (9). Intermediate and high scoring patients (>18 and >27, respectively) were associated with increasing cardiac mortality, major adverse cardiac events, and were ultimately found to be best served by CABG as opposed to PCI (10). The FREEDOM trial showed that patients with diabetes and multivessel coronary artery disease treated with CABG surgery had significantly lower rates of death from any cause, nonfatal MI, or nonfatal stroke when compared with diabetic patients treated with PCI. When the SYNTAX score was applied to these patients the absolute difference in the primary end-point persisted between PCI and surgery with low, intermediate, and high SYNTAX scores, thus implying that surgery conferred a benefit in all diabetic patients regardless of the severity or complexity of coronary disease. It is well recognised, that the rate of fatal coronary heart disease is higher in patients with diabetes than in those without (11) and so from this it can be inferred that as seen in the SYNTAX trial CABG is most beneficial in higher risk patients, making those that have diabetes qualify automatically regardless of other risk factors and coronary anatomy.

1.2.2 Valvular heart surgery

Although valvular heart surgery, is less frequently performed than CABG surgery, it is still common despite the decline in rheumatic heart disease in developed countries. Other than in developing countries rheumatic heart disease is now rare, and although some glory goes to the ready availability of penicillin, the truth is that better hygiene and living conditions have resulted in reduced transmission of Group A streptococci (12). This means that valvular

heart disease is now largely a degenerative disease of the elderly with aortic stenosis and mitral regurgitation being the forerunners and aortic regurgitation and mitral stenosis trailing behind but in equal frequency (13).This means that the majority of patients requiring valvular heart surgery will have comorbidities contributing to an increased operative risk.

1.2.3 Cardiac surgeries are now higher risk

In essence, cardiac surgery is increasingly being performed in higher risk patients. This is due to increased life expectancy resulting in an older cohort of patients with more comorbidities (14). Similarly, valvular heart disease has a more elderly patient demographic. In addition, higher risk patients are being selected out for CABG over PCI through the use of scoring systems. These risks need to be offset and this can be done by the development of existing or new therapeutic strategies that improve cardioprotection.

<u>1.3 Perioperative myocardial injury and infarction</u>

1.3.1 Defining perioperative myocardial injury

Up until the 1990s, there was no consensus on the definition of perioperative myocardial infarction and its significance on long-term prognosis was debated (15). Despite a lack of evidence, a joint European and American task force put forward an expert consensus in 2007 that biomarker values >x5 of the 99th percentile of the normal reference range during the first 72 hrs following CABG with new pathological Q-waves or new left bundle branch block (LBBB), angiographically documented new graft or native coronary artery occlusion, or imaging that demonstrates new loss of viable myocardium,

should be considered as diagnostic of CABG-related myocardial infarction or Type 5 MI (16). It is now believed that 2 to 15% of patients undergoing cardiac surgery develop postoperative myocardial infarction resulting in decreased short and long-term survival (17). Obviously, the degree of myocardial injury that is encountered after surgery lies on a spectrum with some patients having lesser insults not fulfilling the definition criterion for myocardial infarction. This is illustrated by studies showing prognostically significant low-level troponin elevations in high-risk cardiac patients using current highly sensitive troponin assays (18).

1.3.2 Causes of perioperative myocardial injury

The triggers for perioperative myocardial injury are many. Perhaps the most common and important of these triggers is myocardial oxygen supply and demand mismatch, with tachycardia being the most common mechanism of this imbalance (19). The preoperative causes of tachycardia are many and include light level of anesthesia, endotracheal intubation and extubation, hypovolaemia, fever, anaemia, congestive heart failure, and postoperative pain. Other contributors to inadequate oxygen supply include hypertension, hypotension, blood loss, drugs, systemic vasodilatation, and left ventricular hypertrophy. Inadequate ventilation may also account for mismatch with hypoxaemia and hypercarbia aggravating any resultant ischaemia.

As well as the direct trauma of surgery, there are also extreme stresses involved in inducing a hypercoaguable state. These include pain, anaesthesia, analgesia, bleeding and hypothermia. The stress state results in increased

catecholamines and cortisol, which in turn results in increases in heart rate, blood pressure, relative insulin deficiency and free fatty acid levels, all of which increase oxygen demand.

The hypercoaguable state promotes increased procoagulants such as plasminogen activator inhibitor-1, factor VIII and platelet reactivity, as well as decreases in endogenous anticoagulants such as protein C and antithrombin III (20).

The inflammatory state that is produced releases tumour necrosis factor-alpha, interleukin (IL)-1, IL-6 and C-reactive protein (21). All of these changes can result in increased coronary artery sheer stress and induce coronary artery thromboses. A local and systemic inflammatory response is also created by blood being exposed to the foreign surfaces of the cardiopulmonary bypass circuit (22). Cardiopulmonary bypass will be discussed later but this allows surgery to be performed in a motionless, bloodless field while providing physiological support through an extracorporeal circuit.

Other mechanisms involved in perioperative myocardial ischaemia involve technical problems associated with placing coronary bypass grafts and valves. These include graft kinking, closure, anastomotic stenosis and spasm (23). Inadequate placement or selection of valves with resultant regurgitation or failure will also produce myocardial injury (24).

Another factor involved in myocardial injury is the length of time for aortic cross-clamp and cardiopulmonary bypass times. Aortic cross clamping is used to isolate the heart from the systemic circulation so that surgeons can operate in a bloodless field during valve and coronary bypass surgery. The clamping and declamping of the aorta causes a global ischaemia-reperfusion injury. The longer the aortic cross clamp is applied the worse the injury and prognosis. Similarly, cardiopulmonary bypass times show a close correlation with postoperative mortality and morbidity (25).

1.3.3 Risk factors for perioperative myocardial injury

As well as the wide variety of mechanisms resulting in perioperative myocardial injury it is important that preoperative risk factors be noted as these patients are more likely to have postoperative complications and increased mortality. Higher risk patients will be more susceptible to myocardial injury and so identifying these patients may yield greater benefit from cardioprotection strategies. The following preoperative risks are taken from the new EuroSCORE II (26) risk model (European System for Cardiac Operative Risk Evaluation) which was first announced in 2011 and superseded the original EuroSCORE first published in 1999 (27):

- Age >70 years old
- Female sex
- Renal failure
- Diabetes
- Peripheral artery disease
- Emergency or redo surgery

- Severe LV dysfunction (LVEF<35%) or cardiogenic shock

- Preoperative MI

- Chronic lung disease

- Poor mobility

- Surgery on thoracic aorta

- Surgery other than isolated CABG

- Pulmonary hypertension

- NYHA and angina class

- Active endocarditis

1.4 The need for cardioprotection in cardiac surgery

1.4.1 Development of cardiopulmonary bypass

Cardiopulmonary bypass was first developed in the 1950s as it was recognised that there was a need for surgery to be performed in a motionless and bloodless field. The most credit lies with a surgeon from Boston named John Gibbon who made the most pioneering advances during that decade and developed cardiopulmonary bypass machinery with many automated and safety features (28). Blood is typically drained by gravity from the heart and lungs through tubing to a reservoir where it is oxygenated via an oxygenator or gas-exchanger and then returned to the cannulated arterial system. The bloodless field is created by the application of a cross-clamp to the aorta proximal to the arterial cannula but distal to the coronary ostia thereby isolating the coronary arteries and cardiac chambers. It was from this that a need for myocardial protection was realised due to the risk of myocyte cell death from cross clamping the aorta and cardiac bypass itself.

1.4.2 Crystalloid cardioplegia

In the 1960s, cold crystalloid cardioplegic solution was developed to provide diastolic electromechanical arrest and hypothermia thereby reducing metabolic demands (29). Crystalloid solutions are infused at 4°C antegradely down the coronary arteries via the aortic root or coronary ostia, or retrogradely from the coronary sinus or both antegradely and retrogradely. Where coronary stenoses are severe, antegrade perfusion may not be sufficent. Retrograde perfusion is also believed to offer poor perfusion of the right ventricle. For these reasons, surgeons may use simultaneous antegrade and retrograde infusion. Potassium is used to induce cardiac arrest at a concentration of 10 to 20 mmol/L. Crystalloid solutions are differentiated into two types: intracellular which contains low sodium, calcium and magnesium concentrations, and extracellular which contains these elements at higher concentrations. Small amounts of calcium in the cardioplegia solution reduce the risk of its intracellular accumulation during ischaemia and reperfusion. Due to the risk of calcium paradox, phenomenon complete elimination of calcium is not recommended because rapid calcium accumulation during reperfusion leads to acute hypercontracture known as 'stone heart'. The addition of magnesium provides a protective antiarrhythmic effect on the heart, which also inhibits entry of calcium and decreases uptake of sodium into the myocytes during ischaemia. Magnesium is then exchanged for calcium during reperfusion. The volume and rate of infused cardioplegic solution varies depending on the type of solution and the institution but around 1L at 50mmHg to 80mmHg for antegrade or 40mmHg for retrograde delivery is adequate in most adults to induce cardioplegic arrest with some surgeons

allowing continued infusion for a few minutes afterwards. If the heart resumes electrical activity or the cross-clamp time is prolonged then further doses up to 500 mls may be given at a time (30).

1.4.3 Blood cardioplegia

Crystalloid cardioplegia has largely been superseded by blood cardioplegia because of its oxygen carrying capabilities. Blood cardioplegia involves infusion of native oxygenated blood mixed with crystalloid solution in the ratio of 4:1, which is passed through a heat exchanger prior to being applied to the heart. Standard blood cardioplegia uses cold solution at 8-12°C delivered antegradely and retrogradely with reinfusions at 20 minutes and a final 'warm' or normothermic substrate-enriched infusion prior to aortic unclamping. Variations on the traditional cold blood cardioplegia have seen surgeons use warm blood cardioplegia induction for patients that have poor LV function and acute myocardial infarction. Warm blood cardioplegia is seen to maximise the kinetics involved in 'cell repair' and the solution is supplemented to replenish the compromised hearts with much needed amino acids from the Kreb's cycle. Tepid blood cardioplegia has also evolved as a technique and is felt to combine the benefits of warm and cold blood cardioplegia. Intermittent antegrade blood cardioplegia has also developed as a method of cardioprotection to allow surgery in a bloodless operative field (31), and is the preferred technique at our institution and worldwide.

Over the last two decades, there has been considerable debate as to which solution and technique for cardioplegia is the best. A meta-analysis of trials

found that the only difference was that blood cardioplegia had less low cardiac output syndrome and reduced CKMB but no significant difference in endpoints of myocardial infarction and death when compared to crystalloid cardioplegia (32). The CABG patch trial investigated higher risk surgical patients with poor LV function, and found that operative deaths (2% vs. 0.3%), postoperative myocardial infarctions (10% vs. 2%) and shock (13% vs. 7%), were significantly higher in the cold crystalloid cardioplegia group, although there was no difference with early and late survival when compared to the blood cardioplegia group (33). In practice, the choice of cardioplegic solution and technique ultimately lies with the personal experience of each individual surgeon but in the UK antegrade, intermittent cold blood cardioplegia has emerged as the choice for surgeons because of its more physiological constitution and lower morbidity.

1.4.4 Cross-clamp fibrillation

An alternative to cardioplegia is cross-clamp fibrillation where the aortic cross-clamp is applied intermittently after induction of ventricular fibrillation. In the UK, around 15% of surgeons prefer this method for CABG surgery (34). Reperfusion is allowed after every distal anastomosis in CABG while the proximal end of the conduit is anastomosed to the aorta, as it is felt that there is no detriment to myocardium with up to 15 minutes of ischaemia. In fact, it is proposed that with each subsequent graft there is protection of the myocardium afforded by IPC (IPC) (35), a mechanism of cardioprotection, which will be discussed later. Experimental models have added weight to this by showing that blockade of reputed IPC cascade factors attenuated any

cardioprotective efficacy afforded by intermittently cross-clamped ('preconditioned') and fibrillated myocardium (36).

1.4.5 Off-pump versus On-pump CABG

Off-pump CABG was developed to reduce the risk of stroke from cardiopulmonary bypass. The main prerequisite of performing surgery on a beating and blood-filled heart are that the surgeon has the necessary skills to anastomose grafts; however, there are devices available to immobilise the relevant areas of the heart requiring suturing. Studies have shown that off-pump CABG is comparable to on-pump CABG with excellent results (37).

1.4.6 Hypothermia

Hippocrates a Greek physician first noted the therapeutic effects of hypothermia in wounded soldiers packed with snow and ice. William Osler another great physician placed his typhoid fever pateints in a cold bath in the 1890s. Systemic hypothermia during cardiac surgery lowers the metabolic rate and oxygen demands of the heart and thereby conferring protection. Typically, systemic temperature in cardiac surgeries is reduced to between 25°C and 34°C. More challenging operations may require a deep hypothermia to as low as 15°C. For each decrement in temperature by one degree Celsius, there is an approximate 5-7% reduction in metabolism. Hypothermia also minimises disruption to cell haemostasis by increasing cell membrane stability, which prevents the influx of unwanted ions during ischaemia. Low temperatures may also reduce reperfusion injury (RI) by decreasing free radical production due to oxidative stress and dampening down the various

inflammatory immune responses (38). Reperfusion injury will be discussed in more detail later on.

1.4.7 Volatile anaesthetics

The reader is asked to refer to the sections on ischaemia and reperfusion injury and IPC and ischaemic postconditioning prior to reading this section to gain an understanding of the mechanisms described. Volatile anaesthetics are gases used to rapidly induce and maintain general anaesthesia. The most commonly currently studied volatile anaesthetics are sevoflurane, isoflurane, enflurane and halothane. Animal studies have shown a direct cardioprotective role of these agents from ischaemia and reperfusion injury as shown by decreased infarct size and more rapid postoperative recovery of contractile function. These experimental studies have also helped elucidate some but not all of the complex signalling factors involved in anaesthetic preconditioning but clinical studies have shown equivocal results in cardiac surgery (39-41).

The most recently studied volatile gases are sevoflurane, isoflurane and halothane. Experimental studies have shown that volatile anaesthetic agents modulate the K+ adenosine triphosphate (ATP)-sensitive channel or K_{ATP} channel and the mitochondrial permeability transition pore (mPTP) which have both been shown to be cardioprotective mediators in IPC. The opening of mitochondrial K_{ATP} channels leads to the generation of reactive oxygen species (ROS) activating downstream kinases, resulting in cardioprotection (42). Another pathway involved in cardioprotection and volatile anaesthetic agents is Phosphatidylinositol-3-Kinase and Protein Kinase B (Akt/PI3k)

pathway which is a key intracellular signal pathway involved in apoptosis. Evaluation of the Akt and phosphorylated-Akt (active Akt) expression during ischaemia and reperfusion revealed higher expression in ischaemia-reperfusion and volatile anaesthetic (isoflurane) pre-treated groups with administration of PI3K inhibitors leading to inhibition of phosphorylated Akt and repeated inhibitor administration abolishing the cardioprotective effects of anaesthetic pre-treatment (43). Similarly the extracellular-signal kinases (ERK) pathway have been linked to myocardial protection elicited by volatile anaesthetics but this is dose dependent with an experimental study showing that a second dose of desflurane diminished cardioprotection and that although the ERK1/2 are downstream effectors of PKC their activation (i.e. phosphorylation) is independent (44).

It is also well recognised that reperfusion injury is a result of the accumulation of Ca^{2+} that leads to activation of nuclear factor-κB (NF-κB), which causes release of inflammatory mediators. Experimental studies have shown that rats pre-treated with sevoflurane had reduced infarct size and that by inhibiting NF-κB led to even greater protection from ischaemia than sevoflurane administration alone (45).

In the clinical setting for volatile anaesthetics, there have been conflicting results with trials only showing some protection with respect to cardiac function and troponin release but no evidence of reduced risk of myocardial infarction or cardiovascular mortality (46-48). Despite this, the

recommendations from the ACC/AHA guidelines state that patients at risk for myocardial infarction during *non-cardiac* surgery would benefit from pretreatment with gas anesthesia, if hemodynamically stable (49). Future investigations need to evaluate the most optimal anaesthetic agent, concentration and administration protocol for the best cardioprotective effects as studies have noted a difference in benefit based upon pretreatment (50). This probably best explains why experimental models have not translated their results in clinical studies.

1.4.8 Intravenous glyceryl trinitrate and IPC

Intravenous glyceryl trinitrate (GTN) is often given during cardiac surgery after anaesthetic induction as a means to control blood pressure. Its benefits are that it has a rapid onset of action, and a short-half life so blood pressure can be controlled tightly through careful titration of this drug. GTN is also given on the assumption that it results in coronary vasodilatation pre-operatively and may maintain graft patency post-operatively. GTN may play a role in reducing IR injury. It is known that short-term exposure to GTN has protective properties similar to IPC (51). Damage and dysfunction to the vascular endothelium can influence outcomes after IR injury but nitrates may protect against this (52). The exact mechanisms remain poorly defined, but it is postulated that nitrates and other nitric oxide donors trigger signalling cascades and induction of protective genes and pathways mentioned earlier (53). Bolli et al. describe a mechanism for late preconditioning and suggested that NO and secondary species activate PKCε either directly or via reactive by products. PKCε leads to further signalling through various kinases and

transcription factor NF-κB and other unknown components resulting in increased inducible nitric oxide synthase (iNOS) activity, which is responsible for protection (53). Others have proposed that instantaneous mitochondrial ROS release induced by GTN amongst other mediators, leads to increased expression of protective molecules and enzymes such as superoxide dismutase, haem-oxygenase and calcitonin gene related peptide (53, 54, 55). A human *in vivo* study by Gori et al. in 2010 showed that in subjects that received transdermal GTN for 2 hours there was a prevention of the endothelial dysfunction caused by IR injury in the arm. However, after 1 week of chronic administration of GTN the protective effect seen was lost. In the chronic administration group, the endothelial responses were blunted before IR and subsequent IR did not cause further dysfunction. The same investigators also used a separate experimental model to show that in isolated human endothelial cells, short-term incubation with GTN caused up-regulation of haem oxygenase, an effect that was lost after prolonged GTN administration. They therefore proposed that a short duration of GTN therapy is able to protect the endothelium from IR injury endothelial dysfunction but that this protection was lost upon prolonged administration possibly through an oxidative mechanism. Further to this, animal and human studies have documented the non-haemodynamic cardioprotective effects of GTN resulting in a reduced myocardial sensitivity to IR injury (51, 55, 56).

1.5 Cardiac Troponins

1.5.1 Description

Cardiac troponin (cTn) is a complex of three regulatory troponins (troponins C, T and I) which produce myocardial contraction through control of the calcium-mediated interaction between actin and myosin. The troponin regulatory proteins are integral to muscle contraction in skeletal and cardiac muscle but not in smooth muscle. During myocardial cell death these cardiac cTn proteins are released into the blood. Since the early nineties highly sensitive and cardiospecific immunoassays for cTn T and cTn I have come into regular clinical use. These superseded the use of the cardiac biomarker creatinine kinase MB (CK-MB) and have become the cornerstone of assessment in acute coronary syndromes (57, 58). cTn C is not useful for detecting cardiac muscle injury as its isoform is shared by skeletal muscle. Raised troponin levels are therefore indicative of myocardial necrosis but they do not determine the mechanism for their elevation. Troponins are internationally recommended as the cardiac biomarker of choice in the diagnosis of acute coronary syndromes and myocardial infarction as well as acting as an adverse prognostic marker. The 2012 Third universal definition defined myocardial infarction after CABG as requiring a troponin rise >10-times 99% upper reference limit (URL) from a normal pre-operative level (59). In fact, various studies have in fact shown that the true inflection point for cTn elevations to be deemed prognostic is actually higher. Indeed cTn elevations should always be interpreted with caution as many factors contribute to elevated cTn readings in post-operative patients on intensive care units, other than perioperative myocardial injury. For example, even the type of cardiac surgery performed has a bearing with valve only surgery releasing less troponin than CABG (60). Post-operative complications can also increase

troponin such as pulmonary embolus, acute renal failure, hypotension, sepsis, pericarditis and heart failure (61). With this in mind, studies have shown that raised troponin is prognostic in postoperative cardiac surgery with greater elevations associated with delayed recovery, increased stay on intensive care as well as short and longer-term mortality.

1.6 Myocardial Ischaemia-reperfusion injury

1.6.1 Ischaemia

Myocardial ischaemia develops when there is inadequate supply of oxygen to an area of myocardium usually through diminished blood flow. This results in a cascade of metabolic and biochemical events causing ionic changes within cells. Within the cell low oxygen levels result in ATP depletion which cause cell swelling through the accumulation of sodium (Na^+) and water and expulsion of potassium (K^+). Metabolism switches to anaerobic respiration causing the accumulation of toxic lactate, which lowers the pH to below 7.0 resulting in acidosis. This causes activation of the sodium-hydrogen (Na^+-H^+) exchanger removing H^+ in place of Na^+, which becomes intracellularly overloaded (62). Further Na^+ overloading occurs within the cell through the resultant decline in $3Na^+$-$2K^+$ ATPase function through depleted ATP. Due to rising intracellular Na^+ levels the $2Na^+$-Ca^{2+} ion exchanger that normally imports Na^+ into cells works in reverse resulting in intracellular Ca^{2+} overloading. Ca^{2+} overload results in electrical instability, mitochondrial dysfunction and mechanical dysfunction with further usurping of ATP and resultant cell death.

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1.6.2 Reperfusion injury

Initially myocardial cell death was felt to be due to ischaemia only but increasingly, reperfusion has been shown to play a role, although with some controversy (63). Reperfusion was seen to replenish blood supply and therefore prevent further ischaemic damage and so cells that died during the reperfusion phase were considered as already irreversibly injured. The best way to prevent ischaemic cell death was to replenish or reperfuse the myocardium as soon after the onset of ischaemia as possible. Reperfusion strategies such as primary percutaneous coronary intervention (PPCI) have always focused on prompt intervention and resulted in the well-known adage that time is myocardium (64). Despite this evidence emerged that reperfusion could damage myocardium and this was termed 'lethal reperfusion injury' and defined as being a period after ischaemia where an intervention could reduce the reperfusion injury (65). Despite these strategies to reduce reperfusion injury by varying reperfusion, conditions have proved successful in experimental models but have eluded researchers in clinical trials (66).

Two reversible forms of myocardial reperfusion injury exist which are malignant ventricular arrhythmia and myocardial stunning. Myocardial arrhythmia during reperfusion is a well-recognised phenomenon which is either self-terminating or can be reversed by various therapeutic measures or indeed can result in death if there is prolonged haemodynamic compromise. The observation that ventricular arrhythmia occurred more frequently during reperfusion than during coronary artery occlusion itself was first made in 1960 (67). 'Myocardial stunning' as a term, was first coined in 1982, and is defined

as a delay in the recovery of function of myocardium after reperfusion despite no myocardial damage and the restoration of blood flow (69). Various mechanisms have been postulated in the pathogenesis of stunning but current thinking lends itself to calcium overload and oxygen-derived free radicals (69, 70).

The third form of reperfusion injury is irreversible and termed microvascular obstruction (MVO). This is an inadequate reperfusion of the ischaemic myocardial territory despite removal of coronary artery obstruction and occurs at the level of the microvasculature – structural changes at this level impede flow. During PCI, this phenomenon is seen as sluggish blood flow at the time of coronary angiography down a visually normal coronary artery and is termed as no-reflow (71). The major contributor to MVO is the massive neutrophil activation and infiltration of platelets that adhere to capillary endothelium as well as forming aggregates, which cause mechanical obstruction to blood flow (72). Activated neutrophils also release oxygen free radicals, inflammatory mediators and proteolytic enzymes that can cause cellular oedema and further endothelial damage. Swollen myocytes caused by the oedema and extravasated red blood cells also extrinsically compress the microvasculature contributing to further MVO. Microemboli from artherosclerotic plaque are also sent downstream causing further mechanical obstruction and vasoconstriction of the coronary microcirculation caused by release of vasoconstrictors from damaged endothelial cells, neutrophils and platelets play a role (73). The fourth recognised form of reperfusion injury is termed 'lethal reperfusion injury' and refers to death of cardiac myocytes that were considered to have

reversible injury at the end of ischaemia. The proposed contributors to reperfusion injury are oxidative stress; restoration of physiological pH from the acidosis caused by ischaemia and mitochondrial transitional pore (mPTP) opening, Ca2+ overload and associated hypercontracture (74).

1.6.3 The mitochondrial permeability transition pore

The mPTP is a nonselective, voltage-dependent channel within the inner mitochondrial membrane, that is closed under normal physiological conditions, and is impermeable to solutes. Research over the last two decades has shown that during ischaemia, the pore remains closed probably due to acidic and toxic lactate-induced conditions (75) created during ischaemia but during the first few minutes of reperfusion the pore opens resulting in inner mitochondrial membrane permeability, leading to matrix swelling and subsequent cell death. Oxidative stress and calcium influx during reperfusion are seen to have an influence on mPTP opening directly. As mentioned above during ischaemia oxidative stress occurs resulting in ionic changes culminating in intracellular Ca2+ overloading. Acidosis prevents mPTP opening during ischaemia, however, during reperfusion there is a burst of ROS formation, a return to physiological pH with washout of lactate, which all restores mPTP opening. There is also uncoupling of mitochondrial oxidative phosphorylation and further aggravation of calcium influx further propagating the opening of the mPTP leading to myocyte death (74, 76). The mPTP has therefore led to renewed interest as a target for cardioprotection during reperfusion with inhibition of its opening in experimental models yielding promising results but this is yet to be translated into clinical trials.

Cyclosporin A is known to be a direct inhibitor of mPTP and Piot et al. showed that in 58 patients undergoing PCI there was a decrease in infarct mass after 5 days quantitated by cardiac MRI when cyclosporine A was given (46g vs 36g) but no significant difference in cardiac enzymes (77). Subsequent to this the Cyclosporine before PCI in Patients with Acute Myocardial Infarction (CIRCUS) trial published in 2015 failed to show better clinical outcomes in patients with anterior ST-elevation myocardial infarctions that received an intravenous bolus injection of cyclosporine when compared to those patients that did not (78). This international, multicenter, randomized, double-blinded, placebo-controlled trial recruited 970 patients. The primary outcome of the trial, a composite end-point including all-cause mortality, worsening heart failure during the admission, rehospitilisation for heart failure and adverse ventricular remodeling at 1 year occurred in 59% of the 395 patients randomised to cyclosporine and 58.1% of the 396 patients randomised to placebo. This was not statistically significant and yielded neutral results. However, the results of the trial were questioned in an editorial by Hausenloy and Yellon, as the findings for the end point of left ventricular remodeling were not robust and also a completely different preparation for Cyclosprin was used compared to that which had been used in previous experimental studies and yielded positive data (79). Therefore, IPC may possibly exert its cardioprotective effects through the mPTP, resulting in inhibition of its opening during reperfusion. IPC refers to short burst of sublethal ischaemia prior to the actual ischaemic insult, which may protect against reperfusion injury and will be discussed in the next section.

1.7 IPC

1.7.1 Background and conception

Initial studies into therapeutic interventions at the time of coronary artery occlusion lacked control for baseline variables and had poor models for quantification of infarct size (80). The story of IPC (IPC) therefore actually begins in 1986 with Murry et al. who presented this cardioprotective phenomenon using the canine model (81). They used anaesthetised dogs and found that by instigating four cycles of 5 minutes of ischaemia with intervening 5 minute periods of reperfusion there was a significant decrease in infarct size in the intervention arm compared to controls after the circumflex coronary artery was ligated for 40 minutes. Since then research has focused on elucidating some of the signal transduction pathways involved in this phenomenon. Yellon and Downey (82) provide a useful division of labours here by dividing, signalling into triggers which occur after the preconditioned stimulus and before a memory element which keeps the heart 'preconditioned'. Signalling that occurs after the memory element involves mediators and includes the end-effectors, which exert their protective effect on the myocardium during the index ischaemia or reperfusion.

1.7.2 Two windows of protection

Preconditioning affords itself two distinct time zones for protection and although the triggers are shared, there is a difference in the potential mediators. The first zone or window for protection lasts up to 2 to 3 hours after the preconditioning stimulus and this is referred to as the acute or classical preconditioning window (81). There then lies a second window of

preconditioning (SWOP) and this begins 12 hours after the stimulus and can last up to 72 hours (83). The SWOP allows for the possible mediators of cardioprotection to be because of protein biosynthesis, post-translational protein modification (manipulation of polypeptide structure prior to it becoming its mature protein product) or movement of protein between compartments (80).

1.7.3 Triggers

Proposed triggers of IPC include adenosine, bradykinin, prostaglandins, nitric oxide, Ca2+, opioids and noradrenaline (84). Receptors for these triggers act in tandem to precondition the heart and result in activation of G_i protein. Observation that these receptors work in parallel comes by virtue of the fact that blockade of a single receptor in experimental models requires an increase to the IPC stimulus to afford protection (85). Free radicals may also act as triggers for preconditioning (86) by activating kinases with their origin being the result of xanthine oxidase on purine metabolism or perhaps more complex mechanisms (80). Animal models also suggest another trigger, which acts without receptor, may also be Ca2+ which itself may cause a preconditioned state or be involved in IPC (87). Nitric oxide (NO) may have a novel dual role in IPC as trigger or mediator but notably evidence for its role is considered to be in late preconditioning rather than early (88). There are even reports that suggest its role may be simply to lower the threshold for preconditioning rather than be involved in signalling (89).

1.7.4 Mediators

The Protein Kinase C (PKC) family of enzymes are involved a variety of physiological processes and their role in cardioprotection has been investigated with vigour. PKC is split into three groups dependent on their responsiveness to diacylglycerol (DAG) or Ca2+ for enzyme activity. The α, βI, βII, and γ isozymes of PKC are both DAG- and Ca^{2+}-dependent. The ε, δ, θ, and η isoenzymes are only DAG-dependent and do not require Ca^{2+} for activity. Isoenzymes, which are neither DAG- nor Ca^{2+}-dependent and require lipid-derived molecules for activity are referred to as the atypical PKCs; ζ and λ (90). Evaluation of the individual isoforms roles in cardioprotection has involved isozyme-specific modulators as well as transgenic and knockout mice of specific PKC isoenzymes. PKC isoenzymes each anchor to unique docking sites called receptor for activated C-Kinase (RACK), which results in isozyme activation and therefore phosphorylation of any local substrate (91). Various studies have shown that PKCε has a major role in IPC resulting in reduced cell death (92-94). Conversely, the isoenzyme PKCδ has been found to cause cell death by promoting damage from ischaemia and its inhibition is cardioprotective (95). ROS, which is harmful to cardiac myocytes, has been identified as an activator of PKCδ (96).Unfortunately despite these findings the signalling mechanisms that directly link PKC to preconditioning have not been completely characterised. Postulated mechanisms include the generation of free radicals, changes in the levels of pro- and anti-apoptopic proteins of the Bcl-2 family and activation of the mitochondrial ATP-sensitive potassium channel (mitoK$_{ATP}$)

PKCε has been shown to directly interact in the inner mitochondrial membrane and is required for the opening of mK_{ATP} (97). mK_{ATP} channels have been shown to potentiate cardioprotection through use of channel-activating drugs or inhibitors which reverse the protective effects seen. Initially studies had shown that exogenous cyclic GMP (cGMP) and protein kinase G (PKG) activates mK_{ATP} and was reversed by inhibitors of PKC (98) which strongly lends itself to PKG transmitting its cardioprotective signal to through a PKC pathway. Current thinking for signal transduction in IPC resulting in mK_{ATP} is through the PI3K–Akt–eNOS–cGMP–PKG pathway (99). mK_{ATP} then goes on to generate ROS which activate survival kinases. The K_{ATP} channel is normally inhibited by ATP and is modulated by pH, NO, fatty acids, G proteins and ligands such as adenosine (100). They have been shown to be important mediators in cardioprotection and downstream to adenosine receptor activation as this can be blocked by glibenclamide, which is a K+channel blocker (100). PKC has been shown to activate sarcolemmal K_{ATP} channels in experimental models (101).

1.7.5 Reperfusion injury salvage kinase (RISK) pathway

Other potential mediators of IPC are conceptualised into groups of pro-survival anti-apoptotic protein kinases termed the RISK pathway (102). The first members of this cardioprotective unit were PI3-kinase/Akt and the p42/p44 mitogen-activated kinase Erk1/2 which conferred cardioprotection during reperfusion. Activation of both pathways is through G-protein coupled receptors (G-PCR) or growth hormones and descends onto downstream kinases such as p70S6 kinase and glycogen synthase kinase 3 β (GSK3-β).

The RISK pathway also includes other reperfusion salvage kinases mentioned above: PKC and PKG. In conflict to this the RISK pathway is the already mentioned PKCδ and rho-kinase which is deleterious to myocytes and counteracts RISK pathway protection (103). Mitogen activated kinases (MAPK) p38 and c-Jun N-terminal (JNK) controversially exhibit beneficial and harmful effects (104). An alternative to the RISK pathway is the surviving activating factor enhancement (SAFE) pathway involving tumour necrosis factor α (TNF–α), PKG, sphingosine and the Janus Kinase/Signal Transducer and activator of transcription JAK/STAT3 system (105, 106). Janus Kinase/Signal Transducer and activator of transcription JAK/STAT3 has mainly been implicated in delayed preconditioning through its activation of nuclear transcription factors.

1.7.6 End-effectors

The survival kinase pathways appear to converge on the mitochondrion and specifically the mPTP which is described above. Its role here as an end-effector is realised by its inhibition of opening to limit infarct size in ischaemic preconditioning and postconditioning but unfortunately the exact mechanism underlying this phenomenon remains elusive.

1.8 Ischaemic postconditioning

1.8.1 Conception

Ischaemic postconditioning (IPost) is a method for protecting the myocardium by interrupting early reperfusion with short-lived episode of ischaemia, and it may have partially been borne from observation that slow reperfusion was

beneficial over rapid reperfusion (107). The actual technique involves several brief periods of ischaemia during reperfusion such that reperfusion is halted periodically and then allowed to resume. In 2003, Zhao et al. (108) demonstrated the concept by applying a protocol of IPC in reperfusion (i.e. postconditioning). Their protocol involved LAD ischaemia in anaesthetised open-chest dogs with the preconditioned arm involving 5 minutes occlusion followed by 10 minutes reperfusion and then 60 minutes ischaemia. In the postconditioning arm, there were three cycles of 30-second reperfusion and 30-second reocclusion. They showed that preconditioning and postconditioning similarly and significantly reduced infarct size and tissue oedema while preserving endothelial function when compared to controls.

1.8.2 Mechanistic insight

Initially it was believed that IPost was simply exerting its effect mechanically by causing a gradual low-pressure reperfusion thereby limiting the negative effects of reperfusion injury such as oxidative stress, calcium accumulation, myocardial oedema and inflammation (109). Indeed some felt that IPost and IPC had different pathways as the interventions lay at either end of ischaemia (108). However, subsequent studies have found that many of the signalling pathways recruited in IPost are similar to those seen in IPC. In fact, the first data that prosurvival kinases seen in IPC were also involved in IPost came from the Yellon group in 2004 who showed the involvement of PI3K/Akt pathway (110). Other prosurvival kinases shown to be involved in a common pathway include ERk1/2 thereby implicating the RISK pathway (111) Now after much research it is accepted that preconditioning and postconditioning

activate the same key pathways beginning with upstream GPCRs including adenosine, bradykinin, opioid and sphingosine-1-phosphate (SIP) receptors. As well as this, experimental studies have shown that IPost activates the JAK/STAT3 pathway, which is known to also be involved in the SWOP (112, 113). Another common mediator not yet mentioned is Hydrogen sulphide (H_2S) which activates RISK (114) and is seen in IPC and IPost. In the end, all of these signalling pathways appear to converge on the mPTP as in IPC.

1.8.3 Evidence

Human studies have had conflicting results with the first landmark study by Staat et al. investigating postconditioning in patients that underwent direct stenting during PCI for acute myocardial infarction with an intervention group having 4 cycles of 1-minute angioplasty balloon inflation and 1-minute deflation before full reperfusion. Results showed that there was a reduction in CK release quantified by area-under-the-curve at 72 hours. There was also significant improvement in blush grade at angiography, which is a marker of reperfusion (115). Further studies showed IPost infarct-limiting capabilities by a variety of assessment modalities, which included troponin release and left ventriculography with follow up at 1 year (116) and cardiac MRI (117). In stark contrast to this, there have been negative clinical studies, which also used the accepted postconditioning algorithm of 4-minute cycles of ischaemia and reperfusion within actual reperfusion. Friexa et al. looked at 79 patients undergoing PCI for a first STEMI, found that there was no difference in infarct size as assessed by MRI at 1 week, and 6 months and in fact

postconditioning was associated with lower myocardial salvage and salvage index along with an enhancement of troponin release (118). Although results are conflicting there is still optimism regarding IPost being a genuine phenomenon and failure of clinical studies has been put down to confounding factors that may attenuate its protection in studies. For example, age is known to reduce cardioprotective signalling as seen in mice but not rats (119). Various co-morbidities are known to interfere with cardioprotection and IPost such as hypercholesterolaemia, diabetes, obesity and hypertension. Drugs may also play a role and affect IPost by recruiting cardioprotection in control groups. These include adenosine, GTN, B-blockers, angiotensin converting enzyme (ACE) inhibitors and statins that are all given around the time of PCI (120). However, our current understanding is insufficient to quantify the extent of the roles played by these confounders.

A few conclusions can be drawn from the above experimental and clinical research bearing in mind the potential reasons why a translation to bedside results has not been seen. Firstly, the extent of protection in IPost is dependent on the duration of ischaemia and postconditioning protocol used with species and inter-individual variations being clearly apparent. Secondly, there is a need for the actual clinical benefit of IPost to be seen with large multicentre studies showing outcome data to build on the smaller human studies that have shown non-prognostic benefit through cardiac enzyme release and infarct size reduction using imaging modalities.

1.9 Remote IPC (RIPC)

1.9.1 Conception

IPC has had limited clinical application because of the inability to predict the onset of ischaemia in myocardial infarction, which has restricted its application to elective cardiac surgery. IPost has been investigated in small trials at the time of PCI but conflicting results, concerns over interrupting reperfusion, lengthening procedural time and the issues surrounding funding due to limited incentive for industry, have also meant that large multicentre trials have not been realised (121). From this, the concept of RIPC has evolved in the last decade as a mechanism for cardioprotection from IR injury through the preconditioning stimulus being performed at sites remote to the heart. As a concept, its origins lie in a canine model experiment by Przyklenk et al. in 1993 (122). Anaesthetised dogs underwent four 5-minute cycles of ischaemia and reperfusion in the circumflex artery territory which resulted in cardioprotection in the subsequently infarcted LAD territory. This suggested that IPC performed in one vascular bed afforded protection in another coronary artery territory. It was postulated that this was mediated by factors created, activated or transported through the heart. Since then inter-organ protection has been demonstrated by a preconditioning stimulus being applied to small bowel (123) or kidney (124) and this resulting in decreased infarct size in the heart. After this Birnbaum et al. (125) showed that by reducing femoral artery blood flow and pacing the gastrocnemius muscle followed by 30 minutes of coronary artery flow occlusion and 4 hours of reperfusion resulted in significant reduction in infarct size when compared to controls. From this a simple, safe, cheap and easily instituted stimulus to investigate

cardioprotection was born. The preconditioning stimulus could be applied to the arm or leg by performing cycles of ischaemia and then reperfusion using blood pressure cuffs inflated to suprasystolic blood pressure and then deflated. This resulted in experimental RIPC studies in humans showing cardioprotection and laid the platform for a large number of clinical trials.

1.9.2 Mechanistic insight – Humoral pathway

RIPC appears to confer two distinct phases of protection like IPC. The first phase vanishes within 4 hours whereas the second prolonged phase lasts for at least 48 hours (126, 127). There is also a growing body of evidence that reduction in infarct size can be induced by applying the ischaemic stimulus after the index ischaemia during reperfusion and this is termed Remote ischaemic postconditioning (RIPost). Signalling is believed to be through both a humoral and neural pathway. The potential humoral factor is blood-borne and represents a mode of communication between the site of the protective stimulus and the target tissue or organ. Evidence for a humoral factor in mediating systemic spread is supported by two studies in 1999 by Dickson et al. that showed when the transfusion of serum from rabbits that had undergone an IPC protocol to rabbits which had not resulted in cardioprotection (128, 129). Konstantinov et al. showed that a pig, which had undergone RIPC of a limb and then received a denervated donor heart exhibited cardioprotection strongly suggesting a humoral role rather than a neural role (130). The humoral factor is as yet unidentified but appears to be a small hydrophobic molecule which is heat labile, dialysable, less than 15 kDa and blocked by pre-treatment with the opioid antagonist naloxone (131, 132).

Studies have also looked at whether endogenous mediators such as bradykinin, adenosine, opioids, endocannabinoids and CGRP are released from the remote organ during the preconditioning ischaemia and are carried via the blood to the heart where they activate intracellular signalling pathways or they may activate afferent neural pathways within the remote preconditioned organ (133).

1.9.3 Mechanistic insight – Neural pathway

Neurogenic mechanisms have been explored by virtue of autonomic ganglion blockade. Hexamethonium abrogated RIPC protection of infracted rat hearts achieved by mesenteric artery occlusion had no effect on IPC of the heart (134). In 2005, MaCallister's group also showed that in humans the autonomic ganglion blocker trimethaphan inhibited RIPC. They measured endothelial IR injury following 20 minutes arm ischaemia by flow mediated dilation and protection conferred by RIPC of three 5 minutes IR cycles in the contralateral arm was attenuated by trimethaphan (127).

1.9.4 Mechanistic insight – Systemic response

There is also some evidence of a systemic response to RIPC involving cross talk with immune cells (135) and suppression of an inflammatory response with Konstantinov's group suggesting that RIPC modifies myocardial gene expression through the upregulation of cardioprotective genes and suppression of genes involved in the pathogenesis of IR injury (136). They also showed that brief forearm RIPC suppressed pro-inflammatory genes in

circulating leucocytes expressing cytokine synthesis, leucocyte chemotaxis, adhesion, migration and apoptosis (137).

1.9.5 Signalling

Once the signal that RIPC has taken place and reached the myocardium then the intracellular transduction mechanisms are activated which are similar to those seen in IPC and IPost. As mentioned previously these include the G-protein cell surface coupled receptors such as adenosine and opioids. Again, the prosurvival kinases of the RISK pathway are implicated as well as other signalling components such as NO, heat shock proteins, iNOS and the mK_{ATP} channel. Ultimately, it is felt that this all converges on the mPTP with the signalling cascade causing its opening at reperfusion and therefore resulting in cardioprotection.

Unfortunately despite much compelling data in experimental models and some promising results in initial studies there is a gap in our knowledge of the fundamental science involved in RIPC protection. Firstly, the optimal algorithm involved in creating the ischaemia reperfusion stimulus has yet to be elucidated with the number and length of cycles still needing to be fully established. Another issue with studies in RIPC is there is no consensus on whether the magnitude of cardioprotection is greater with arm ischaemia rather than leg ischaemia and whether two simultaneous limb sites are better than one. Despite this RIPC has the potential to provide a prognostic yet cost effective means to improve outcomes in patients at risk of myocardial IR injury

and what is needed from here is large multicentre clinical trials to provide proof of concept (138).

1.10 Studies of RIPC

1.10.1 RIPC in cardiac surgery

The first clinical trial in this area was in 2000 and this was a small study of eight patients who were randomised to receive RIPC or be controls. The protocol involved two 3-minute cycles of forearm blood pressure cuff ischaemia inflated to 300mmHg with intervening 2 minutes reperfusion (139). This study demonstrated an increase in lactate dehydrogenase (LDH) in the preconditioned group suggestive of the myocardial cells maintaining their ability for anaerobic metabolism. Creatinine phosphokinase (CPK-MB) values were not different between the two groups but this was attributed to the fact that the study was grossly underpowered and CPK-MB levels were only taken 5 minutes after unclamping the aorta. It was also felt that the RIPC protocol was inadequate (138). Following this in 2006 Cheung et al. were the first to show a potential benefit of RIPC in the setting of 37 paediatric surgery patients having corrective congenital cardiac surgery. Four cycles of 5 minutes lower limb ischaemia prior to surgery were effective in reducing cTn levels, postoperative inotropic requirements and airway resistance at 6 hours (140). Another study involving cardiac congenital heart disease patients having ventricular septal defect repair demonstrated that preconditioning resulted in an improvement in lung compliance and a decrease in LDH, CK and cTn and the cytokines IL-6, IL-8, IL-10, and TNF-α (141).

Subsequent to this Hausenloy et al. in 2007 showed in 57 adult patients undergoing CABG surgery that serum troponin T levels were significantly reduced at 6, 12, 24 and 48 hours when undergoing 3 cycles of upper arm ischaemia lasting 5 minutes by cuff inflation to 200mmHg with subsequent 5 minutes reperfusion. Total area-under-the-curve for troponin at 72 hours was reduced by 43% (142). Patients in this study underwent both cross-clamp fibrillation and cardioplegia. The cardioprotective effects of RIPC in CABG patients was also demonstrated in the presence of cold blood cardioplegia (143). Similar results were found in a study published in 2010 by Thielmann et al., which looked at 53 non-diabetics with triple vessel disease who underwent CABG with crystalloid cardioplegia (144). They found a 45% reduction in cTn I 72 hour area-under-the-curve after left upper arm RIPC with 3 cycles of 5 minute transient cuff inflation to 200mmHg with 5 minute reperfusion. A meta-analysis published in 2012 looked at nine studies totalling 704 patients that had undergone CABG due to conflicting data and small sample size. They found that RIPC reduced cTn release, even when excluding trials that used the infrequently practiced method of cross-clamp fibrillation, off-pump CABG, and studies using isoflurane as an anaesthetic agent (which is believed to precondition myocardium itself). Postoperative creatinine and hospital stay were not influenced (145).

In contrast to the above results, a slightly larger study of 162 patients by Rahman et al. was published in 2010, which did not show reduced troponin area-under-the-curve at 72 hours (146). They also found that there was no

correlation with blood haemodynamics, renal dysfunction, lung injury or total hospital intensive care unit stay (ICU) stay. This was a single centre, prospective randomised placebo-controlled trial with 3 cycles of 5 minutes upper arm ischaemia and 5 minutes reperfusion. This study was praised because of its use of a scrupulous technique to remove inadvertent bias by blinding the surgeon, anaesthetist and investigators to the group allocation by using a dummy arm placed under the surgical drapes next to the left arm, which had a cuff placed on it. A cuff was also sited on the patient's left arm and the cuff to be inflated was covertly operated through a switch mechanism. Isoflurane was also specifically avoided in this study. Limitations of this study and potential confounders were that there was a higher use of B-blockers pre-operatively. Bypass and cross-clamp times were also higher when compared to other studies. Non-elective patients were also included that may have had angina in the preceding 30 days or an acute coronary syndrome. It is possible that subtle differences in preconditioning may occur in these groups.

This was followed up with a study published in 2013 enrolling 329 patients over 4 years undergoing elective CABG with cold crystalloid cardioplegia and cardiopulmonary bypass in Germany also used the same RIPC protocol of three 5-minute cycles of ischaemia and reperfusion in the upper arm (147). They showed that troponin area-under-the-curve was lower at 72 hours and in the preconditioned patients. The secondary end points comprised all-cause mortality, major adverse cardiac and cerebrovascular events (MACCE) and repeat revascularisation at 30 days, 1 year and completion of follow-up ranging from 1 to just over 4 years with a mean of 1.54 years. They found that

there was also lower all-cause and cardiac mortality as well as MACCE in the RIPC group. They also found that RIPC reduced sepsis, stroke and non-cardiac deaths adding weight to the theory that RIPC has a multitude of systemic benefits.

In contrast to this a study in 2014 published by Hong et al. did not show improvement in clinical outcome in 1280 patients undergoing cardiac surgery however the patients were randomised into a control group or a group that received RIPC and Remote ischaemic postconditioning (RIPost) (148). In the intervention arm, four cycles of 5-minute ischaemia and 5 minute reperfusion were administered twice to the upper limb before cardiopulmonary bypass or coronary anastomoses for RIPC and after cardiopulmonary bypass or coronary anastomoses for RIPost. This group did not show a reduction in the primary endpoint, a composite of major adverse outcomes, including death, myocardial infarction, arrhythmia, stroke, coma, renal injury or multiorgan failure. Lengths of intensive care unit and duration of hospital stay showed no difference. Notably there was a significantly increased composite endpoint in the off-pump subgroup. The strengths of this study were that randomisation involved a large population recruited over a short period from June 2009 to November 2010. Limitations included patients undergoing different cardiac surgeries encompassing CABG, valve surgery, combined CABG and valve surgery, ascending or transverse aortic surgery and congenital heart defect repair were included so that the heterogeneity probably introduced bias. As well as this, the higher risk patients who may have been more likely to benefit from preconditioning such as those with poor left ventricular function were

excluded. Another factor may have been the addition of RIPost to the RIPC protocol. Furthermore, every patient received propofol in their anaesthetic regimen, which has been shown to interfere with the activation of the signal transducer and activation of STAT5 pathway and hence abrogate the cardioprotection of RIPC (149).

Therefore, although several small trials have shown benefit in RIPC with improvement in cardiac enzymes and sometimes outcome data there is also conflicting data suggesting no benefit from RIPC. What was needed at this point was some larger randomised controlled trials to clearly define the role of RIPC in cardiac surgery. Two large-scale, prospective, randomized, sham-controlled trials of RIPC have since been undertaken with results published after completing my own studies. Both studies delivered the same RIPC stimulus involving four cycles of 5 minutes ischaemia interspersed with 5 minutes of reperfusion in anaaesthetised patients. The Remote Ischaemic Preconditioning for Heart Surgery (RIPHeart) Study comprised 1385 patients all deemed higher risk through pre-operative Euroscore assessment with an average score for all recruited patients being 4.2 (150). This study included a small number of patients that had ascending-aorta replacements as well as CABG and valve surgery. The results showed that RIPC did not reduce major adverse cardiac and cerebral events. In the Effect of Remote Ischaemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Surgery (ERICCA) trial recruitment totalled 1612 patients with a Euroscore of 5 or higher who were undergoing CABG with or without valve surgery (151). This trial, which was led by our institution also failed to show a

benefit in reducing major adverse cardiac and cerebral events assessed 12 months after surgery.

Another trial in progress is the (Effect of Remote Ischaemic Conditioning on Atrial Fibrillation and Outcome) RICO trial. This study is a randomised, controlled, double blinded muticentre study. Elective CABG patients will be randomised to one of the four following groups: 1) control 2) RIPC 3) RIPost or 4) RIPC and RIPost. The primary endpoint is the incidence of AF in the first 72 hours after surgery detected using Holter monitoring and is looking to see a reduction of 40 to 50% by RIPC or RIPost or both. Some may question why AF as chosen as an endpoint but it is a strong predictor of outcome. Secondary endpoints are MACCE at 30 days 3 months and 1 year as well as length of stay in hospital and ICU. The benefits of investigating RIPost is that most ischaemic episodes such as acute coronary syndromes are actually unpredictable and therefore RIPC is not possible but RIPost may therefore have greater clinical implications (152). A summary of the main trials in RIPC in CABG is in table 1, 2 and 3.

Table 1: RIPC studies in CABG showing cardioprotection

Author	Year	Patient cohort	RIPC Protocol	Outcome in RIPC group
Gunayadin	2000	8	2x3 mins	↑LDH, CK-MB↔
Hausenloy	2007	57	3x5 mins	↓cTn AUC 72 hrs
Venugopal	2009	45	3x5 mins	↓cTn AUC 72 hrs
Thielmann	2010	53	3x5 mins	↓cTn AUC 72 hrs
Ali	2010	100	3x5 mins	↓CK-MB 8,6,12,48 hrs

Wagner	2010	91	3x5 mins	↓cTn at 8hrs only. ↔cTn at 16&24 hrs
Hong	2010	130	4x5 mins	↓cTn AUC 72 hrs
Krawczyk	2011	19	3x5 mins	↓cTn at 24 hrs
Kottenberg	2012	72	3x5 mins	↓cTn AUC 72 hrs
Hong	2012	70	4x5 mins	↓cTn AUC 72 hrs

Table 2: RIPC studies in CABG failing to show cardioprotection

Author	Year	Patient cohort	RIPC Protocol	Outcome in RIPC group
Rahman	2010	162	3x5 mins	↔cTn AUC 72 hrs
Karuppasamy	2011	54	3x5 mins	↔cTN AUC 48 hrs
Lucchinetti	2012	55	4x5 mins	↑cTn AUC 72 hrs
Hausenloy	2015	55	4x5 mins	↔cTN AUC 48 hrs
Meybohm	2012	55	4x5 mins	↔cTn AUC 72 hrs

Table 3: Latest RIPC studies in CABG looking at MACCE

Author	Year	Patient cohort	RIPC Protocol	Outcome in RIPC group
Thielmann	2013	329	3x5 mins	↓cTn AUC 72 hrs ↓MACCE at 30 days, 1yr and 1.58 yrs (median) follow up
Hong	2014	1280	3x5 mins	↔ short term MACCE
Hausenloy	2015	1612	4x5 mins	↔ 12 month MACCE
Meybohm	2015	1385	4x5 mins	↔ 90 day MACCE

1.10.2 Remote ischaemic Conditioning in elective PCI

One of the first trials in RIPC was by Iliodromitis et al. in 2006 and this looked at 41 consecutive normotensive patients with stable angina and single-vessel disease (153). RIPC was induced by three cycles of 5 minute ischaemia reperfusion of both upper limbs. Troponin [0.255(0.059) v 0.804 (0.232) ng/ml p<0.05] and CK-MB [1.33(0.27) v 3.57(0.97) ng/ml, p<0.01] was significantly increased in the RIPC group when compared to controls at 24 hours. Interestingly, a subgroup of patients on statins in the RIPC group had a significantly lower increase in troponin than those patients not on statins [23.8(3.71) versus 11.4(3.0) mg/l, p<0.01] which suggests that if RIPC induced an inflammatory response then statins may abate this.

The Cardiac Remote IPC in coronary stenting (CRISP) study was a prospective, randomised controlled trial, which investigated RIPC in 242 consecutive patients undergoing elective PCI (154). Patients were randomised to receive RIPC with the standard 3 cycles of 5 minutes forearm ischaemia and 5 minutes reperfusion. This study showed that RIPC reduced median troponin levels at 24 hours after PCI when compared to control. Subjects that received RIPC also experienced less chest discomfort (p=0.0006) and ECG ST segment deviation (p=0.005). At 6 months, the major adverse cardiac and cerebral event rate was also lower but not statistically significant. Notably the cardioprotection from RIPC was also seen in groups, which have previously shown to reduce the effect of preconditioning such as elderly patients, those with diabetes, hypertension and previous history of myocardial infarction. Longer-term follow-up at 6 years confirmed that the

reduction in MACCE was maintained by RIPC (155). Since the publication of this meta-analysis, the CRISP stent trial initially published by Hoole et al. and mentioned above has published its outcomes at 6 years after longterm follow up. MACCE was defined as all-cause mortality, nonfatal myocardial infarction, transient ischaemic attack or stroke and heart failure requiring hospital admission adjudicated by the medical notes and national database review. MACCE rate at 6 years remained lower in the remote IPC group (HR 0.58; 95% CI, 0.35-0.97; p=0.039 ARR=0.13 and NNT=8) and in keeping with this mean troponin was elevated in the patients with MACCE. The investigators therefore concluded that RIPC conferred a MACCE-free survival benefit at both short and longterm follow-up (155).

However, the CRISP investigators also applied the same RIPC protocol to 42 patients with single vessel disease, which did not diminish the degree of LV dysfunction during coronary balloon occlusion, nor did it improve contractile recovery during reperfusion (156). Similarly, another study by the same group also published in 2009 failed to show an improvement in coronary microvascular resistance or coronary flow in humans from RIPC in elective PCI (157). These finding showed promise but other studies in elective PCI showed varying results. Table 4 below summarises all the trials in elective PCI to date. The main problem with these studies has been that characterized by non-standardised protocols and had small numbers with recruitment from a single centre. Where these studies have been negative they have often had small numbers and so were underpowered or have had a very low ischaemic

stimulus which has been felt to be to too weak to produce a cardioprotective effect.

Table 4. Main studies of Remote ischaemic conditioning (RIC) in elective PCI

Author	Year	Patient cohort	RIPC Protocol	Outcome in RIC group
Iliodromitis	2006	41	3x5 mins Both arms Before elective PCI	↑cTn AUC 48 hrs ↓cTN on statins
Hoole	2009	242	3x5 mins Before elective PCI	↓cTn at 24 hrs ↓chest discomfort ↓ST segment deviation ↓MACCE but not significant
Ghaemian	2012	80	2x5 mins Lower limb	↓cTN mean ↔MACCE
Prasad	2013	95	3x3 mins Upper limb	↔cTN mean
Ahmed	2013	149	3x5 mins Upper limb	↓cTN at 16 hrs ↔procedural MI
Luo	2013	205	3x5 mins Upper limb	↓cTN ↓PCI-related MI
Davies	2013	192	3x5 mins Elective PCI	↓MACE short and longterm
Liu	2014	200	3x5 mins Upper limb 18 hrs before PCI	↓median troponin ↓MACE at 6 months
Zografos	2014	94	1x5mins Upper limb	↓median troponin ↓PCI-related MI
Xu	2014	200	3x5 mins Upper limb diabetics	↔cTN ↔PCI-related MI

1.10.3 Remote ischaemic Conditioning (RIC) in PPCI and STEMI

There are also a small number of studies looking at the role of RIC in STEMI patients and similarly the results of these studies are not consistent. Munk et al performed a randomised controlled trial in patients with first time

presentation of STEMI (158). Results suggested that RIPC during transfer for PPCI consisting of four 5-minute cycles resulted in no significant differences in LV function but there was a substantial improvement in left ventricular function in high-risk patients prone to developing large infarcts. In the subset of patients, having LAD infarcts there was preserved LV function at 1 day and 1 month with significantly reduced LV volumes suggestive of decreased LV remodeling after RIPC. The results only being found in a subgroup led to criticism though that the effects had arisen by chance because of multiple testing. In addition, there was no data on LV function prior to infarction so the differences observed may have existed prior to the index event.

Botker et al. used the same RIPC protocol in a study of 251 patients undergoing PCI for a first acute myocardial infarction. This study found that preconditioned patients had a greater myocardial salvage index assessed by single-photon emission CT (SPECT) after PCI compared with controls (0.75 vs 0.55) at twenty days. Similarly, to the study by Munk et al. the effect was more robust in anterior infarcts and patients where vessels were totally occluded. However, left ventricular ejection fraction and troponin release were not significantly different between the two groups (159).

Another study was by Rentoukas et al in 2010 which enrolled 96 patients undergoing PPCI and srandomised them to one of three groups, which were A) RIPC B) RIPC and morphine C) Control group. Upper arm ischaemia was achieved by inflating the cuff to >20mmHg systolic arterial pressure for three cycles each lasting 4 minutes with intervening reperfusion. A higher proportion

of patients in Groups A and B (73 and 82% respectively) achieved ST-segment resolution after PCI when compared to controls (53%). Group B also achieved significantly lower troponin and ST segment deviation resolution. These findings demonstrated that overall RIPC and morphine demonstrated a cardioprotective effect, which is in keeping with the role of opioids in signal transduction in RIPC and is in keeping with the finding that IPC is blocked by the opioid antagonist naloxone (160). Criticisms of this study were that RIPC was performed using a new protocol that had never been used before involving three cycles of 4 minutes as mentioned earlier with reperfusion 10 minutes before the estimated time of first balloon inflation. Furthermore very little is known about clinical confounders such as age, comorbidity or co-medication on the effectiveness of RIPC (161).

Another study published in 2014 by Sloth et al. recruited 333 patients for 18 months up until November 2008. This study randomised patients having STEMI and PPCI to remote ischaemic conditioning with 4 cycles of 5 minutes ischaemia followed by 5 minutes reperfusion. Patient follow up was for a median of 3.8 years and the primary endpoint was MACCE events. MACCE occurred in 17 (13.5%) in the intervention group compared with 32 (25.6%) patients in the control group. This study therefore reinforces RIPC as being of benefit in long-term clinical outcomes in patients treated with PPCI for STEMI (162). This study provides grounds for optimism but should be viewed with caution due to its size.

Yamanaka et al. published in 2013 performed a study with positive findings in favour of RIC (163). This study looked at RIC in 105 STEMI patients and used an automated blood pressure cuff to perform three cycles of 5 minutes alternating reperfusion and ischaemia. Although RIC had no effect on ST-segment resolution, peak CPK values or MACCE during hospitalisation, there was a reduction in reperfusion arrhythmias after PCI and the incidence of contrast-induced nephropathy.

A study from our own institution by White et al. randomly assigned 197 STEMI patients with a completely occluded artery (TIMI flow zero) to receive RIC consisiting of four cycles of 5 minutes of upper arm cuff inflation and deflation or control (164). Using cardiac magnetic resonance they showed that when patients were treated by RIC initiated prior to PPCI they reduced infarct size and oedema while increasing myocardial salvage by imaging 83 subjects 3 to 6 days after admission.

The LIPSIA trial by Eitel et al (165) investigated both RIC and IPost in STEMI patients. They evaluated whether intra-hospital RIC and IPost had a more powerful effect on myocardial salvage compared with either IPost alone or control. This prospective, controlled single centre study recruited 696 STEMI patients to these three groups and showed that myocardial salvage index assessed by CMR was significantly greater in the combined RIC and Post-C group (p=0.02). Clinical follow-up at 6 months revealed no significant difference in the combined clinical endpoints of death, reinfarction and new congestive heart failure (p=0.44).

Table 5. Main studies of Remote ischaemic conditioning (RIC) in PPCI

Author	Year	Patient cohort	RIPC Protocol	Outcome in RIC group
Botker	2010	251	4x5 mins Before PPCI	↓cTN at 90-102 hrs ↑Myocardial salvage index by SPECT
Rentoukas	2010	96	3x4 mins During PCI	↓Peak cTn ↓ST segment deviation ↑ St segment resolution
Munk	2010	242	4x5 mins Before PPCI	↔LV function Subset of LAD infarcts had preserved LAD function with RIPC
Sloth	2014	333	4x5mins Before PPCI	↑MACCE at median of 3.8 yrs
Yamanaka	2014	109	3x5mins Before PPCI	↔ST resolution ↔CPK levels ↔MACCE ↓reperfusion arrhythmia ↓CIN
White	2015	333	4x5mins Before PPCI	↓myocardial infarct and oedema

Yetgin et al. (161) have provided an overview of the current state of play regarding RIC in PCI with their meta-analysis. Notably they analysed data from trials of elective PCI and PPCI. They found that there was no significant decrease in myocardial injury biomarkers although the trend was towards a cardioprotective effect with RIC. They also found that the greatest decrease in cardiac biomarkers in patients receiving RIC during PPCI with no presence of heterogeneity. When excluding the Iliodromitis et al. study there was significant pooled data standardised mean differences in favour of RIC. The use of both arms to apply RIC may in part, have explained the neutral results of this study. Notably subgroup analysis of trials seemed to indicate that the most benefit from RIC in PCI was reached when there was a large myocardial area at risk from the index ischaemia. If results show that RIC is of prognostic

benefit then this will enable the routine use of a simple, cheap and low-risk intervention to further benefit patients. The CONDI2/ERIC-PPCI trial is currently underway to investigate the prognostic benefit of RIC following PPCI.

1.11 Diabetes

1.11.1 Background

In the UK, diabetes mellitus (DM) has a prevalence of 4.7% and affects around 3 million people. This has risen sharply from 1.9 million in 1996 and is expected to reach 5 million by 2025 in the UK (166) and worldwide an expected 300 million (167). The increase in this figure has been attributed to the fact that the population is becoming increasingly aged, more sedentary and there is a rise in obesity. The metabolic cause of DM is a combination of insulin resistance in the periphery as well as a deficiency of insulin secretion by pancreatic B cells. Insulin resistance develops from being overweight and physical inactivity and is coupled with genetic susceptibility. The decline in insulin secretion also has a genetic basis but mainly declines with increasing age (168). As well as this diabetes often coexists with other cardiovascular risk factors such as hypertension and hypercholesterolaemia. The complications of diabetes are many and include coronary heart disease, stroke, peripheral arterial disease, nephropathy, retinopathy and possible neuropathy and cardiomyopathy. Of these, cardiovascular morbidity and mortality is perhaps the most serious with a significantly higher mortality in these patients from acute myocardial infarction due to more extensive atherosclerotic lesions, diffuse disease and also a hypertrophied and

dysfunctional left ventricle (169). Rates of re-infarction and heart failure after the first acute myocardial infarction are also higher as well as the incidence of multivessel disease in DM (170, 171). Haffner et al. showed that cardiovascular event risk in diabetic patients with no history of infarction was the same as those seen in non-diabetic patients with prior myocardial infarction (172). From large scale trials it is recognised that chronic glycaemic control is unlikely to play a role in modulating mortality from myocardial infarction and so the risk from diabetes is absolute (173). Therefore, there is an opportunity to develop novel strategies for cardioprotection from IR injury in DM patients.

1.11.2 The myocardium in diabetes

The results of infarct size studies in diabetic animal models have been contradictory, and the reason for differences remains unclear. There exists controversy as to whether the diabetic heart is more or less susceptible to myocardial IR injury. However, clinical studies have shown that there is an increased myocardial susceptibility to IR injury (174).

Studies in animal models have shown that infarct size in diabetic models have never varied in comparison to non-DM patients. Animal models have included rat, mice, dog and rabbit with rodents being the most popularly studied because of the ability to inbreed and reproduce quickly with inherited diabetes and obesity. Infarct size has been similar, smaller, or larger in DM animals and the reasons for this have not been fully understood, but there has always been a significant difference in experimental preparations and protocols. The

duration of diabetes appears to influence myocardial tolerance against infarction and experiments have therefore yielded different results between the two. For example in a study by Ravingerova et al., type I DM was induced by injection of streptozotocin (STZ) into rats which is a toxic glucose analogue that accumulates in pancreatic B cells thereby rendering them inactive. Infarcts were induced by 30 minutes ischaemia but notably infarct size limitation was seen at 1 week but not later at 8 weeks (175). This has been reinforced by other studies which have shown the resistance of diabetic hearts to IR injury was seen in the early phase of DM but disappeared with time (176) but has also been contradicted by studies that have shown enlargement of infarct size at around 1 week after STZ injection (177). This all indicates that duration of diabetes is not the only factor responsible for these inconsistencies. Possible explanations include other co-existent unfavourable metabolic states such as dyslipidaemia. Other factors for consideration also include whether studies used low-flow ischaemia or zero-flow ischaemia, which have different effects on glycolysis, acidosis and lactate production and other metabolism within myocardial cells (178).

A clinical study by Marso et al in 2007 of 501 patients of which 62 (12%) has shown increased myocardial damage in infarction determined by technetium-99m single proton emission computed tomography (SPECT) imaging after reperfusion therapy. As well as this, there was greater development of heart failure, 30-day mortality, 6-month mortality and impaired myocardial perfusion evidenced by blush grade in diabetic patients (174). A study by Algeria et al. in the same year enrolled 2948 patients that underwent SPECT and 6-month

follow up. This study found a slightly larger infarct size in the diabetic cohort of patients and a slightly poorer ejection fraction. There was a substantially increased risk of mortality by 4 to 6-fold in diabetic patients but this was not explained by the small changes in infarct size and implied that DM itself, associated co-conditions or diabetic cardiomyopathy played a significant role in risk (179). Ruber et al. first introduced diabetic cardiomyopathy as a term 30 years ago when he described four patients with heart failure and normal coronary arteries (180). Several factor probably underlie diabetic cardiomyopathy and these are coronary atherosclerosis, prolonged hypertension, chronic hyperglycaemia, microvascular disease, glycosylation of myocardial cell proteins and autonomic neuropathy (178). At a cellular level, potential contributors are reduced glucose and lactate metabolism with increased free fatty acids activating PPAR-α signalling leading to the transcription of genes that modulate fatty acid oxidation. This leads to the formation of ROS at the level of the electron transport chain. ROS, which can also be generated by extramitochondrial mechanisms such as NADPH oxidase plays a critical role in several pathways thought to mediate diabetic cardiomyopathy. These include toxicity from lipids, cell death and mitochondrial uncoupling (183).

1.11.3 Defects in intracellular signalling in diabetic hearts

Experimental and clinical studies have both shown that diabetes interferes with the cardioprotective strategies of preconditioning and postconditioning. The majority of these studies have shown that diabetes interferes with the protective signalling pathways involved in cardioprotection that all appear to

converge on the mPTP. Experimental studies have shown that the infarct limiting size effect of IPC or IPost was lost or diminished in DM or may require an increased stimulus. The Yellon group showed that one, two and three cycles of IPC entailing 5 minutes of global ischaemia followed by 10 minutes of reperfusion each resulted in cardioprotection when infarction was induced. However in type 2 DM Goto-Kakizaki (GK) rats, only 3 cycles of IPC cardioprotected from the infarction protocol of 35 minutes regional ischaemia followed by 120 minutes reperfusion (182). This strongly suggested that the threshold for stimulating IPC in diabetic hearts needed to be increased. Impairment of preconditioning in DM has been observed across the board with species to include rats, rabbits and dogs and using different models of diabetes such as type I DM (STZ rats) and type 2 DM genetic rodent models (OLETF, GK, db/db, Zucker diabetic fatty rats (ZDF) and ob/ob mice).

Various mimetics of IPC and IPost have confirmed that intracellular signalling is impaired in DM by being ineffective at limiting infarct size in diabetic hearts and these include diazoxide, erythropoeitin and isoflurane (183). Other steps involved in cytoprotective signalling have also been impaired or blocked by DM. For example, PI3K/Akt was shown to be defective in DM resulting in less AKT phosphorylation, which was shown in the same study performed by our group mentioned above (183). As well as this, other mediators such as ERK and STAT3 and other various components of the RISK pathway have also been shown to be impaired (184). As mentioned previously GSK-3β has been considered a pivotal step in IPC and IPost cardioprotection. Studies have shown that as well as RISK inactivation that diabetes induced GSK-3β plays a

crucial role in diabetic myocardial oxidative damage and remodelling. Indeed some studies have reported that GSK-3β activity is doubled in diabetes (185). Various studies have also looked at whether hyperglycaemia itself has a role in cardioprotection afforded from IPC or IPost. Acute hyperglycaemia either made no difference or resulted in impaired protection such that infarct size limitation was reduced. This was also seen in other studies using other conditioning stimuli such volatile anaesthetics and K+ channel openers (186).

In various human studies, it has been shown that those patients with pre-infarct angina had reduced infarct size determined by CK release, improved left ventricular function and reduced mortality and a better prognosis (187). Pre-infarct angina is recognised as a natural form of IPC. However, in patients with DM these benefits were not seen suggesting an attenuated response to IPC in these patients (188). In vitro experiments by the Yellon group who established this technique using atrial trabeculae have further supported this finding using atrial trabeculae taken at the time of cardiac surgery, which has shown contractile dysfunction after hypoxia and reoxygenation to be significantly suppressed by preconditioning but not in diabetic hearts (189). To restore cardioprotection in diabetic tissue the ischaemic stimulus needed to be increased from 4 minutes to 7 minutes and was also associated with a downregulation of PI3K/Akt axis. Similarly prior to this Ghosh et al. showed the failure of IPC to protect the diabetic human myocardium using right atrial appendage with one 5 minute cycle of ischaemia followed by one 5 minute cycle of reperfusion (190).

1.11.4 Diabetic medications and preconditioning

A hot topic for some time has been whether sulphonylureas result in adverse effects on diabetics. In the UGDP study published in the 1970s there was an increased mortality in patients taking tolbutamide (191). These drugs have been shown to inhibit protection from IPC and IPost by blocking the K_{ATP} channel in cardiac myocytes raising concerns that sulphonylureas may be detrimental in myocardial infarction, however, the effects of different sulphonylureas are not the same in IPC and IPost. Glibenclamide a non-specific K_{ATP} blocker and has been found to block effects of IPC and IPost in non-diabetic animals (192). However, Nieszner et al. reported the restoration of the infarct sparing effect of preconditioning with low does glibenclamide in type I DM alloxan-treated rabbits. This was not seen with high dose glibenclamide. The postulated mechanism is that the benefit of glibenclamide was due to tight glycaemic control possibly influencing PI3K/Akt signalling which could override the partial inhibition of cardiac K_{ATP} channels (193).

Glimepiride showed the reverse effect of glibenclamide when tested by the Yellon group. Glimepiride a second-generation sulphonylurea was reported to affect the non-pancreatic K_{ATP} channels less than glibenclamide. This study looked at normal rat hearts and showed that glimepiride did not block the infarct-size limiting effects of IPC by not inhibiting the beneficial effects of mitochondrial K_{ATP} channels in the heart (194). Similar results were seen with intravenous infusion of glimepiride in a double-blind placebo controlled study of humans during PCI. This study also reinforced the potential of glibenclamide to block preconditioning (195). In rabbit myocardium,

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glimepiride infusion during reperfusion was shown to have an IPost-mimetic effect by activation of PI3K/Akt (196). Notably these few studies have been performed in non-diabetics as IPC and IPost have failed to show an infarct-limiting effect in DM.

Metformin is an insulin-sensitising agent that reduces hepatic glucose production and increases peripheral uptake. In a study by Ye et al. metformin reduced infarct size by activation of AMP-activated protein kinase (197). The infarct size-limiting effect of metformin remains highly controversial with conflicting results from two studies (198, 199). One of these studies was performed by our group and showed that metformin given during reperfusion protected myocardium during infarction in diabetic and non-diabetic hearts and this was mediated by PI3/Akt and inhibited mPTP opening (199). Kravchuk et al. found that infarct size was reduced in type 2 DM rats and metformin did not induce a cardioprotective effect in an IR model (198). This result is not in keeping with metformin's known beneficial cardiovascular effects in humans.

Pioglitazone and Rosiglitazone are Thiazolidinediones administered before ischaemia and were both found to reduce infarct size in normal and diabetic animals. However, the impact of the cardioprotective effect of thiazolidinediones on clinical benefit and adverse events is unclear with Rosiglitazone having been shown to increase the risk of myocardial infarction and Pioglitazone appearing to reduce cardiovascular events. It is speculated

that this is because of the differing properties and specificities for these drugs on the peroxisome proliferator-activated receptor (PPAR) receptors (199).

Recently glucagon-like peptide (GLP-1) receptor agonists and inhibitors of dipeptidyl peptidase-4 (DPP-4) inhibitor have demonstrated clinical cardiovascular benefits. However, there is little data on the effects of these two drugs on IR injury (200). One study by Huisamen et al (201) has reported that enlargement of infarct size in obese pre-diabetic rats was decreased at 4 weeks by treatment with a DPP-4 inhibitor. Our group have shown that the active metabolite GLP-1(7-36) can limit infarct size by decreasing IR injury in isolated and in-situ rat hearts when given with a DPP-IV inhibitor [which prevents the breakdown of active GLP-1(7-36) to GLP-1(9-36)] before IR (202). It has also been shown to protect against myocardial IR injury when given as a preconditioning-mimetic or at reperfusion (203). Interestingly a study using pigs failed to show a benefit with GLP-1 but this study was carried out without co-administration of a DPP-IV inhibitor (204). Interestingly these initial studies described above suggest a beneficial effect in non-diabetic and diabetic patients. Further studies of these drugs are required on intracellular protective signalling and their beneficial cardiovascular effects (205).

1.11.5 Diabetes and cardioprotection

In summary, it appears that DM increases myocardial susceptibility to IR injury through various mechanisms, which results in worse outcomes for diabetic patients in the context of myocardial infarction. DM also changes the response of myocardium to IPC and IPost by disruption of signalling cascades

involved in cardioprotection. At a cellular level cardioprotection may occur through manipulation of mechanisms upstream from GSK-β and mPTP or blockade of mPTP opening. Preconditioning appears to be a potential mechanism for affording cardioprotection potentially through altering cytoprotective signalling but this may only be realised with an increased preconditioning stimulus.

Chapter 2: Aims and Hypotheses

2.1 A study of RIPC in cardiac bypass patients with diabetes in our institution

A previous study at our institution investigating three cycles of arm RIPC (5 minutes ischaemia with 5 minutes reperfusion intervals) did not show benefit in a diabetic cohort of CABG patients. However, when the RIPC stimulus was intensified by using simultaneous inflation/deflation of cuffs placed on upper arm and leg, there was a cardioprotective effect but only 35 patients were recruited. In my thesis, I have added patients to this cohort.

2.1.1 Aims 1

To further evaluate the role of RIPC in diabetic CABG patients using 2 cycles of simultaneous leg and arm RIPC in a sufficient sample size to provide adequate statistical power.

2.1.2 Hypothesis 1:

Increasing the intensity of the RIPC stimulus will confer cardioprotection in diabetic patients undergoing CABG surgery.

2.2 A study of RIPC in cardiac surgery patients that received intravenous GTN at our institution

A retrospective analysis of 180 patient cohort suggested that those patients receiving IV GTN were not cardioprotected by two cycles of 5 minutes ischaemia and 5 minutes reperfusion with simultaneous arm and legs RIPC. In 2014, our institution began a study to determine whether GTN reduces

injury to the heart during heart-lung bypass surgery in combination with the newer technique of RIPC. This is called the Effect of Remote IPC and Glyceryl Trinitrate on Peri-operative Myocardial Injury in Cardiac Bypass Surgery Patients (ERIC-GTN Study). There are four arms in this study with a control group receiving Sham Remote IPC (RIPC) with IV normal saline infusion. Patients will also be administered an RIPC protocol of three 5 minute cycles of ischaemia with intervening reperfusion with simultaneous upper arm and thigh cycles prior to surgery. A third group will receive GTN and sham simulated RIPC at 2-5 ml/hr during surgery. A fourth group will receive RIPC and IV GTN. By increasing the stimulus to three cycles of multi-limb RIPC but with the same protocol I hope to see whether this will result in cardioprotection in those patients receiving IV GTN.

2.2.1 Aims 2

To evaluate whether an increased stimulus of 3 cycles of simultaneous arm and leg RIPC overcomes the potential effect of confounding factors such as administration of intravenous GTN and provides further myocardial preservation to those patients receiving RIPC where it had not been seen previously at the lower 2 cycle RIPC stimulus.

2.2.2 Hypothesis 2:

Increasing the intensity of the RIPC stimulus will confer cardioprotection in patients undergoing CABG surgery who are administered IV GTN.

Chapter 3: General Methodology

3.1.1 Experimental design

The two main clinical studies in diabetics patients and in the presence of GTN were already designed originally in 2001 and various amendments had been made over the years by investigators at our institution because of variations in their study. I therefore provided the amendments to the previous study protocol and obtained ethical approval for these changes. The specific amendments for each study are described in their individual results chapter. Below is the general methodology which was generic for both studies.

3.1.2 Inclusion criterion

a. Age more than 18 years old.

b. Patients undergoing CABG surgery and/or valve surgery at the Heart Hospital at University

c. Patient to be given informed consent with an information sheet which should ideally be given 24 hours prior to agreement to participate.

d. The current WHO diagnostic criteria for diabetes was used to see if patients fulfilled the criterion for being diagnosed with having diabetes. Patients needed to have a fasting plasma glucose ≥ 7.0mmol/l (126mg/dl) or 2–h plasma glucose ≥ 11.1mmol/l (200mg/dl. Although it is understood that there are limitations with this definition the current criteria distinguish a group with significantly increased premature mortality and increased risk of microvascular and cardiovascular complications.

3.1.3 Exclusion criterion

a) Cardiogenic shock or cardiac arrest preceding surgery where cardiogenic shock was defined as systolic BP <90mmHg for 30 minutes before inotrope/vasopressor administration or an intra-aortic balloon pump (IABP) was used to maintain systolic BP>90mmHg.

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b) Positive baseline serum hsTnT and/or CK-MB as these patients may already be preconditioned by a recent event with a subsequent potential impact on RIPC prior to cardiac surgery.

c) Pregnancy.

d) Significant peripheral artery disease affecting any of the limbs.

e) Significant liver dysfunction with an International Normalised Ratio (INR) greater than 2.0.

f) Significant pulmonary disease with a Forced Expiratory Volume of less than 40% predicted.

g) Renal failure with an estimated glomerular filtration rate (eGFR) <30ml/min/1.73m2 as troponin excretion via the kidney is altered in the presence of kidney disease thereby making hsTnT evaluation unreliable.

h) Concomitant therapy with glibenclamide or nicorandil as these medications may interfere with RIPC.

3.1.4 Randomisation

Randomisation was carried out on the day of surgery as soon as patients were sent to the theatres for anaesthesia. Randomisation was carried out using a free online tool, which randomly allocated patients to either control or intervention arms.

3.1.5 Intervention: RIPC and sham treatment protocols

RIPC and control protocols were applied after anaesthesia induction and prior to transfer to theatre for draping. RIPC was delivered with a standard arm BP cuff placed on the contralateral arm where the arterial line was sited and a standard thigh BP cuff. The cuffs were simultaneously inflated to 200mmHg and then left inflated for 5 minutes. They were then deflated to 0mmHg and left deflated for 5 minutes. This cycle was repeated three times so that the

total duration of the intervention was 30 minutes. For the control protocol the two cuffs were simultaneously placed on the upper arm and the upper thigh and left uninflated for 30 minutes.

3.1.6 Anaesthetic procedure

Patients received pre-medication with oral temazepam 10 to 20mg approximately one hour prior to surgery. The patient was then taken the anaesthetic room where IV access was gained through a peripheral venous cannula a central venous and continuous invasive BP monitoring was achieved with the insertion of an arterial line. Anaesthesia induction was obtained with a combination of midazolam, etomidate, propofol, fentanyl, rocuronium, vecuronium, or pancuronium. Following anaesthesia induction the trachea was intubated and mechanical ventilation commenced with oxygen with or without air. Anaesthesia maintenance was achieved with volatile anaesthetic agents including isoflurane or sevoflurane and propofol infusion with or without fentanyl. Intravenous glyceryl trinitrate infusion (GTN) was administered at the discretion of the anaesthetist in order to optimise BP control and improve intraoperative coronary vasodilatation.

3.1.7 Surgical procedure

Following anaesthesia and either RIPC or sham protocols the patient was transferred to theatre where mid-line sternotomy was performed. Standard non-pulsatile cardiopulmonary bypass (CPB) was performed using a membrane oxygenator and cardiotomy suction with cannulation of the aortic root and the right atrial appendage: for coronary artery bypass surgery the

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proximal end of each vein graft anastomosis was created during CPB with the distal end to the coronary arteries being constructed during cardiac standstill which was achieved through either injection of cardioplegic solution or induction of ventricular fibrillation with aortic root cross clamp. Ventricular fibrillation was induced through the application of an alternating current to the epicardium following clamping of the aortic root and was reverted using a direct current shock.

Cardioplegic myocardial preservation was achieved through two different methods.

1) Antegrade cardioplegia: 1 litre of cold blood cardioplegic solution (1 part of St John's Cardioplegia solution mixed with 4 parts of cold blood) was delivered to myocardial cells through the aortic root further to aortic cross-clamp followed by a maintenance cold blood cardioplegia which was given down the grafts in occluded arteries and also into the aortic root every 20 to 30 minutes. Systemic temperature in these patients was 28-34 C.

2) Antegrade and retrograde cardioplegia: an initial 800ml dose of antegrade cardioplegia was administered into the aortic root followed by 400mls of retrograde cardioplegia solution given through the coronary sinus. Following each anastomosis maintenance was achieved with a further administration of 100mls of retrograde cardioplegia. On completion of the anastomosis CPB was discontinued and rewarming was initiated and protamine was given to reverse any heparin.

3.1.8 Baseline characteristics for intravenous GTN

Both groups matched well and had no significant differences between the baseline characteristics.

3.1.9 Statistical analysis

Standard statistical methods were used for analysis with comparison between the sham and intervention groups being made using unpaired Student-T test for approximately distributed variables or Wilcoxon-Mann-Whitney test for non-normal data and categorical data using the Chi-square test. For outcomes collected at different time points a repeated measures linear regression model was used to estimate the difference at each point and 95% confidence intervals. The post-hoc analysis of associations between RIPC and intravenous GTN was performed using an interaction test in a linear regression model.

3.1.10 Imputation of missing values

Data sets that had missing troponins at time point 0 or 72 hours had to be excluded. This totalled 9 patients or 10% of the total recruited. I then excluded 6 (8%) of patients that had more than 2 troponin values missing from time points 6, 12 ,24 and 48 hours as no meaningful analysis could be performed. For those that remained 7 patients (11%) had two time point values missing and 11 patients (17%) had had a single value missing. Using SPSS missing values software the multiple imputation procedures provided analysis of patterns of missing data and multiple imputation of missing values. Multiple

versions of the dataset were produced each containing its own set of imputed values. When statistical analyses were performed, the parameter estimates for all of the imputed datasets were pooled providing estimates that are generally more accurate than they would be with only one imputation. For unbiased results it is essential that the assumption that missing data is completely at random which should be the case here.

3.2 Study endpoints

3.2 Primary endpoint

3.2.1. Periprocedural myocardial infarction (PMI)

This was periprocedural myocardial infarction or PMI measured by total area-under-the-curve (AUC) of the total release of hsTnT over the 72 hours pours operatively using 0, 6, 12, 24, 48 and 72 hour blood troponin samples being taken. My research predecessor showed that an increased stimulus of RIPC of 2 cycles of simultaneous arm and leg ischaemia for 5 minutes afforded significant cardioprotection but not in the presence of patients that received intravenous GTN (3). We therefore intended to establish whether an enhanced RIPC stimulus of 3 cycles would reduce periprocedural myocardial infarction and overcome confounding factors such as f those patients that had received intravenous GTN. HsTnT was measured using the one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche, Switzerland). Study primary end-point: PMI. The threshold level of 0.0014 ug/L was cut off for the indication of significant myocardial necrosis and was the 99th centile for the reference population with a risk of <10%.

Absolute hsTnT release over the 72 hour post-operative period was calculated with AUC using the following Excel office 2010 formulas:

1) AUC between two specific time-points (for example time-points 1 and 2):

   AUC **t1-t2** = [(hsTnT at t1 hours + hsTnT at t2 hours)/2] x (t2-t1)

2) Total AUC over the post-operative 72 hours:

   AUC-72 hours=AUC0-6+AUC6-12+AUC12-24+AUC24-48+AUC48-72

3.3 Study secondary endpoints

3.3.1 Acute Kidney Injury (AKI)

In the context of cardiac surgery AKI can occur in up to 30% of patients and impairs the function of other organs such as the brain, lungs and gut. The risk of death is increased by 5-fold during hospitalization. Around 2-5% of patients following cardiac surgery need renal replacement therapy and this causes a steep rise in mortality of up to 50% (206). Preventative strategies are limited and centre around maintain renal perfusion and metabolism and so RIPC could potentially lend itself to benefit in this area. Acute Kidney Injury is the abrupt loss of kidney function resulting in the retention of urea and other nitrogenous waste products and in the dysregulation of extracellular volume and electrolytes. For the purposes of this study AKI was derived using an AKI score over the first 3 post-operative days through looking at serum creatinine and urine output. AKI score as modified from Rifle's criteria (207 and 208) with creatinine measure at 0, 24, 48 and 72 hours and urine volumes measured at 24,48 and 72 hours.

Table 6. Rifle's criterion for AKI

AKI Grade	Creatinine criteria	Urine output criteria
1	Creatinine rise >26.4 µmol/L or 150-200% baseline	<0.5ml/kg/hr for >6 hours
2	Creatinine rise 200-300% of baseline	<0.5ml/kg.hr for >12 hours
3	Creatinine rise>300% or >354 umol/L with an acute rise of at least 44 umol/L	<0.3ml/kg/hr for >24 hours or anuria for 12 hours.

3.3.2 Inotrope requirements

The amount of cardiovascular support in the first 72 hrs after heart surgery with cardiopulmonary bypass predicts eventual mortality and morbidity (209, 210). The degree of support is best characterised by a maximum vasoactive-inotropic score adapted from a score by Ko et al.(211) and provides an objective measurement of the requirement of inotropes in the immediate postoperative period. Data was collected every 24 hours for as long as the patients were on inotropes and calculated using the formula below.

Inotrope score=Dosages (**in ug/**kg/min) of:

[Dopamine + Dobutamine + Dopeximine] + [(Adrenaline + Noradrenaline + Isopreterenol) x 100] + [(Enoximone + Milirinone) x15].

The inotrope score was calculated for time '0' for the dose of individual inotropes used at the time of coming off bypass and the other time points used the maximum dose of individual inotropes used in the previous 24 hour period.

3.3.3 Intensive Care Unit (ICU) and hospital stay

Post-operatively all patients had a planned admission to ICU as this is recognised to reduce morbidity and mortality (212). Increased lengths of stay of these two parameters is known to be associated with more 'adverse' peri-operative conditions such as longer anaesthesia, cardiopulmonary bypass and postoperative intubation time (213). Lengths of stay were therefore collected to see if RIPC had any impact on burden. The length of ICU and hospital stay was taken as the total number of days from operation to transfer or discharge respectively. ICU stay was counted up to when it was clearly documented in the notes that transfer was appropriate as this was often delayed due to beds not being available. Similarly hospital stay was counted up to the date where it was clearly documented that discharge was appropriate as frequently there were delays because of hospital inefficiencies or social reasons.

3.3.4 New onset of post-operative AF

New onset of post-operative AF occurs in up to 33% of patients undergoing coronary artery bypass surgery (214) and is even higher in those that have had valve surgery. Post-operative AF is associated with an increased risk of stroke and thromboembolism as well as greater morbidity and mortality (215). We therefore collected data on incidence of new AF to see whether AF may protect patients from this arrhythmia. An episode of new AF was counted as any episode noted by telemetry or ECG that required treatment by pharmacological intervention or direct current cardioversion. Patients with known permanent AF or paroxysmal AF were excluded from the evaluation of the secondary end-point.

Chapter 4 – Results for RIPC in a diabetic cohort of patients undergoing cardiac surgery

4.1 Introduction

4.1.1 A study of RIPC in cardiac bypass patients with diabetes in our institution

We have shown in our institution that three cycles of 5 minutes of upper limb RIPC cardioprotected in an unselected cohort of patients undergoing cardiac surgery, however this effect was not seen in a susbsequent study of diabetic patients. A total of 60 patients with type 2 diabetes completed this later study over a two year period. 30 patients were in the RIPC group and 30 in the control group. Both groups were well-matched in terms of baseline characteristics and intra-operative parameters. With three cycles of upper limb ischaemia there was no statistical difference in mean cTnT levels between the RIPC group and control groups at any time point collected over 72 hours. The mean cTnT AUC over 72 hours in the control group was 24.55 ug/L with the RIPC group value being 22.38 ug/L which gave rise to $p=0.52$. Similarly, there was no difference between post-operative ventilation times (7.74 hours control group vs. 7.93 hours RIPC group, $p=0.87$), post-operative ITU stay (2.77 days control vs. 2.8 days RIPC group, $p=0.98$), post-operative inotrope score (7.4 ug/kg min control group vs 9.98 ug/kg/min RIPC group, $p=0.55$), post-operative atrial fibrillation (6 patients control group vs 4 patients in the RIPC group, $p=0.73$) and post-operative acute kidney injury (4 patients control group vs. 2 patients in the RIPC group $p=0.67$).

As discussed earlier, in diabetic hearts the myocardium behaves differently in relation to IR injury and this is felt to be through differences in metabolism or intracellular signalling when compared to normal myocardium. The RIPC effect is either attenuated or to achieve cardioprotection a greater stimulus is needed in the diabetic heart. This study looked at an intensified RIPC stimulus using two cycles of 5 minutes simultaneous inflation and then 5 minutes of deflation of cuffs placed on upper arm and leg. This multi-limb RIPC is in effect an increase of one cycle in those patients that had undergone 3 upper limb cycles. 48 patients in total were recruited in this cohort with 23 in the control group and 25 patients in the RIPC group. My predecessor had recruited 36 patients and I added 12 further patients to this cohort with 6 in the control group and 6 in the RIPC group.

Figure 4.1 Study profile for cohort of diabetic patients recruited and added to a predecessor's study

449 patients screened for eligibility in total.

3 refused to participate

217 recruited into other studies

181 fulfilled exclusion criterion

48 patients randomised

25 patients randomised to control and 26 patients randomised to RIPC

3 patients excluded as 0 and 72 hour timepoint values missing

23 patients control

25 patients randomised to RIPC

Table 7. Baseline characteristics in patients undergoing cardiac surgery and randomised to either sham or two cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

Patient Characteristic DM cohort	Control (n=23)	RIPC (n=25)
Age (years)	71±9	66±8
Gender		
Male	18 (78%)	16 (64%)
Female	5 (22%)	9 (36%)
Ethnicity		
Caucasian	15 (65%)	18 (72%)
Asian	3 (13%)	6 (24%)
Turkic	3 (13%)	0 (0%)
Middle-Eastern	2 (9%)	1 (4%)
Body Mass Index	28.2±5.9	29.9±4.7
Smoking history		
Smoker	5 (22%)	7 (28%)
Ex-smoker	15 (65%)	16 (64%)
Non-smoker	3 (13%)	2 (8%)
Co-morbidities		
Hypertension	20 (87%)	18 (72%)
Hypercholesterolaemia	19 (83%)	20 (80%)
Atrial Fibrillation	3 (13%)	3 (12%)
Previous MI	6 (26%)	9 (35%)
Previous CVA/TIA	1 (4%)	2 (8%)
Previous cardiac surgery	0	0
LVEF		
>50%	22 (96%)	23 (92%)
30-50%	1 (4%)	2 (8%)
<30%	0	0
NYHA	2.2±0.9	2.5±0.7
CCS	2.6±0.8	2.3±0.9
Family History of IHD	15 (65%)	16 (64%)
Euroscore	3.59±1.67	3.22±1.92
Bypass time (mins)	82.4±38.2	84.8±33.8
Cross-clamp time (mins)	60.5±22.7	68.4±18.3

Patient Characteristic	Control (n=23)	RIPC (n=25)
Drug history		
Aspirin	20 (87%)	24 (96%)
Clopidogrel	5 (22%)	5 (20%)
Prasugrel	0 (0%)	0 (0%)
Ticagrelor	1 (43%)	0 (0%)
Warfarin	3 (13%)	4 (16%)
Beta-blocker	17 (78%)	22 (88%)
Calcium channel blocker	8 (35%)	4 (16%)
Statin	20 (87%)	25 (88%)
Ace-i/A2RB	17 (74%)	20 (80%)
Long acting nitrate	4 (17%)	2 (8%)
Nicorandil	3 (13%)	1 (4%)
Insulin	4 (17%)	2 (3%)
Biguanide	3 (23%)	2 (8%)
Sulphonylurea	4 (17%)	2 (8%)
Thiazolidinediones	5 (21%)	4 (16%)
Diuretics	5 (22%)	4 (16%)
Cardioprotection		
Blood cardioplegia	19 (84%)	20 (80%)
Cross-clamp fibrillation	4 (17%)	5 (20%)
Operation		
CABG	14 (61%)	17(68%)
AVR	3 (13%)	4 (16%)
MVR or repair	3 (13%)	2 (8%)
CABG+AVR	3 (13%)	2 (8%)
Grafts		
1	3 (13%)	2 (8%)
2	7 (30%)	6 (24%)
3	10 (43%)	14 (56%)
4	3 (13%)	3 (4%)
Anaesthetic agents		
Induction		
Rocuronium	19 (84%)	22 (85%)
Pancuronium	4 (17%)	3 (15%)
Propofol	23 (92%)	24 (96%)
Fentanyl	20 (87%)	23 (92%)
Etomidate	2 (7%)	4 (16%)
Midazolam	14 (61%)	14 (56%)
Maintenance		
Propofol	23 (100%)	25 (100%)
Isoflurane	23 (100%)	24 (96%)
Sevoflurane	0 (0%)	1 (4%)
Intra-operative Glyceryl trinitrate	19 (83%)	20 (80%)

Table 8. Mean high-sensitivity Troponin T at various time points and calculated Total Area-under-the-curve at 72 hours post-operatively

Time endpoint	Control (n=23) Mean SD hsTnT (ug/L)	RIPC (n=25) Mean SD hsTnT (ug/L)	Difference (95% CI)	P value
Pre-operatively	0.020 (0.022)	0.048 (0.163)	-0.0969 to 0.0411	0.417
6 hours	0.643 (0.348)	0.520 (0.276)	-0.0569 to 0.3070	0.178
12 hours	0.604 (0.309)	0.447 (0.214)	0.0036 to 0.3103	0.050
24 hours	0.392 (0.279)	0.309 (0.154)	-0.0465 to 0.2125	0.207
48 hours	0.340 (0.290)	0.223 (0.105)	-0.0077 to 0.2417	0.069
72 hours	0.334 (0.245)	0.172 (0.111)	0.0535 to 0.2716	0.007
Total AUC 72 hours	28.38 (17.44)	20.07 (9.28)	0.2852 to 16.334	0.050

4.1.2 RIPC reduced periprocedural myocardial infarction in a cohort of diabetic patients undergoing cardiac surgery.

Two cycles of multi-limb RIPC reduced peri-operative myocardial infarction with total area-under-the-curve at 72 hours for HsTnT levels from 28.38 ug/L to 20.07 ug/L (p=0.050). This study therefore just achieved significance. Notably two individual time points for mean troponin levels also achieved significance at 12 hours and 72 hours with p values of 0.050 and 0.007 respectively.

Fig. 4.2. Mean high-sensitivity Troponin T levels at 0, 6, 12, 24, 48 and 72 hours in diabetic patients undergoing cardiac surgery (mean±SEM*)

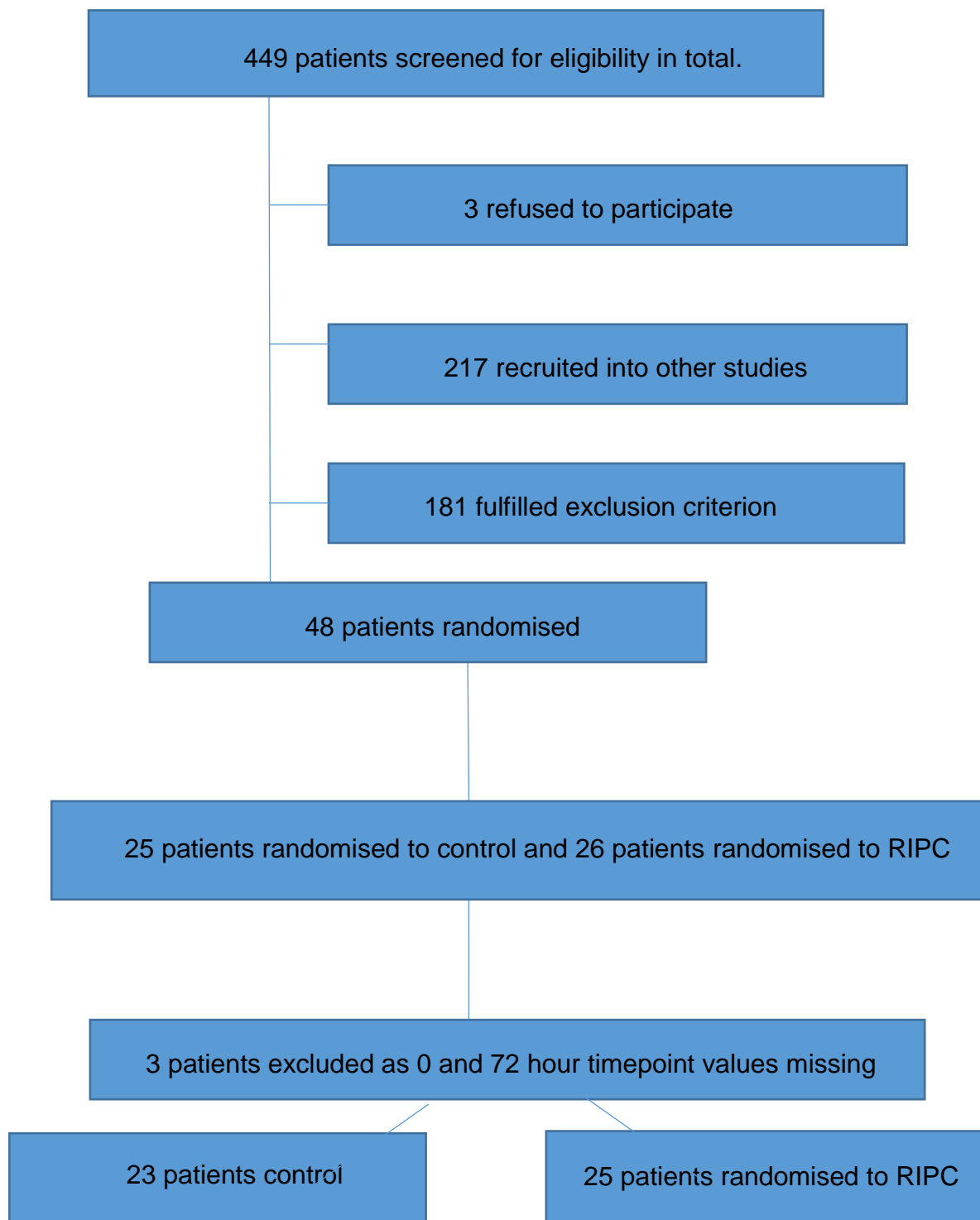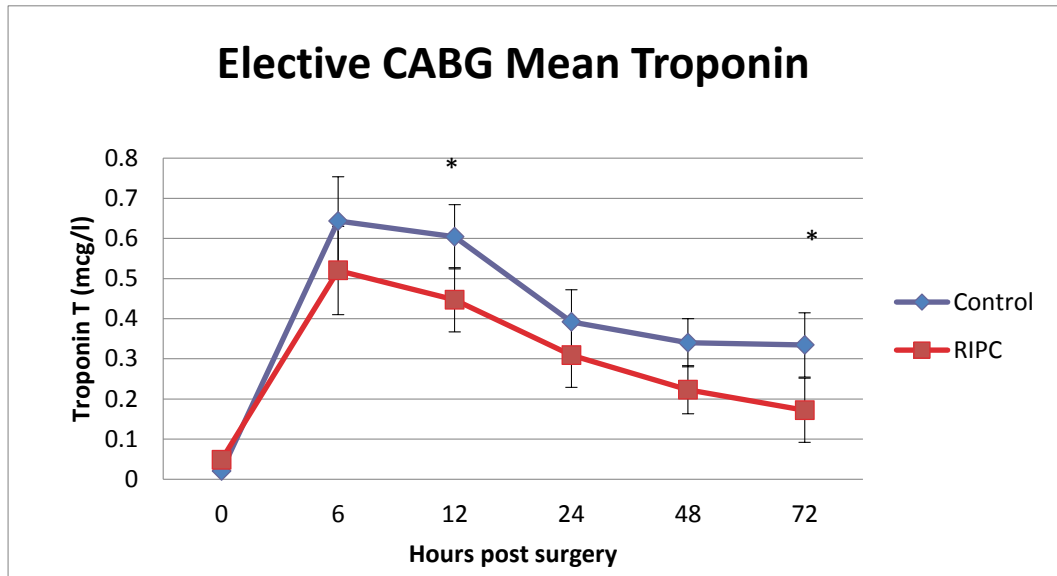**Figure 4.3 Calculated total AUC at 72 hours post-operatively in diabetic patients undergoing cardiac surgery and randomised to either sham or two cycles of 5 minutes of upper and lower limb ischaemia and reperfusion**
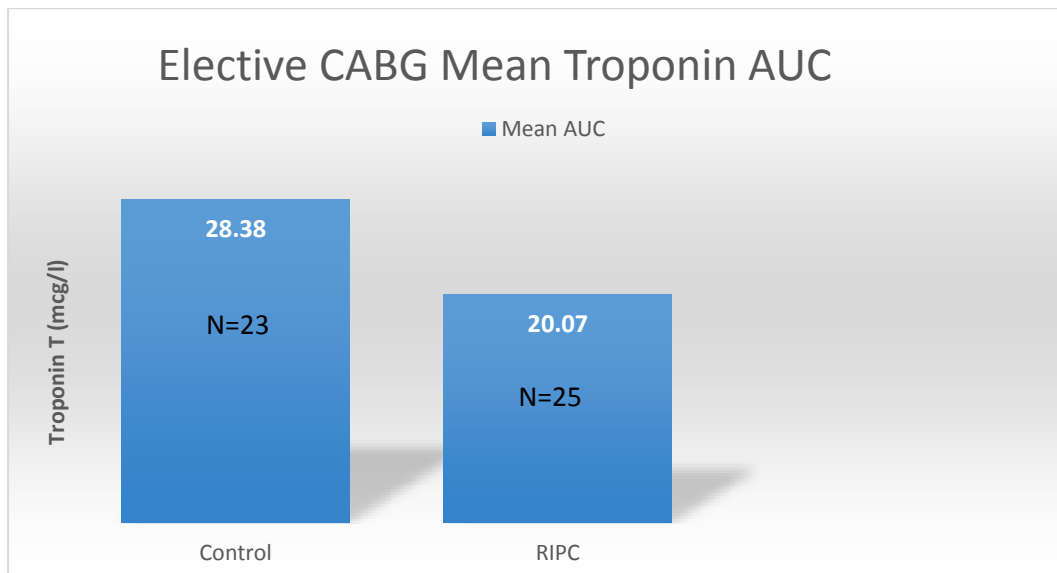
4.2 Secondary Outcomes

4.2.1 Post-operative Acute Kidney Injury (AKI)

The amount of acute kidney injury using the scoring system outlined above was similar between both groups and did not show statistical significance (p=0.8673)

Table 9. AKI scores in diabetic patients undergoing cardiac surgery and randomised to either sham or two cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

AKI score	Control n=23	RIPC n=25	P value
1	2	2	
2	2	1	
3	1	2	
Totals (%) and SD	5 (22%) 0.421	5 (20%) 0.408	p=0.8673

4.2.2 Post-operative atrial fibrillation

There were 3 cases out of 23 in the control group (13%) and 5 cases out of 25 in the RIPC group (20%). This was not statistically significant p=0.525

4.2.3 Length of ICU stay and hospital discharge

The average length of stay in the control group was 2.1 days and the average length of stay in the RIC group was 2.4 days. This did not achieve statistical significance p=0.763**.**

The total length of hospital stay was 8.5 days in the control group and 7.8 days in the RIC group. This did not achieve statistical significance p=0.485.

4.2.5 Inotrope score

The inotrope scores were calculated for 72 hours after coming off bypass in both groups. The control group had an inotrope score total of 8.8. and the RIPC group had a score of 9.1. This was not statistically significant p=0.928.

<u>**4.3 Discussion**</u>

This study of 48 patients showed that an increased stimulus of two cycles of 5 minutes multi-limb RIPC with two intervening cycles of reperfusion cardioprotected diabetic cardiac surgery patients when compared to control patients. This increased stimulus showed benefit over a previous study from our institution which showed that 3 cycles of 5 minutes single-limb RIPC with intervening cycles of reperfusion did not cardioprotect in diabetics. Statistical significance was achieved for the primary outcome which showed that troponin area-under-the-curve at 72 hours was reduced to 20.07 ug/L with the control value being 28.38 ug/L [CI 0.2852 to 16.334, p=0.050] which represented a 29% reduction. Statistical significance was also achieved for the mean troponin values taken at two time points. These were the 12 hour time point mean troponin value with the RIPC value being 0.447 ug/L vs 0.604 ug/L in the control group [CI 0.0035 to 0.3103, p=0.050] and also the 72 hour time point troponin value with the mean RIPC troponin value being 0.172 ug/L with the control value being 0.334 ug/L [CI 0.0535 to 0.2716, p=0.007]

It is known that diabetic hearts are less well protected by preconditioning as the myocardium behaves differently to towards IR injury. From animal studies in diabetic rats preconditioning with a standard ischaemia stimulus fails to

demonstrate the reduction in infarction. Studies have shown that a prolonged ischaemic stimulus is needed to protect animal diabetic hearts in these models (216, 217). We have demonstrated that this increased RIPC stimulus overcomes this normally attenuated effect seen in diabetic patients.

Clinical studies have had discrepant results regarding RIPC with diabetic and non-diabetic patients but there are no other studies looking at this intervention in just diabetic patients. Literature search shows that 7 randomised control trials excluded diabetic patients (218-224) completely with 10 randomised control trials including both diabetic and non-diabetic patients (225-234). This is therefore the first study that shows that an enhanced stimulus cardioprotects in diabetic patients. Notably in our study, we excluded patients taking the sulphonylurea glibenclamide, which has been demonstrated to interfere with IPC, induced cardioprotection by blocking K-ATP channels in animal (235, 236) and human models (237,238). The only other sulphonylurea used was gliclazide, which does not abrogate the effect of IPC.

Unfortunately, no statistically significant effect was seen in the secondary outcomes of post-operative atrial fibrillation, post-operative acute kidney injury, inotrope score and intensive care and hospital stay. The most likely explanation for this being that the study is underpowered to show a benefit in these outcomes and further recruitment may make differences between the two groups more apparent. Another reason is that the time of my study there was competing recruitment from the multicentre ERICCA study and then later the ERIC-GTN study and so achieving statistical significance was challenged

by this as higher risk patients such as those that had poor left ventricular function and concomitant valve surgery were preferentially recruited into these studies. These groups of higher risk patients should exhibit a more profound cardioprotective effect from preconditioning which may also have contributed towards achieving statistical significance. Despite this the primary outcome has been achieved which shows that an enhanced RIPC stimulus is potentially able to overcome the higher preconditioning threshold required in order to achieve significant cardioprotection as measured by troponin release.

Chapter 5. Results for all-comers undergoing cardiac surgery and then post-hoc analysis for patients that received intravenous GTN

5.1 Introduction

5.1.1 A study of RIPC in cardiac bypass patients with or without GTN in our institution

A retrospective analysis of 178 patient cohort suggested that those patients receiving IV GTN were not cardioprotected by two cycles of 5 minutes ischaemia and 5 minutes reperfusion in simultaneous arm and legs RIPC. 118 patients received GTN intra-operatively and had been randomised to control (n=65) or RIPC (n=53). Baseline characteristics were similar between both groups as were the intra-operative parameters. HsTnT levels were lower in the RIPC group at all post-operative time-points, however, with a statistical significance only at 72 hours. RIPC reduced total AUC at 72 hours when compared to control (26.69 ± 13.93 ug/L vs 30.81±17.56 ug/L respectively). Although there was trend to cardioprotection in this group this was not statistically significant p=0.179. Similarly, there was no significant difference between any of the major secondary endpoints although detailed statistical analysis showed that there was a significantly improved urine output at 24 and 72 hours post-operatively and as a total amount over the three days post-surgery. There was therefore no difference between AKI scores which were identical at 5 between both groups, inotrope score which totaled 30.28 in the control group at 72 hours and 25.70 in the RIPC group (p=0.663), new onset atrial fibrillation which was identical between both groups at 3 new cases

(p=0.661), length of ICU stay which was 2.0 days in both groups, length of hospital stay (9 days control group vs 8 days in RIPC group, p=0.068).

By increasing the stimulus for RIPC to three cycles of the same protocol we hoped to see whether this will result in cardioprotection in those patients receiving IV GTN intra-operatively. Use of IV GTN for cardiac surgery in our institution is common with up to 83% of patients receiving this intra-operatively. Its use is primarily dependent on the attending anaesthetist but is ubiquitous because of its rapid hypotensive effect and reduction in preload through vasodilatation.

Therefore, to study patients that had received this drug we initially recruited cardiac surgery all-comers and analysed the data in this cohort. As IV GTN use was dependent on the anaesthetist and clinical need, we then performed a post-hoc analysis of those patients that had received IV GTN intra-operatively.

Figure 5.1. Study profile for all-comers undergoing cardiac surgery

Total number of patients screened 173

87 patients recruited for other studies

3 patients refused consent

4 patients had an eGFR <30ml/min.1.73m2

79 patients randomised

44 patients randomised to control and 45 patients randomised to RIPC

9 patients excluded as 0 and 72 hour timepoint values missing

6 patients excluded as more than 3 timepoint values missing missing

34 patients control

30 patients randomised to RIPC

Table 10. Baseline characteristics in patients undergoing cardiac surgery and randomised to either sham or three cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

Patient Characteristic	Control (n=34)	RIPC (n=30)
Age (years)	67±10	69±9
Gender		
Male	25 (74%)	20 (67%)
Female	9 (26%)	10 (33%)
Ethnicity		
Caucasian	23 (68%)	18 (60%)
Asian	8 (23%)	9 (30%)
Turkic	2 (6%)	1 (3%)
Middle-Eastern	1 (3%)	2 (7%)
Body Mass Index	30.1±5.5	28±4.4
Smoking history		
Smoker	6 (18%)	8 (27%)
Ex-smoker	18 (53%)	15 (50%)
Non-smoker	10 (30%)	7 (23%)
Co-morbidities		
Diabetes Mellitus	8 (24%)	8 (28%)
Hypertension	28 (82%)	22 (73%)
Hypercholesterolaemia	27 (79%)	22 (73%)
Atrial Fibrillation	2 (6%)	3 (10%)
Previous MI	12 (35%)	8 (27%)
Previous CVA/TIA	1 (3%)	2 (6%)
Previous cardiac surgery	0	0
LVEF		
>50%	32 (94%)	27 (90%)
30-50%	2 (6%)	3 (10%)
<30%	0	0
NYHA	2.3±0.8	2.4±0.8
CCS	2.8±0.9	2.9±0.8
Family History of ischaemic heart disease	20 (58%)	22 (73%)
Euroscore	3.62±1.99	3.88±2.01
Bypass time (mins)	92.3±28.7	88.9±31.8
Cross-clamp time (mins)	61±27.8	64.4±22.2

Patient Characteristic	Control (n=34)	RIPC (n=30)
Drug history		
Aspirin	31 (91%)	26 (87%)
Clopidogrel	8 (21%)	7 (23%)
Prasugrel	1 (3%)	0 (0%)
Ticagrelor	3 (10%)	1 (3%)
Warfarin	4 (12%)	5 (17%)
Beta-blocker	22 (73%)	25 (83%)
Calcium channel blocker	8 (24%)	5 (16%)
Statin	28 (93%)	25 (83%)
Ace-i/A2RB	22 (73%)	20 (67%)
Long acting nitrate	5 (15%)	6 (20%)
Nicorandil	3 (9%)	2 (7%)
Insulin	2 (6%)	4 (13%)
Biguanide	7 (21%)	4 (13%)
Sulphonylurea	5 (15%)	6 (20%)
Thiazolidinediones	2 (7%)	1 (3%)
Diuretics	8 (24%)	6 (20%)
Cardioprotection		
Blood cardioplegia	28 (82%)	25 (83%)
Cross-clamp fibrillation	6 (18%)	5 (17%)
Operation		
CABG	19 (74%)	23 (77%)
AVR	5 (14%)	4 (15%)
MVR or repair	4 (12%)	3 (10%)
CABG+AVR	0 (0%)	0 (0%)
Grafts		
1	2 (6%)	1 (3%)
2	10 (29%)	10 (33%)
3	15 (44%)	15 (50%)
4	7 (21%)	4 (13%)
Anaesthetic agents		
Induction		
Rocuronium	28 (82%)	26 (87%)
Pancuronium	6 (18%)	4 (13%)
Propofol	32 (94%)	26 (87%)
Fentanyl	28 (82%)	27 (90%)
Etomidate	3 (9%)	4 (13%)
Midazolam	15 (44%)	15 (50%)
Maintenance		
Propofol	34 (100%)	30 (100%)
Isoflurane	32 (94%)	28 (97%)
Sevoflurane	2 (6%)	2 (3%)
Intra-operative Glyceryl trinitrate	22 (73%)	25 (83%)

5.1.2 RIPC did not reduce periprocedural myocardial infarction in an unselected cohort of patients undergoing cardiac surgery.

Although there was only a single time point of troponin taken at 24 hours that achieved statistical significance, the RIPC group had a mean value of troponins that were lower at each time point. As well as this, the 72 hour troponin AUC was also lower in value in the RIPC group, and approached statistical significance but did not achieve it between the two groups (p=0.074).

Table 11. Mean high-sensitivity Troponin T at various time points and calculated Total Area-under-the-curve at 72 hours post-operatively

Time endpoint	Control (n=34) Mean SD hsTnT (ug/L)	RIPC (n=30) Mean SD hsTnT (ug/L)	Difference (95% CI)	P value
Pre-operatively	0.014 (0.019)	0.018 (0.016)	-0.0795 to 0.0715	0.841
6 hours	0.669 (0.338)	0.512 (0.275)	-0.0128 to 0.0048	0.364
12 hours	0.620 (0.324)	0.413 (0.244)	0.0017 to 0.3122	0.047
24 hours	0.498 (0.423)	0.314 (0.181)	0.0621 to 0.3518	0.058
48 hours	0.453 (0.498)	0.308 (0.196)	-0.0489 to 0.3338	0.140
72 hours	0.402 (0.421)	0.260 (0.118)	-0.0170 to 0.3010	0.071
Total AUC 72 hours	34.29 (25.26)	23.00 (20.23)	-1.145 to 23.723	0.074

Figure 5.2 Mean high-sensitivity Troponin T levels at various time points in patients undergoing cardiac surgery (mean±SEM)

Elective CABG Mean Troponin
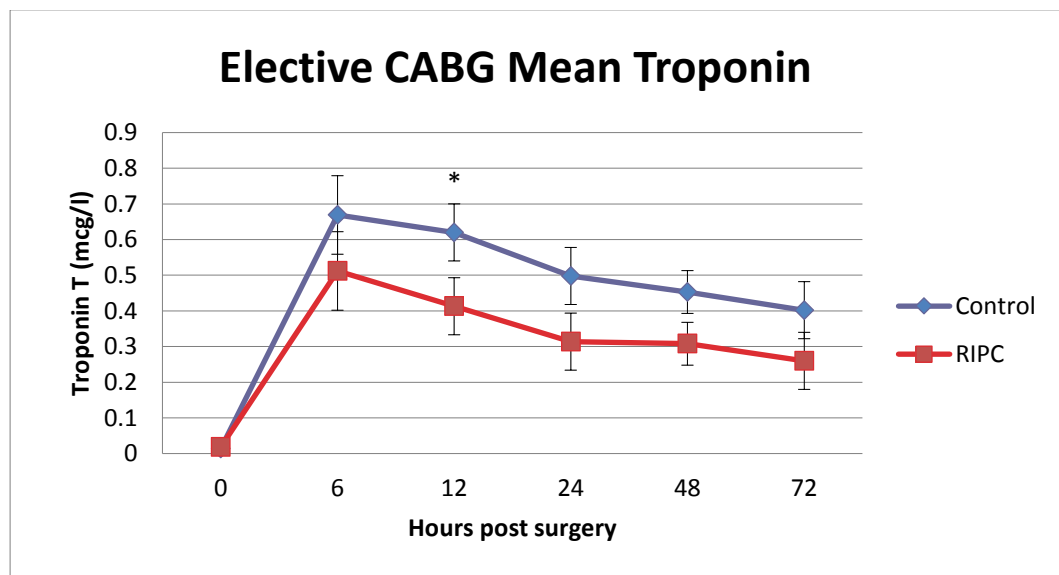
Figure 5.3. Calculated total AUC at 72 hours post-operatively in patients undergoing cardiac surgery and randomised to either sham or three cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

Elective CABG Mean Troponin AUC
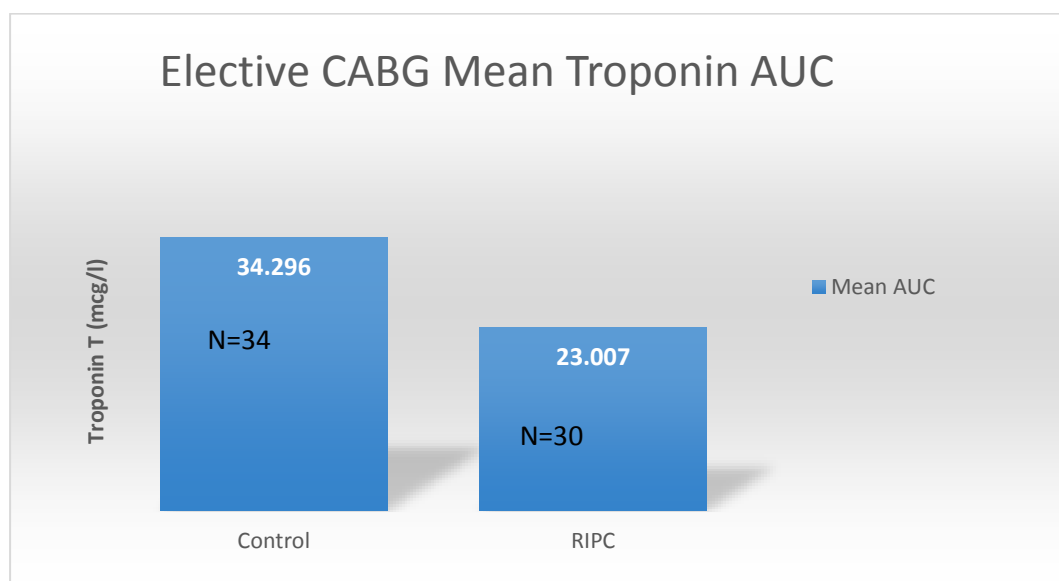
5.2 Secondary Outcomes

5.2.1 Post-operative Acute Kidney Injury (AKI)

The amount of acute kidney injury using the scoring system outlined above was similar between both groups and did not show statistical significance (p=0.7186)

Table 12. AKI scores in patients undergoing cardiac surgery and randomised to either sham or three cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

AKI score	Control n=34	RIPC n=30	P value
1	2	3	
2	1	0	
3	0	1	
Totals (%) and SD	3 (8.8%) 0.404	4 (13.3%) 0.584	p=0.7186

5.2.2 Post-operative atrial fibrillation

There were 5 cases out of 34 in the control group (14.7%) and 3 cases out of 30 in the RIPC group (10%). This was not statistically significant p=0.5722.

5.2.3 Length of ICU stay and hospital discharge

The average length of stay in the control group was 2.3 days and the average length of stay in the RIC group was 2.0 days. This did not achieve statistical significance p=0.662.

The total length of hospital stay was 7.5 days in the control group and 8.1 days in the RIC group. This did not achieve statistical significance p=0.535.

5.2.4 Inotrope score

The inotrope scores were calculated for 72 hours after coming off bypass in both groups. The control group had an inotrope score total of 8.1 and the RIPC group had a score of 11.8. This was not statistically significant p=0.278.

5.3 Post-hoc analysis of patients that received intravenous GTN

Table 13. Baseline characteristics in patients undergoing cardiac surgery that had received intravenous GTN and randomised to either sham or three cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

Patient Characteristic	Control (n=22)	RIPC (n=25)
Age (years)	65±9	71±8
Gender		
Male	18 (82%)	17 (68%)
Female	4 (18%)	8 (32%)
Ethnicity		
Caucasian	16 (73%)	14 (56%)
Asian	5 (23%)	5 (20%)
Turkic	1 (5%)	0 (0%)
Middle-Eastern	0 (0%)	2 (8%)
Body Mass Index	30.8±5.1	29.2±4.9
Smoking history		
Smoker	4 (18%)	4 (16%)
Ex-smoker	12 (55%)	12 (48%)
Non-smoker	6 (27%)	9 (36%)
Co-morbidities		
Diabetes Mellitus	3 (14%)	6 (24%)
Hypertension	16 (73%)	16 (64%)
Hypercholesterolaemia	15 (68%)	17 (77%)
Atrial Fibrillation	1 (5%)	1 (4%)
Previous MI	8 (36%)	3 (12%)
Previous CVA/TIA	1 (3%)	2 (8%)
Previous cardiac surgery	0	0
LVEF		
>50%	21 (95%)	23 (92%)
30-50%	1 (5%)	2 (8%)
<30%	0	0
NYHA	2.2±0.6	2.4±0.7
CCS	2.7±0.8	2.8±0.7
Family History of IHD	14 (64%)	20 (80%)
Euroscore	3.58±1.89	3.92±2.05
Bypass time (mins)	90.6±25.7	85.9±35.8
Cross-clamp time (mins)	58±25.9	66.3±25.2

Patient Characteristic	Control (n=22)	RIPC (n=25)
Drug history		
Aspirin	20 (91%)	20 (80%)
Clopidogrel	4 (18%)	5 (20%)
Prasugrel	0 (0%)	0 (0%)
Ticagrelor	1 (5%)	0 (0%)
Warfarin	1 (5%)	3 (12%)
Beta-blocker	18 (82%)	20 (80%)
Calcium channel blocker	6 (27%)	4 (18%)
Statin	20 (91%)	21 (84%)
Ace-i/A2RB	17 (77%)	18 (72%)
Long acting nitrate	3 (14%)	3 (12%)
Nicorandil	2 (9%)	0 (0%)
Insulin	2 (9%)	2 (8%)
Biguanide	5 (23%)	3 (12%)
Sulphonylurea	3 (13%)	6 (24%)
Thiazolidinediones	2 (9%)	1 (3%)
Diuretics	6 (27%)	4 (16%)
Cardioprotection		
Blood cardioplegia	20 (91%)	20 (80%)
Cross-clamp fibrillation	2 (19%)	5 (20%)
Operation		
CABG	17 (77%)	18 (72%)
AVR	3 (14%)	5 (20%)
MVR or repair	2 (9%)	2 (8%)
CABG+AVR	0 (0%)	0 (16%)
Grafts		
1	2 (6%)	1 (3%)
2	10 (29%)	10 (33%)
3	15 (44%)	15 (50%)
4	7 (21%)	4 (13%)
Anaesthetic agents		
Induction		
Rocuronium	28 (82%)	26 (87%)
Pancuronium	6 (18%)	4 (13%)
Propofol	32 (94%)	26 (87%)
Fentanyl	28 (82%)	27 (90%)
Etomidate	3 (9%)	4 (13%)
Midazolam	15 (44%)	15 (50%)
Maintenance		
Propofol	22 (100%)	25 (100%)
Isoflurane	20 (91%)	23 (92%)
Sevoflurane	2 (9%)	2 (8%)
Intra-operative Glyceryl trinitrate	22 (100%)	25 (100%)

Table 14. mean high-sensitivity Troponin T at various time points and calculated Total Area-under-the-curve at 72 hours post-operatively

Time endpoint	Control (n=22) Mean SD hsTnT (ug/L)	RIPC (n=25) Mean SD hsTnT (ug/L)	Difference (95% CI)	P value
Pre-operatively	0.015 (0.032)	0.012 (0.008)	-0.0111 to 0.0158	0.732
6 hours	0.589 (0.348)	0.419 (0.133)	0.0180 to 0.3210	0.028
12 hours	0.559 (0.320)	0.399 (0.258)	-0.0099 to 0.3299	0.064
24 hours	0.403 (0.204)	0.308 (0.188)	-0.0201 to 0.2101	0.103
48 hours	0.289 (0.575)	0.260 (0.229)	-0.0222 to 0.280	0.817
72 hours	0.220 (0.201)	0.209 (0.166)	-0.096 to 0.1100	0.830
Total AUC 72 hours	25.44 (18.34)	19.52 (12.24)	-3.1404 to 14.982	0.194

5.3.1 RIPC did not reduce periprocedural myocardial infarction in a retrospective analysis of patients undergoing cardiac surgery that had received intravenous GTN.

No single time point of troponin taken achieved statistical significance although they were all lower in the RIPC group. Although the RIPC group had a mean value of troponins that were lower and the 72 hour troponin AUC was also lower in value this did not achieve statistical significance between the two groups (p=0.194).

Figure 5.4. Mean high-sensitivity Troponin T levels at various time points in patients undergoing cardiac surgery (mean±SEM)
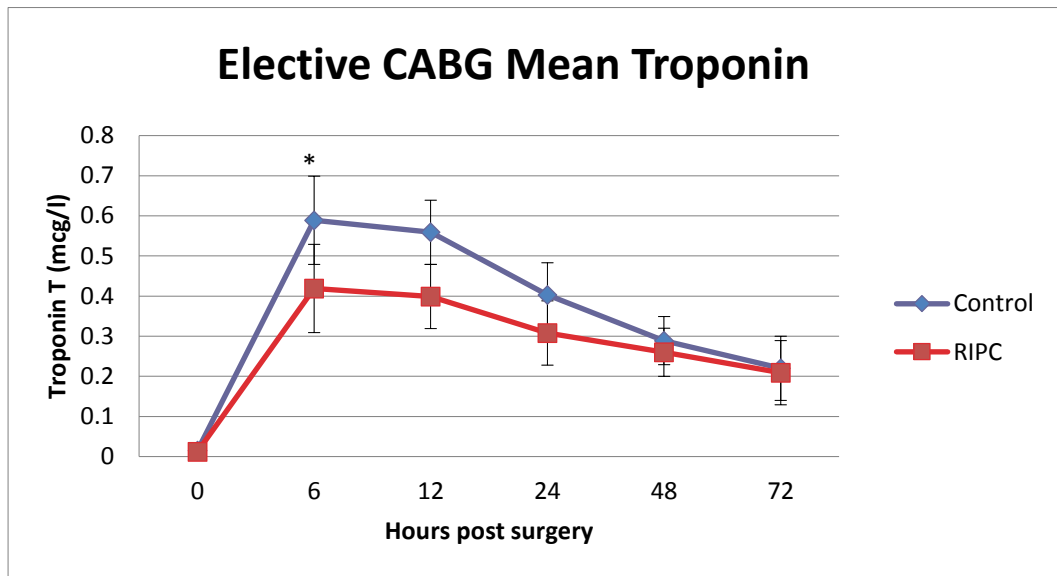

Figure 5.5. Calculated total AUC at 72 hours post-operatively in patients undergoing cardiac surgery and randomised to either sham or three cycles of 5 minutes of upper and lower limb ischaemia and reperfusion
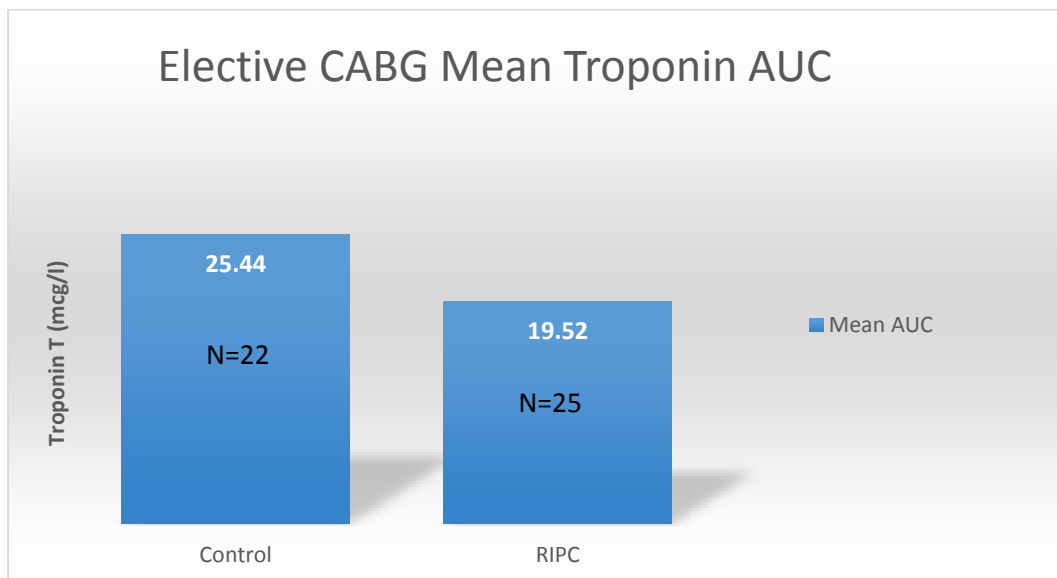
5.4 Secondary Outcomes

5.4.1 Post-operative Acute Kidney Injury (AKI)

The amount of acute kidney injury using the scoring system outlined above was similar between both groups and did not show statistical significance (p=0.7186)

Table 15. AKI scores in patients undergoing cardiac surgery that had received intravenous GTN and randomised to either sham or three cycles of 5 minutes of upper and lower limb ischaemia and reperfusion

AKI score	Control n=22	RIPC n=25	P value
1	1	2	
2	1	0	
3	0	0	
Totals (%) and SD	2 (5.9%) 0.236	2 (6.7%) 0.638	p=0.956

5.4.2 Post-operative atrial fibrillation in patients receiving intravenous GTN

There were 3 cases out of 22 in the control group (13.6%) and 2 cases out of 25 in the RIPC group (8%). This was not statistically significant p=0.522.

5.4.3 Length of ICU stay and hospital discharge in patients receiving intravenous GTN.

The average length of stay in the control group was 2.2 days and the average length of stay in the RIC group was 2.1 days. This did not achieve statistical significance p=0.862.

The total length of hospital stay was 7.3 days in the control group and 7.9 days in the RIC group. This did not achieve statistical significance p=0.431.

5.4.4 Inotrope score in patients receiving intravenous GTN

The inotrope scores were calculated for 72 hours after coming off bypass in both groups. The control group had an inotrope score total of 7.8 and the RIPC group had a score of 11.1. This was not statistically significant p=0.322.

5.5 Discussion

The results from this study show that although there was a trend towards RIPC resulting in cardioprotection in cardiac surgery in the presence of GTN there was no statistically significant reduction in perioperative myocardial injury as measured by hsTnT (ug/L) area-under-the-curve at 72 hours. There was also no significant reduction in the onset of new acute kidney injury, atrial fibrillation, inotrope score, intensive care stay and hospital stay. Similarly, there was also a trend to cardioprotection in my subgroup post-hoc analysis of those patients that had received intravenous GTN intra-operatively, but this did not achieve a statistically significant reduction in perioperative myocardial injury. Although the RIPC group had lower mean values of troponins in this study, this was not reflected in the total area-under-the-curve at 72 hours. The secondary endpoints of new acute kidney injury, atrial fibrillation, inotrope score, intensive care stay and hospital stay were not significant.

This study which has the greatest RIPC stimulus afforded to patients and consisted of three cycles and 2 limb ischaemia failed to show cardioprotection

in all-comers and the post-hoc analysis of those patients receiving IV GTN with study size being probably the greatest hindrance here. Another possibility is that at the time of my study there was competing recruitment from the multicentre ERICCA study and then later the ERIC GTN study and so achieving statistical significance was challenged by this as the ERICCA study preferentially recruited higher risk patients such as those that had poor left ventricular function and concomitant valve surgery. These groups of higher risk patients should exhibit a more profound cardioprotective effect from preconditioning which may also have contributed towards achieving statistical significance. Other problems affecting recruitment included the high number of recruited elective patients that were cancelled on the day of surgery, which totalled 48%, and this was due to bed pressure. In addition, a significant number of patients had to be excluded from my study due to incomplete troponin data sets. Difficulties mainly arose from ensuring that blood samples were taken and that to in a timely fashion. Other less frequent but important problems included blood forms going missing and samples being lost on the way to the laboratory. Another important consideration is that missing data values were imputed using software, but hopefully the effect of this would be minimal as data that had more than two missing time points were excluded. Similar problems were seen in the ERICCA trial, which had only 45.2% of patients with complete data sets.

Another factor is that it remains unknown what the exact optimal preconditioning stimulus is to achieve cardioprotection. Previous studies and meta-analyses have shown that three cycles of 5 minutes of upper arm

ischaemia provide cardioprotection (239-242). Subsequent to this a shortened RIPC protocol but more intense stimulus involving two cycles of upper and lower limb ischaemia from a study from our own institution has also shown to reduce perioperative myocardial injury. The optimal conditioning protocol for RIPC to elicit organ protection in human remains unknown. Only one laboratory study from Xin et al (243) found that 3–4, but not 1 to 2 cycles of 5-minutes ischaemia with 5 minutes (5minutes/5minutes) reperfusion could provide additive cardioprotection to local postconditioning, and that similar results were obtained in 4 cycles of 3-minutes/3-minutes or 1-minute/1-minute. How this applies clinically is still unknown.

Although there is a trend towards significance the most likely contributing factor as to why that this was not achieved was most likely due to the study being underpowered it is also possible that the effect of RIPC is simply not pronounced enough to produce a clinically significant result. My results therefore typify the spectrum of results seen in preconditioning clinical trials. The first clinical trial of RIPC was in the year 2000 with 8 patients undergoing cardiac surgery being randomised to forearm cuff ischaemia or control. This study showed an increase in lactate dehydrogenase in the RIPC group which was attributed to preconditioned cells being able to maintain anaerobic metabolism (244). Further studies with positive findings supporting RIPC followed in paediatric surgery showing benefit in troponin levels, inotrope support and airways resistance at 6 hours (245). Further studies followed from our own institution in 2007 and 2009 with studies from Hausenloy et al (246) and Venugopal et al (247) showing significant reductions in troponin in cardiac

surgery patients undergoing cross-clamp fibrillation and cold blood cardioplegia.

However, in 2010 there were conflicting results to previous studies produced by the Rahman et al. (248) from Birmingham. This was the first large, single-centre double-blind randomised controlled trial with 162 patients undergoing CABG randomised to receive either three cycles of 5 minutes of upper limb cuff inflation to 200mmHg with 5 minutes of intervening reperfusion or sham involving an automated blood pressure cuff placed on a dummy arm under a drape. This was to aid in the blinding of staff. This study showed no difference in troponin between the two groups or cardiac performance, inotrope requirement, echocardiographic function, arrhythmia protection, or renal and lung outcomes. Two possible explanations for the negative data from this study were that all patient received IV GTN and so perhaps the ischaemic stimulus was not great enough to overcome the attenuating effect of this intra-operative medication. Subsequent to this a study from our own institution suggested cardioprotection with a reduction in perioperative myocardial injury by 26% and a fall in acute kidney injury by 48% (249).

 The ERICCA study (249) from our own institution and RIPHeart study (250) followed on from these and in contrast once again they similarly failed to show benefit. These studies were being undertaken at the time of my research and the results only available and published sometime after I had finished recruitment. These were both huge multicentre, prospective, randomised controlled trials of cardiac surgery patients that had been placed on

cardiopulmonary bypass. These studies were both large-scale with 1612 and 1385 patients respectively. Patients were screened and only higher risk patients with Euroscores greater than 4.2 and 5 respectively were recruited in the hope that this would give adequate room to show benefit. Both studies failed to reduce major adverse cardiac and cerebral events with the ERICCA study looking at longer term follow up to 12 months. Similar findings contrasting previous studies with potentially worrisome findings with deleterious outcomes were also seen in other studies (251). The reasons for these negative results have been widely discussed and there are many potential explanations and confounders. Firstly, it is well described that diabetic and infarct remodelled hearts are less well cardioprotected by preconditioning. Up to 30% of patients in my study were diabetic which we know do not respond to preconditioning and many stable angina patients are believed to precondition the heart through repetitive episodes of ischaemia which ultimately result in left ventricular remodelling and cardioprotection. It has been well described that patients experiencing stable angina up to three months prior to acute myocardial infarction have a reduced infarct size (253) and so in this cohort of patients any additional effect from RIPC may be limited as maximal preconditioning has already occurred. As discussed earlier, in diabetic hearts the myocardium behaves differently in relation to IR injury and this is felt to be through differences in metabolism or intracellular signalling when compared to normal myocardium. Either any effect from RIPC is attenuated or to achieve cardioprotection a greater stimulus is needed in the diabetic heart.

Other factors that may further hinder possible RIPC cardioprotection include cardiopulmonary bypass itself through mediation of the inflammatory response, cardioplegic solutions and the cooling of patients which both work metabolically to protect. The administration of concomitant medications is also well known to interfere with RIPC especially anaesthetic agents. Propofol which has been used intra-operatively in approaching 100% of patients in my studies and the volatile anaesthetics sevoflurane and isoflurane are well recognised for their RIPC-attenuating effects. Intravenous GTN which is used ubiquitously by anaesthetists for its vasodilating effects to help lower blood pressure intra-operatively also plays a similar role with a previous study from our institution showing that patients receiving this drug had 39% lower perioperative myocardial infarction. This group of patients are cardioprotected by the nitrate and it is likely that RIPC is not able to overcome this beneficial effect. Even my increased stimulus of three multi-limb cycles failed to show benefit in this group.

Another recognised issue in my studies and other trials is the difficulty with blinding as anaesthetic staff and surgeons would be aware of whether the patients were undergoing intervention with RIPC or sham. Rahman et al managed to overcome this with an elaborate sham method which simulated the intervention as mentioned above. This should be considered in future studies.

This paradigm has been difficult to resolve although what is apparent is that there is an effect from RIPC but it cannot be consistently reproduced and

central to this is the precise ischaemic dose that needs to be delivered remains elusive. My two studies exhibit this with 2 multi-limb cycles cardioprotecting diabetic hearts yet 3 multi-limb cycles failing to show significant cardioprotection in a cohort that received IV GTN albeit showing a trend to reduced periprocedural myocardial infarction. Secondly, there are many confounding factors that may interfere with preconditioning pathways or may precondition the heart itself to an extent where there is no scope left for an additive effect from RIPC. The ERIC-GTN trial being conducted by our own institution hopes to answer some of these questions and elucidate whether IV GTN interferes with RIC protection. This trial is ongoing and will be discussed in the next chapter.

Chapter 6. Future considerations

As mentioned diabetes is a risk factor for coronary artery disease with several postulated mechanisms for the association including abnormal lipid metabolism, nitric oxide levels, platelet function, clotting and autonomic function. Not only is diabetes a risk factor for coronary disease but observational studies show worse outcomes in diabetics undergoing coronary artery bypass surgery (254-259). The increased mortality and morbidity among diabetics after surgery is felt to be due to the more aggressive nature of the atherosclerosis, the diffuse nature of the disease and its effects on the distal artery and on the microvasculature. Adverse outcomes are felt to be due to native vessel disease progression, graft failure, diabetic myocardium and the systemic morbidity associated wth the disease (260,261). Studies have also shown that generally CABG is the preferred mode of revascularisation in diabetics with multivessel disease and also those that have impaired left ventricular function. In patients with diabetes and multivessel disease, PCI with a current generation stent is associated with a lower risk of death and stroke in early follow-up but a higher rate of myocardial infarction and need for repeat revascularization in the long term when compared to CABG (262). The Future Revascularization Evaluation in patients with Diabetes Mellitus: Optimal Management of Multivessel disease (FREEDOM) trial also showed a long-term mortality benefit in CABG patients and it is on this basis that the US and Europe recommend CABG over PCI as a class I indication (263). In light of this new cardioprotective strategies which can help protect diabetic myocardium are needed to improve clinical outcomes in diabetic patients with coronary heart disease undergoing CABG.

Unfortunately, despite IPC showing potential as a strategy to improve outcomes in CABG it appears that in diabetics it faces an even greater challenge. However, with further clinical studies to help deepen our understanding of cardioprotection this defiant group of patients may eventually yield to the benefits of IPC if found.

Intraoperative GTN, which is a nitric oxide (NO) donor, is often used in cardiac surgery as already discussed. Preclinical studies have already well established the cardioprotective effects of NO but we remain uncertain about whether this translates to a benefit in cardiac surgery patients. As discussed, a post-hoc analysis from our institution showed that RIPC failed to show significant cardioprotection in a group of cardiac surgery patients that received IV GTN and when RIPC was compared to a control group. Further post-hoc analysis of those patients in the control group that were administered intra-operative IV GTN during cardiac surgery showed that total 72 hours AUC hsTnT was reduced by 39% in those control patients who had been administered intraoperative IV GTN compared with those control patients that had not (30.8±17.6ug/L vs 50.5±34.2ug/L, p<0.001). To help gain further understanding and potentially answer these questions a study from our own institution is now being conducted looking into this which is a prospective designed randomized clinical trial (ERIC-GTN) mentioned above. The ERIC-GTN trial is a single-site, double-blind, randomised, placebo-controlled study looking at cardiac surgery patients. This study hopes to recruit 260 patients randomised to one of four treatment groups following anaesthetic induction. The groups are (1) RIC alone, a RIC protocol comprising three 5-minute

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cycles of simultaneous upper-arm and thigh cuff inflation/deflation followed by an intravenous (IV) placebo infusion; (2) GTN alone, a simulated sham RIC protocol followed by an IV GTN infusion; (3) RIC + GTN, a RIC protocol followed by an IV GTN infusion; and (4) neither RIC nor GTN, a sham RIC protocol followed by IV placebo infusion. The ERIC-GTN trial should help determine whether intraoperative GTN therapy is cardioprotective during cardiac surgery and whether it affects RIC cardioprotection (264). The four arms should also be able to help answer whether RIC may be unable to confer additional cardioprotection over that provided by GTN itself or whether it was due to GTN reducing RIC cardioprotection.

Interestingly a very recent meta-analysis published in February 2017 of 5262 patients from all studies undergoing RIPC for cardiac surgery with cardiopulmonary bypass these inconsistent findings were all echoed with intervention failing to show reduced incidence of all-cause mortality, myocardial infarction, stroke, and lengths of ITU and hospital stay (265). Despite this, there was a strong trend towards reduction in AKI in patients that had not received propofol. In the subgroup of studies of patients who did not receive propofol which was not many as this is a very commonly used intra-operative drug to maintain anaesthesia, they observed that there were slightly more male patients (60.3%) and the mean or median age ranged from 57.0 to 70.6 years so very similar to baseline characteristics seen in studies from our institution. In this subgroup who did not receive propofol, 71 of 217 patients (32.7%) who underwent RIPC developed AKI compared with 103 of 217 patients (47.5%) treated with a sham procedure. The RR of RIPC versus

sham for AKI was 0.700 and significant statistically (95% CI, 0.527–0.930; p=0.014). Interestingly, studies of patients who had been given propofol showed that of 445 of 1874 patients (23.7%) who received RIPC developed AKI compared with 474 of 1901 (24.9%) who underwent a sham procedure which was not statistically significant (p=0.14). This meta-analysis strongly suggests that future cardiac studies should be performed in the absence of propofol.

Due to these inconsistent results in cardiac surgery and essentially stable patients, RIPC has been used to explore whether it can reduce cardiac and renal events in patients that are unstable. The rationale for this being that the magnitude of ischaemia in a stable patient is not significant enough for RIPC to show a beneficial effect and so current studies are looking at whether acute ST elevation myocardial infarction patients may be the correct substrate. The foundation for this has been laid down in the two studies that are ERIC-LYSIS (Effect of Remote Ischaemic Conditioning in STEMI patients treated by thromboLYSIS) (266) and CONDI (effect of RIC on clinical outcomes in STEMI patients undergoing pPCI) (267). ERIC-LYSIS randomised STEMI patients to receive thrombolysis with or without Remote ischaemic per-conditioning RIPerC and CONDI randomised STEMI patients to receive primary percutaneous coronary intervention (PPCI) with or without in-ambulance RIPerC. Eric lysis showed a median reduction in troponin by 32% troponin T (p=0.020) with RIPerC while CONDI showed a significant reduction in all-cause mortality (p=0.027) at follow up for a median for 3.8 years.

As a consequence of these two proof-of concept studies there are currently two studies underway with results due in 18 months which are the CONDI 2 (Effect of RIC on Clinical Outcomes in STEMI Patients undergoing pPCI) and ERIC-PPCI (Effect of Remote Ischaemic Conditioning in Clinical Outcomes in STEMI Patients Undergoing PPCI) (268). Although the initial ERIC-LYSIS study looked at thrombolysis patients this treatment for acute myocardial infarction has been superseded by PPCI worldwide. Together these studies will recruit around 4300 patients and are both multi-centre, multi-national, double-blinded RCTs. Primary endpoints of hospitalisation for heart failure or cardiovascular mortality at one year will give a definitive answer to this promising area for conditioning. Hopefully, this cheap and easy-to-apply intervention will finally be able to modulate reperfusion injury and affect infarct size so that there can be clinically significant reductions in cardiovascular outcomes.

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