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A Clinical Evaluation of Remote Ischaemic Preconditioning for Organ Protection

Volume 1

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Abbreviations

a/A – arterial alveolar oxygen tension

AAA – abdominal aortic aneurysm

ABPI – ankle brachial pressure index

ACC – American College of Cardiology

ACE – angiotensin converting enzyme

ACEI – angiotensin converting enzyme inhibitor

ACR – albumin creatinine ratio

ACS – acute coronary syndrome

AF – atrial fibrillation

AHA – American Heart Association

AKI – acute kidney injury

AUC – area under curve

BMI – body mass index

CABG – coronary artery bypass graft

CE-CT – contrast enhanced computed tomography

CHF – congestive heart failure

CI – confidence interval

CIN - contrast induced nephropathy

CK – creatinine kinase

CKD – chronic kidney disease

CK-MB – creatinine kinase MB isoenzyme

COPD – chronic obstructive pulmonary disease

CPB – cardiopulmonary bypass

CRP – c-reactive protein

CT – computed tomography

CVA – cerebrovascular accident

CVD – cardiovascular disease

CVS – cardiovascular surgery

cTnI – cardiac troponin I

cTnT – cardiac troponin T

cTnThs – high sensitivity cardiac troponin T

DALY – disability adjusted life year

DES – drug eluting stent

DM – diabetes mellitus

DVT – deep vein thrombosis

eGFR – estimated glomerular filtration rate

ECG – electrocardiogram

EVAR – endovascular aneurysm repair

HD- haemodialysis

HMG-CoA – hydroxylmethyglutaryl coenzyme A

hsTnI – high sensitivity troponin I

ICU – intensive care unit

INR – international normalised ratio

IPC – ischaemic preconditioning

IPostC – ischaemic postconditioning

Abbreviations

IQR – interquartile range
I-R – ischaemia reperfusion
L-FABP – liver-type fatty acid-binding protein
LOS – length of stay
LVEF – left ventricular ejection fraction
MDRD – Modification of Diet in Renal Disesase
M-H – Mantel–Haenszel random effects model
MI – myocardial infarction
MV – mechanical ventilation
NGAL – neutrophil gelatinase-associated lipocalin
NSQIP – National Surgical Quality Improvement Program
NT pro BNP – N-terminal pro b-type natriuretic peptide
NYHA – New York Heart Association
OR – odds ratio
PCI – percutaneous coronary intervention
PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PVD – peripheral vascular disease
RBP – retinal binding protein
RCRI – Revised Cardiac Risk Index
RIPC – remote ischaemic preconditioning
RIPerC – remote ischaemic preconditioning
RIPostC – remote ischaemic postconditioning
RR – risk ratio
SCr – serum creatinine
SD – standard deviation
SEM – standard error of the mean
SOFAc – sequential organ failure assessment score
STEMI – ST elevation myocardial infarction
URL – upper reference limit
VF – ventricular fibrillation

Abstract

Periprocedural complications are an issue for patients with cardiovascular disease. Remote ischaemic preconditioning (RIPC) may offer periprocedural organ protection. Although the proof of concept data underpinning RIPC are encouraging, there are few data regarding clinical endpoints.

The first component of this thesis comprises a literature review that explores the history and current status of RIPC with a focus on cardiovascular interventions. This is followed by two systematic reviews and meta-analyses. The first review examined the role of RIPC in percutaneous coronary intervention (PCI) and found a significant reduction in periprocedural myocardial infarction (MI) rates with RIPC, although the number of included patients was small. The second review examined the role of RIPC in the prevention of major clinical complications following cardiovascular surgery and found no significant effect although MI rates were reduced almost by half with RIPC. Heterogeneity of the studies and small individual sample sizes were likely to have rendered the meta-analysis underpowered. The remainder of the thesis comprises two pilot clinical trials. The first examined RIPC as a renoprotective strategy following contrast-enhanced computed tomography scanning. It demonstrated feasibility and it found that RIPC may have reduced kidney injury in those with impaired renal function but it found no evidence for a benefit across the whole cohort. The second trial examined RIPC in the setting of major vascular surgery in three centres. It found no significant effect on clinical outcomes or on troponin leakage following surgery although it demonstrated feasibility.

The meta-analyses generated new data on the pooled effects of RIPC, thereby encouraging further clinical studies. The trials demonstrated feasibility and yielded data to guide future studies.

RIPC represents an attractive and cheap risk reduction tool. If convincing data on patient important outcomes can be generated, it will become widespread.

Declaration

I wish to submit this thesis in order to be considered for the degree of PhD in Surgery (MD) through the School of Medicine at National University of Ireland Galway. I declare that that the work described in this thesis was driven by my personal efforts and that individual aspects of the thesis were enhanced through collaboration with other researchers. No aspect of the work has been attributed to any other research degree. My contribution to each section of the thesis can be delineated clearly. I have endeavoured to ensure that copyright law has not been breached and that all sources of data unpinning the thesis have been cited and acknowledged within the text. I took care to ensure that my work was original.

The first section of the thesis is a literature review. An article based upon this was published in *International Journal of Surgery.* In this publication, I had four co-authors. Regarding the published article, I contributed to design, literature search, data extraction, writing, revision and approval of the final draft. I received help from collaborators: Dr. Mary Clarke Moloney contributed to design, writing, revision and approval of the final draft, Mr. Seamus M McHugh contributed to writing, revision and approval of the final draft, Professor Pierce A Grace contributed to writing, revision and approval of the final draft and Professor Stewart R Walsh contributed to design, writing, revision and approval of final draft.

The second section of the thesis is a systematic review and meta-analysis on remote ischaemic preconditioning (RIPC) in percutaneous coronary intervention (PCI). An article based upon this was published in *International Journal of Cardiology: Metabolic and Endocrine.* In this article I had six co-authors. Regarding the published article, I contributed to design, literature search, data extraction, analysis including statistical analysis, writing, revision and approval of the final draft. I received help from collaborators: Dr. Patrick Carroll contributed to the literature search and data extraction, Dr. Mary Clarke Moloney contributed to design, writing and approval of the final draft, Mr. Tjun Tang contributed to writing and approval of the final draft, Professor Pierce Grace contributed to design, writing and approval of the final draft, Professor Thomas Kiernan contributed to design, writing and approval of the final draft and Professor Stewart Walsh contributed to design, writing and approval of the final draft.

Declaration

The third section of the thesis is a systematic review and meta-analysis on RIPC in cardiovascular surgery. This was published in *International Journal of Cardiology*. Regarding the published article, I had thirty co-authors. This article represents the largest meta-analysis on RIPC to date and it included a large amount of published and unpublished data. In order to complete this study, Professor Stewart Walsh and I established a collaborative group that included principal investigators from trials on RIPC and cardiovascular surgery. All principal investigators from trials on RIPC in cardiovascular surgery were invited to join the group, provide unpublished data and contribute to design, revision and approval of the final draft. The group was termed "The Remote Preconditioning Trialists' Group" and it finally comprised thirty one members. I contributed to design, literature search, data extraction, statistical analysis, interpretation, writing, revision and approval of the final draft. I received help from the thirty co-authors. Many RIPC researchers agreed to provide unpublished data. These contributors are ME Gaunt, S Chen, S Tehrani, DJ Hausenloy, DM Yellon, RS Kramer, RF Zimmerman, VV Lomivorotov, VA Shmyrev, DN Ponomarev, IA Rahman, JG Mascaro, RS Bonser, Y Jeon, DM Hong, R Wagner, M Thielmann, G Heusch, K Zacharowski, P Meybohm, B Bein, TY Tang. These co-authors also contributed to revising and approving the final draft. Mr. WA Khan, Mr. Michael Wong, Dr. Mary M Clarke Moloney, Professor Pierce Grace, Professor JC Coffey and Professor Colum Dunne contributed to design, revision and approval of the final draft. Professor Stewart Walsh contributed to design, literature search, data extraction, analysis, interpretation, writing, revision and approval of the final draft.

The fourth section of the thesis is a pilot clinical trial that investigated RIPC in the setting of contrast-enhanced computed tomography (CE-CT) scanning. This was published in *Clinical and Investigative Medicine*. Regarding this article, I had eight co-authors. I was involved in design, data collection, analysis and interpretation, writing, revision and approval of the final draft. Mr. Iain Feeley contributed to design and data collection, Dr. Cillian Keogh contributed to data collection, Dr. Timothy Scanlon contributed to design, Dr. Philip Hodnett contributed to design, Professor Austin Stack contributed to design, Dr. Mary Clarke Moloney contributed to design and analysis, Professor Peter Whittaker contributed to writing, design, analysis and interpretation and Professor Stewart Walsh contributed to design, analysis and interpretation. All of the authors of this publication approved the final draft.

Declaration

The fifth section of the thesis is a pilot clinical trial that investigated RIPC in the setting of major vascular surgery. This was published in *Vascular and Endovascular Surgery.* I had twenty one co-authors. I contributed to design, data collection, analysis, interpretation and writing. I received help from collaborators. Ms. Emily Boyle contributed to data collection, Mr. Damian McCartan contributed to data collection, Mr. Michael Burke contributed to data collection, Mr. Mekki Medani contributed to data collection, Dr. John Ferguson contributed to statistical analysis, Dr. John Newell contributed to statistical analysis, Professor Martin O'Donnell contributed to design, Ms. Catriona Canning contributed to data collection, Mr. Morgan McMonagle contributed to data collection, Mr. Joseph Dowdall contributed to data collection, Professor Simon Cross contributed to data collection, Ms. Siobahn O' Daly contributed to design, Mr. Brian Manning contributed to data collection, Mr. Gerard Fulton contributed to data collection, Mr. Eamon Kavanagh contributed to data collection, Mr. Paul Burke contributed to data collection, Professor Pierce Grace contributed to data collection, Dr. Hatim Yagoub contributed to data collection and analysis, Professor Thomas Kiernan contributed to data collection and analysis, Dr. Mary Clarke Moloney contributed to design, data collection and analysis and Professor Stewart Walsh contributed to design, data collection, analysis, interpretation and critical revision. All authors approved the final draft.

Acknowledgements

Data collection for the randomised controlled trials was performed University Hospital Limerick, University Hospital Waterford and Cork University Hospital. Data collection was performed in conjunction with the Graduate Entry Medical School at University of Limerick. I wish to thank all of the clinical team members at University Hospital Limerick, University Hospital Waterford and Cork University Hospital. Particular words of thanks are due to the nursing staff in the surgical wards, the high dependency units and the intensive care units. I also wish to thank the operating theatre staff, especially theatre nursing teams and anaesthesia team members. Analysis, interpretation and writing were performed at the School of Medicine at National University of Ireland Galway following a transfer to National University of Ireland Galway. I wish to thank the staff at the School of Medicine at National University of Ireland Galway for facilitating the transfer of my studies and for the support that I received.

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Chapter 1: Introduction

1.1: Health and life expectancy improvements, the epidemiologic transition and the increasing global burden of cardiovascular disease

Life expectancy at birth is one of the most useful and comparable measures of global health status and dramatic gains have been realised within the last century – in 1950 the worldwide average life expectancy at birth was 46 years and in 1998 it was 66 years [1]. The increase in life expectancy has been consistent across multiple geographic areas (although historical data from much of Africa and Asia are lacking). Table 1.1 is modified from the World Health 1999 report [1] and it summarises the life expectancy improvements that occurred across some selected geographical areas.

Table 1.1: Life expectancy at birth in selected countries around 1910 and 1988. Modified from the World Health Report 1999 [1].

Country	Around 1910		1998	
	Males	Females	Males	Females
Australia	56	60	75	81
Chile	29	33	72	78
England and Wales	49	53	75	80
Italy	46	47	75	81
Japan	43	43	77	83
New Zealand	60	63	74	80
Norway	56	59	75	81
Sweden	57	59	76	81
United States	49	53	73	80

Health profiles of societies have historically been related to levels of economic and social development. In poor countries, infectious diseases and nutritional deficiencies are the predominant causes of death whereas in more developed and industrialised countries non-communicable and degenerative diseases take precedence. The shift in disease burden that occurs with societal advancement has been termed "the epidemiologic transition" – it was first described in 1971 by Omran. [2]. The concept of the epidemiologic transition allows a tangible framework that facilitates increased understanding of regional and temporal health inequalities. At any point, different countries and sometimes different areas within countries are at different stages of the transition [3]. The structure of the epidemiologic transition can be applied to varying disease categories (for example, adult chronic diseases taking precedence from childhood infectious diseases) and within specific disease categories (for example, atherosclerotic heart disease taking precedence from nutritional cardiomyopathies and rheumatic heart disease). Applying the epidemiological transition to cardiovascular disease (CVD) allows us to appreciate how the magnitude of the CVD burden is set to increase further in line with increasing worldwide industrialisation (table 1.2). As India, China and Sub-Saharan Africa develop and industrialise, epidemiologists predict increases in CVD. Figure 1.1 depicts differing causes of death in Chile at points in the last century. The higher prevalence of diseases from the latter part of the epidemiologic transition can be appreciated from figure 1.1.

Table 1.2: The epidemiological transition as it relates to cardiovascular disease.
Modified from Yusuf *et al.* [3].

Stage	Deaths due to CVD	Predominant CVD and risk factors	Regional examples
Age of pestilence and famine	5-10%	Rheumatic heart disease, infections, nutritional cardiomypoathies	Rural India, Sub-Saharan Africa
Age of receding pandemics	10-35%	Additionally hypertensive heart disease and haemorrhagic stroke	China
Age of degenerative and man-made diseases	35-65%	All stokes, ischaemic heart disease in younger people, obesity, diabetes	Urban India, Aboriginal communities, some Eastern European countries
Age of delayed degenerative diseases	<50%	Stroke and ischaemic heart disease at older age	Western Europe, North America, Australia, New Zealand
Age of health regression and social upheaval	35-55%	Re-emergence of disease from earlier in the spectrum and in younger people	Russia

Figure 1.1: Distribution of deaths by cause for two cohorts from Chile, 1909 and 1999. Modified from the World Health Report 1999 [1].
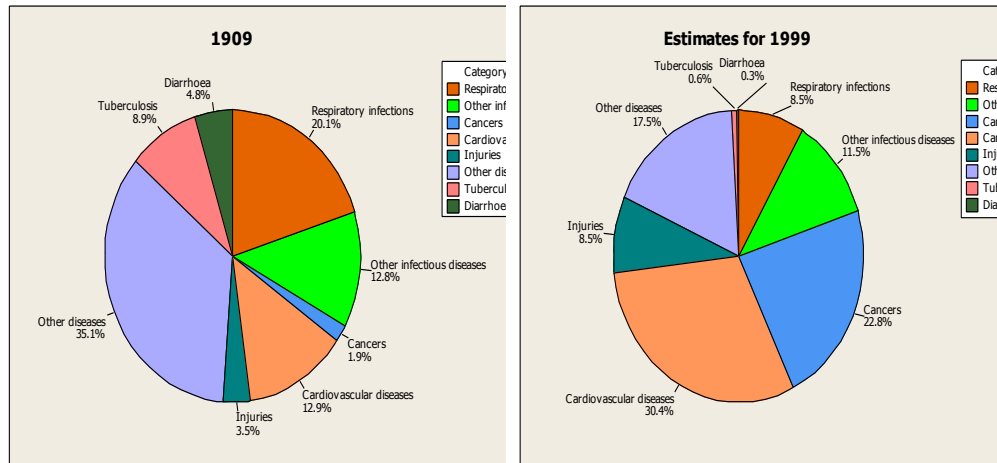
In line with what one might expect based on the degree of recent worldwide development, several studies have provided objective evidence of the expanding burden of CVD. The Global Burden of Disease Study [4] and the World Health Report 1999 [1] estimate that cardiovascular disorders contributed in some way to about 31.7 million deaths in 1998. This corresponds to about 50 % of global mortality and 43% of the global burden of disease – the lower figure for global disease burden in comparison to mortality reflects the fact that CVDs tend to affect middle aged and older people. In terms of the direct contribution, the World Health Report 1999 [1] estimates that CVD was directly related to 30.9% of 1998 worldwide mortality and 10.3% of worldwide disability adjusted life year (DALY) loss. Again, the lower figure for DALY loss compared with mortality reflects older age at disease onset.

Another phenomenon that has emerged in line with the epidemiologic transition is the dominance of low and middle income countries in global CVD – an estimated 85% of the 1998 CVD burden was felt in poorer countries [1, 3]. The evolution of CVD from being a disease of the poor to one of the wealthy has been documented before in studies that have examined socioeconomic status as a health risk factor [5, 6].

There are several potential reasons for the expanding CVD burden in poorer countries [3]. Multiple factors resulting from urbanisation and industrialisation are likely to contribute to an increased prevalence of atherosclerosis in largely younger

populations. Such factors are higher rates of diabetes, smoking, dyslipidaemia and hypertension. The rapid changes in diet, activity and obesity that affect developing countries have been termed "the nutrition transition" [7]. A conservative estimate based on static risk factors levels predicted that cardiovascular mortality in poor and middle income countries would more than double between 1990 and 2020 [3, 4] – it is likely that by 2020 the increase will have been much greater.

In the developed world, cardiovascular disease mortality is finally declining. Data on heart disease and stroke mortality from the American Heart Association (AHA), published in the AHA Statistical Update, confirmed that CVD mortality rates in the USA have declined by 32.7% between 1999 and 2009 [8]. Prior versions of the AHA statistical update also had this finding [9, 10]. However, the disease burden remains persistently high – one in three US deaths in 2009 (one every forty seconds) was attributable broadly to CVD and one in six was attributable directly to coronary disease [8]. One in every nine US death certificates in 2009 mentioned heart failure [8]. The size of the burden in developed countries is further reflected by an examination of the costs of CVD management: in 2009, the direct and indirect costs of CVD in the US totalled $312.6 billion. As a comparison, the 2008 US costs relating to neoplastic disorders were $228 billion.

1.2 The worldwide burden of perioperative cardiovascular disease

In 2002, the World Bank estimated that surgically treatable conditions comprised 11% of the worldwide disease burden and contributed 164 million disability adjusted life years (DALYs) [11]. A conservative estimate from further international research by the Harvard School of Public Health suggested that 234 million operating theatre procedures were performed across the globe in 2004 [12]. These researchers also reported that there were vast differences in numbers of procedures performed in low, middle and high health expenditure countries. Low expenditure countries (<$100 health expenditure per person), comprising 34.8% of the population, undertook only 8.1million procedures (3.5%) during the year. In contrast, middle ($401-1000 per person annually) and high expenditure (>$1000 per person annually) comprised 30.2% of the world's population and conducted 172.3 million procedures (73.6% of the worldwide total) in 2004. The authors of the report concluded that reduced access to surgery in poor countries highlights a large and unaddressed problem. When the epidemiologic transition is considered in this context, it is likely that the future will see vast increases in numbers of operations across the world, and especially in the current low expenditure countries.

Surgery is associated with major cardiovascular complications – it has been estimated that about 1 million people have such complications per year [13, 14]. Studies have found markedly different cardiac complication rates depending on the risk profile of the included patients – when patients with cardiac disease or who are at risk of cardiac disease are evaluated, a 3.9% incidence of a major cardiac complications (a composite of cardiac death, myocardial infarction (MI) or cardiac arrest) was reported [13]. This contrasts with a rate of 1.4% in unselected patients undergoing elective surgery [15]. It is likely that this study on unselected patients has most external validity and is thus most reflective of global perioperative cardiac risk [13]. As global numbers of operations increase over coming years, evidence suggests that the incidence of cardiac complications will increase accordingly.

The significance of cardiac complications on such a scale is visible when one considers the consequences. The in-hospital mortality rate for patients who have perioperative MI can be as high as 25% [13]. Those that survive a perioperative MI are at increased risk of another MI within 6 months, 1 year and 2 years [16]. Death rates are also higher [16]. Those who have perioperative cardiac arrest have a

hospital mortality rate of 65% [17] and those who survive perioperative cardiac arrest are more likely to die of cardiac causes in 5 years following the surgery [18].

In addition to the negative impact that cardiac complications have on hard clinical outcomes, there is also sizeable economic fallout with such complications. Convincing evidence for this is available from a prospective single centre study from Massachusetts that evaluated cardiac complications, non-cardiac complications and length of hospital stay in a cohort of 3790 patients who underwent non-cardiac surgery [19]. There was a cardiac complication rate of 2% across a variety of procedures, thus emphasising the study's external validity. There was an average increase in length of stay of 11 days in those with a cardiac complication and the presence of a cardiac complication increased the likelihood of developing a non-cardiac complication, even after adjustment for baseline clinical factors (OR 6.4%; 95%CI 3.9-10.6). There was an average increase of 15 days hospital stay for those who had both cardiac and non-cardiac complication.

Thus, with increasing numbers of surgical procedures being performed on a population with rising cardiac disease prevalence, it can be seen that perioperative cardiac complications are an enormous healthcare burden in terms of both patient important outcomes and economics. This burden will continue to swell in coming years and addressing it represents a major challenge.

The next section discusses the pathophysiology of such complications and the ensuing section examines some strategies that have been used to reduce perioperative cardiac risk: risk assessment tools, pharmacological cardioprotection, prophylactic revascularisation and cardioprotection by conditioning.

1.3: Pathophysiology of perioperative cardiac complications

Perioperative MI is the most common major cardiac complication and other important complications are cardiac death, nonfatal cardiac arrest, heart failure and arrhythmias [13]. Although the pathophysiology of each of these conditions is well understood outside of the perioperative setting, much controversy exists regarding pathophysiology in the perioperative setting [13].

Coronary plaque rupture is the central step in most MIs outside of the surgical setting [20] but its role in perioperative MI is less clear [13]. The other proposed mechanism for perioperative MI is via haemodynamic disturbance with supply-demand mismatch [13]. These two mechanisms correspond to type 1 and type 2 MIs respectively according to the American College of Cardiology (ACC) definitions of acute myocardial infarction [21]. Proposed underlying mechanisms for perioperative plaque rupture (in type 1 MI) are increased physiological and emotional stress, increased procoagulants and mechanical stresses from tachycardia and hypertension [22]. The proposed mechanisms underlying type 2 perioperative MI are tachycardia (the most important factor), hypotension, hypovolaemia, hypoxaemia, hypervolaemia, hypertension and any other factors that may alter oxygen supply or increase oxygen demand [22]. The distinction is important to make as the treatments of perioperative MI may differ depending on aetiology. ST elevation MI requires medical management with consideration for coronary revascularisation whereas ST depression due to tachycardia with or without hypotension benefits from rate control, volume optimisation and blood pressure control and is less likely to benefit from coronary intervention [22].

Overall, the evidence on the incidence of perioperative type 1 and 2 MI is weak and often conflicting. Two retrospective studies have shown that most patients who have a fatal perioperative MI have underlying severe coronary disease [23, 24]. One of these studies [23] (26 fatal perioperative MIs) found that 46% of MIs were associated with plaque rupture and in only 35% of cases intracoronary thrombus was found. The other study (42 fatal perioperative MIs) [24] found that the severity of pre-existing stenoses was not predictive of the location of the infarct and that 55% of fatal perioperative MIs were associated with unstable plaques with rupture, and 45% were associated with plaque haemorrhage [24]. The suggestion from these retrospective studies is that plaque rupture and flow limiting stenoses both have central roles. In contrast to these reports is a study in vascular patients where most nonfatal perioperative MIs occurred in coronary arteries without significant stenoses [25], suggesting that plaque rupture was the main cause. However, those

who had cardiac death in that study were more likely to have a critical coronary stenosis [25]. A high quality prospective study that examined coronary angiograms in three groups of patients (perioperative MI, spontaneous MI and stable coronary artery disease patients) found that plaque rupture was implicated in 50% of perioperative MIs. Current expert opinion is that type 2 MI resulting from supply demand mismatch is more common than type 1 MI (plaque rupture) [22] – this stems from the observation that ST segment elevation MIs are rare post operatively whereas ST depression associated with tachycardia, hypotension, anaemia, hypovolaemia is more common [22]. It has not been possible to determine the exact incidence of either type of MI based on current evidence and this represents a challenge for future researchers [22].

In relation to perioperative cardiac death, it is thought that about 2/3 of cases are due to MI and 1/3 are due to heart failure and arrhythmias [13]. However, these figures were extrapolated from a relatively heterogenous group of studies without uniform diagnostic criteria or assessment of reliability [13] – therefore there is a need for further high quality studies in this area.

The evidence on the causes of perioperative cardiac arrest is also conflicting – perhaps this is unsurprising given its relatively low incidence, the multitude of potential causes and the level of difficulty when attributing causative factors. One study that retrospectively reviewed 223 cases of perioperative cardiac arrest found that cardiac causes predominated (44%), with contributions from bleeding (35%) and other causes (21%) (e.g. pulmonary embolism, anaesthesia/airway problems etc.) [17]. In contrast, a retrospective study on perioperative cardiac arrests found that only 3/168 (1.8%) arrests were due to perioperative MI. Most were due to sepsis and multiorgan failure (42/168; 25%), trauma (31/168; 18.5%) and major bleeding at operation (24/168; 14.3%). Further prospective high quality studies are needed to accurately establish the important causative factors.

1.4: An overview of strategies for the prevention of perioperative cardiac complications

Several strategies have been employed as methods of reducing perioperative cardiac complications: risk assessment with optimisation, preoperative coronary revascularisation, pharmacological cardioprotection and myocardial conditioning. In this section, each of these strategies will be discussed in turn.

1.5: Cardiac risk assessment as a strategy to reduce perioperative cardiac complications

The use of risk estimation prior to surgery is probably the oldest strategy – the first perioperative cardiac risk tool (the Goldman Risk Index) was proposed in 1977. The goal of the approach of risk assessment is the timely identification of high risk patients who might benefit from further investigations with risk reducing interventions in certain cases [13]. Though there are no published data confirming that this strategy offers benefits in hard endpoints [13], the principle is intuitive and simple and it is therefore appealing. In cases of elective surgery, high risk patients may potentially benefit from postponement of surgery, medical optimisation or other interventions [13]. Furthermore, patients are better able to make informed choices when they can weigh up competing risks – an elderly man who is considering joint replacement surgery for a moderately disabling condition might decide against surgery if he thought the risk was too high.

In 2007 and 2009, the American College of Cardiology and the American Heart Association published guidelines on perioperative cardiac evaluation that highlighted major and minor risk factors for cardiac complications [26, 27]. These predictive factors are presented in table 1.3. There are also several published risk scoring systems – examples include the Lee, Goldman, Larsen, Gilbert, Kumar and Detsky indices [13] and the American College of Surgeons' National Surgical Quality Improvement Program (NSQIP) risk tool [28]. The best validated tool is the Lee index which is also known as the Revised Cardiac Risk Index (RCRI) [13, 15]. Its components are summarised in table 1.4. Its value was shown in a 2010 meta-analysis – it discriminated moderately well between high and low risk patients with a pooled area under the curve (AUC) of 0.75. Some limitations were also identified – it performed less well at determining high and low risk in vascular surgery (pooled AUC = 0.64) and at predicting mortality (median AUC 0.62), however its role is firmly established [29]. The NSQIP risk assessment tool [28] is newer and was developed based on a large (>250 hospitals) north American prospectively maintained database of 211,410 patients who underwent surgery in 2007 and it was validated on 257,385 patients who had surgery the following year. It performed slightly better than the RCRI tool – the 2007 NSQIP AUC was 0.884 and the 2008 NSQIP AUC was 0.874 with corresponding AUC of 0.747 when the RCRI tool was applied to the database [28]. There is a simple online calculator for determining individual NSQIP risk [30].

Procedure specific risk is also worth considering when determining perioperative risk. Emergency procedures were shown to be associated with a higher frequency of cardiac complications [31, 32]. The ACC/AHA 2009 perioperative evaluation guidelines classified procedure specific risk into high, intermediate and low risk categories – these are summarised in table 1.5.

Table 1.3: Summary of the major and minor clinical risk factors for cardiac complications from the 2009 ACC/AHA perioperative evaluation guidelines [27].

Major predictors that require intensive management, delays or cancellation
Unstable coronary syndromes, including severe angina, unstable angina or recent MI
Decompensated, worsening or new heart failure or NYHA Class 4 heart failure
Significant arrhythmias
Severe valvular disease including severe aortic or mitral stenosis
Other factors that necessitate careful assessment
History of ischaemic heart disease
History of cerebrovascular disease
History of heart failure
Diabetes
Renal impairment

ACC – American College of Cardiology; AHA – American Heart Association; MI – myocardial infarction; NYHA – New York Heart Association.

Table 1.4: Summary of the components of the Revised Cardiac Risk Index [15]

Independent predictor	
High risk surgery, for example vascular or open abdominal or thoracic procedures	
History of ischaemic heart disease	
History of heart failure	
History of cerebrovascular disease	
Diabetes with the need for insulin treatment	
Renal impairment with creatinine > 177 µmol/l	
Rate of complications of cardiac death, nonfatal MI and nonfatal cardiac arrest according to number of predictors [13]	
No risk factor	0.4% (95%CI:0.1-0.8)
One risk factor	1.0% (95% CI: 0.5-1.4)
Two risk factor	2.4% (95% CI: 1.3-3.5)
Three or more risk factor	5.4% (95% CI: 2.8-7.9)

MI – myocardial infarction.

Table 1.5: Summary of procedure specific cardiac risk according to the 2009
ACC/AHA perioperative evaluation guidelines .

High risk (cardiac complication rate of >5%)
Aortic and major vascular surgery
Peripheral arterial surgery
Intermediate risk (cardiac complication rate of between 1-5%)
Carotid endarterectomy
Head and neck surgery
Intraperitoneal and intrathoracic surgery
Orthopaedic surgery
Prostate surgery
Low risk (cardiac complication rate of less than 1%)
Ambulatory surgery
Endoscopic procedures
Superficial procedures
Cataract surgery
Breast surgery

ACC – American College of Cardiology; AHA – American Heart Association.

Overall, it can be seen that accurate risk assessment has the potential to improve
outcomes by allowing focused intensive management for those at high risk.
However, hard evidence is lacking and even high risk patients were shown to not
benefit from prophylactic coronary revascularisation [33]. Nonetheless, as most
operations are performed electively [34], it is likely that risk assessment has at least
some benefit. Future challenges in the area of risk assessment are to improve upon
the current discriminative abilities of the RCRI and NSQIP tools and to perform
further high quality validation studies of these tools. It would be difficult to provide
hard evidence for benefits in clinical outcomes with risk assessment tools –
nonetheless this is another potential goal for future researchers.

1.6: Preoperative coronary revascularisation as a strategy to reduce perioperative cardiac complications

Preoperative coronary revascularisation has been used as a perioperative cardiac risk reduction strategy but is only recommended occasionally at present [35, 36] as it has been shown to be ineffective in randomised trials [33, 37-39]. This is likely to be because surgical revascularisation is an operation with high morbidity and mortality and because percutaneous revascularisation cannot treat all atheromatous lesions [36]. Furthermore, percutaneous revascularisation often necessitates dual antiplatelet therapy which increases the risk of perioperative bleeding [36]. Finally, considering the pathophysiology of perioperative MI, we cannot accurately predict which plaques are vulnerable – rupture does not always occur at the tightest stenosis [40].

In 2004, McFalls *et al.* reported on a randomised controlled trial on prophylactic revascularisation on 510 patients who were scheduled for major vascular surgery [37]. Those in the revascularisation group had either surgical (59%) or percutaneous (41%) revascularisation and 12% of the revascularisation group patients had an MI within 30 days postoperatively compared with 14% in the control group (p=0.37). There was no mortality difference after 2.7 years of follow up (22% mortality in the revascularisation group and 23% in the control group; p=0.92). A further analysis of this study using the revised cardiac risk index found that revascularisation did not improve outcomes in any risk group [33]. The DECREASE-V Pilot Study tested a similar hypothesis in high risk vascular surgery patients (n=101) who had evidence of stress induced ischaemia on cardiac stress testing [39]. The primary outcome was a composite of all-cause death or MI at 30 days and one year. No difference was shown at 30 days (21/49 in revascularisation developed the composite outcome versus 17/52 in control group; p=0.3) or at one year (24/49 in revascularisation group versus 23/52 in control group; p=0.48). Long term follow up at 2.8 years found no survival difference (61% intervention vs 64% control; hazard ratio 1.18, 95%CI 0.63-2.19, p=0.61) [38]. Although the DECREASE-V trial was not adequately powered to answer the question about high risk patients, the result obtained was consistent with the previously published literature [35].

1.7: Pharmacology as a strategy for reducing perioperative cardiac risk

The aims of pharmacological cardioprotection are the stabilisation of coronary plaques and the improvement of the balance between myocardial oxygen supply and demand [36]. As the perioperative withdrawal of aspirin, statins and beta blockers has been associated with a rebound effect, current best practice advises continuation of such therapy or discontinuation for the shortest period possible [36, 41].

Aspirin is widely used for its analgesic, anti-inflammatory and antiplatelet properties. In patients with established cardiovascular disease, it dramatically reduces the risk of vascular death, stroke and MI (relative risk reduction 22%) [42]. Though it irreversibly inhibits platelet function and is associated with an increased rate of surgical bleeding, evidence suggests that the severity of bleeding and the resulting complications are not increased when aspirin is continued perioperatively [43, 44]. A study found that patients who stopped taking aspirin had a twofold increase in the short term risk of death when they were compared to patients who did not stop taking aspirin and nonusers of aspirin, indicating that a strong rebound thrombotic effect is associated with its discontinuation [41]. Specifically in relation to surgery, evidence suggests that the premature withdrawal of antiplatelet therapy prior to surgery leads to a 10% increased rate of vascular events [45]. If there has been recent percutaneous coronary intervention (PCI) and stenting, stopping antiplatelets may cause a 5-30% mortality rate, depending on the timeframe since stent deployment [36]. Notably, there is no evidence of an important role for aspirin in cardiovascular disease primary prevention [46]. However, perioperative prophylactic aspirin therapy has been shown to prevent venous thromboembolism in patients who are undergoing hip surgery [47] although aspirin is not the preferred agent for this purpose [48]. Although in general evidence suggests strongly that aspirin should be continued perioperatively, there are some particular operations in which the bleeding risk associated with aspirin continuation outweighs the benefits of its continuation [41]. In relation to transurethral resection of the prostate (TURP) the evidence is conflicting because some studies report no significant differences in bleeding and others report significantly higher bleeding rates and transfusion rates with aspirin continuation [41]. One large retrospective series found no increased rate of cardiovascular complications when aspirin was discontinued [49]. No guidelines specific to urological surgery exist although guidelines are needed – a recent cross-sectional survey of United Kingdom

urologists demonstrated that practice is highly variable [50]. For most urological procedures, about 50% of urologists discontinue aspririn and about 50% advise its continuation. Other operations where aspirin should be discontinued are those in which bleeding may occur within a confined space with disastrous consequences. Examples of this include ophthalmological surgery, cranial surgery and spinal surgery [41].

Beta blockers are another class of drug that may offer cardioprotection. They work by partially or fully blocking the effect of beta adrenoceptor activation and are helpful in the management of hypertension, arrhythmias, heart failure and myocardial infaction [36]. Several small studies showed a benefit when beta blockers were used for perioperative cardioprotection and this led to widespread interest [36]. However, a large multicentre trial (POISE) that aimed to evaluate the cardioprotective role of extended release metoprolol in non-cardiac surgery contradicted the earlier reports [14]. POISE found that although myocardial infarction rates were reduced with perioperative metoprolol treatment (4.2% with metoprolol versus 5.7% with control, p = 0.0017), stroke rates were increased (1% versus 0.5%, p=0.0053) and mortality was increased (3.1% versus 2.3%, p=0.0317). The primary endpoint (cardiovascular death, nonfatal MI or nonfatal cardiac arrest) favoured metoprolol (5.8% versus 6.9%, p=0.0399) although this was largely driven by the reduced MI rate and did not include strokes. A meta-analysis that combined POISE with other studies found similar conclusions relating to adverse events [51]. The conclusion from POISE and these smaller trials is that initiating perioperative beta blockade is not beneficial to the majority of patients. However there is evidence that selective perioperative beta blockade may be beneficial in high risk groups and this strategy is recommended in the 2009 American College of Cardiology/American Heart Association update on the use of perioperative beta blockade [27] – several small randomised studies support this conclusion [52-58]. There is a level B recommendation for initiation of perioperative beta blockade in vascular surgery patients with coronary artery disease. There is a corresponding level C recommendation for vascular surgery patients who are deemed to be at increased risk based on the presence of two or more risk factors. There is a level B recommendation for patients with two or more risk factors who are undergoing intermediate risk surgery. Other important conclusions from the report are that there is uncertain benefit to beta blockade initiation in patients with a single risk factor and that initiating beta blockade without careful dose titration is of uncertain benefit. There is a recommendation

that beta blockers should be continued perioperatively in patients who take them routinely.

Statins are lipid lowering drugs that work via inhibition of the enzyme hydroxymethyglutaryl coenzyme A (HMG-CoA) reductase. They improve serum lipid profile and have several other systemic vascular effects, including beneficial effects on endothelial function, vascular inflammation and plaque stability [27]. They have an established role in secondary prevention of cardiovascular disease [27] and the concept of perioperative statin use has also been explored. Most of the studies evaluating perioperative statin use are observational in design and most show a plausible reduction in cardiac events with statin use [27]. There are current AHA/ACC level A recommendations that patients with established cardiovascular disease and patients with abnormal lipid profiles in the absence of established cardiovascular disease should be on statin therapy unless there is a problem with intolerance [59]. Furthermore, there is a level B recommendation that statin use should be continued perioperatively if patients are already taking statins [27]. Thus, it seems that for high risk patients, the question of perioperative statin use has been answered. However, there is uncertainty regarding the optimal statin strategy in low risk patients and this question can probably only be definitively answered in a large randomised controlled trial.

1.8: An overview of the concept of reperfusion injury and myocardial conditioning

In 1972 it was shown for the first time that reperfusion of ischaemic myocardium could reduce infarct size and thereby allow salvage of functional myocardium [60]. Since then, reperfusion has become a cornerstone of the management of MI [61], initially via thrombolysis and subsequently via percutaneous coronary intervention (PCI). Following the discovery of the benefit of reperfusion, it was recognised that reperfusion can cause injury – this was termed "reperfusion injury" or "ischaemia-reperfusion injury" [61]. Several factors combine to generate ischaemia-reperfusion injury – these include reactive oxygen species [61, 62], intracellular calcium overload [61, 63], mitochondrial dysfunction [61, 63], intracellular proteolysis [61]and excessive contractile activity [61, 63], platelet activation [64], complement activation [62] and pro-apoptic signalling [65]. Ischaemia-reperfusion injury can manifest as myocardial stunning, reperfusion arrhythmias, lethal reperfusion injury [63] and the "no reflow" phenomenon at angiography following PCI [62, 66].

Although the underlying mechanisms are well understood, there are no established treatments for ischaemia-reperfusion injury [67]. There are several potential reasons for the lack of clinically effective therapy despite our knowledge of the pathopyhsiology: multiple simultaneous mechanism must be targeted, there may be comorbidities and there may be difficulties with timing of treatments [68]. At present, the only established way to attenuate ischaemia-reperfusion injury is to allow gentle reperfusion [61]. However, over recent years there has been much interest in myocardial conditioning techniques and these phenomena hold much promise in relation to organ protection from ischaemia-reperfusion injury [61].

The myocardial conditioning techniques can be subdivided into direct and remote subtypes (figure 1.2 and table 1.6). The direct subtypes comprise ischaemic preconditioning (IPC) and ischaemic postconditioning (IPostC).The remote subtypes comprise remote ischaemic preconditioning (RIPC), remote ischaemic perconditioning (RIPerC) and remote ischaemic postconditioning (RIPostC). Direct conditioning involves the concept that brief episodes of ischaemic and reperfusion of an organ can confer organ-specific resistance to ischaemic events that follow (as in the case of IPC) or have already taken place (as in the case of IPostC). Remote conditioning involves the concept that brief episodes of ischaemia and reperfusion of a tissue can confer resistance to ischaemia of distant organs and tissues. This resistance can be conferred before a major ischaemic event (termed RIPC), during

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a major ischaemic event (termed RIPerC) or after a major ischaemic event (termed RIPostC). Figure 1.2 illustrates these concepts and table 1.6 provides concise definitions of each of the conditioning subtypes. It is noteworthy that direct ischaemic perconditioning does not exist – this would require brief episodes of ischaemic-reperfusion of an organ at the time that the organ was undergoing sustained ischaemia, which is not possible. It is also worth mentioning that any organ may theoretically derive protection from conditioning although cardioprotection is the most frequent goal of the conditioning techniques.

The remainder of the introductory section of this thesis discusses aspects of the conditioning techniques with a particular focus on RIPC and its potential applications. The following chapters of the thesis deal with questions that are specifically related to clinical applications of RIPC.

Figure 1.2: Diagram highlighting the nature of ischaemic conditioning and remote ischaemic conditioning and the importance of the timing of the brief ischaemia-reperfusion stimulus. Preconditioning requires that an ischaemia-reperfusion stimulus occurs before the index ischaemic event. Perconditioning requires an ischaemia-reperfusion stimulus at the time of the index ischaemic event. Postconditioning requires an ischaemia-reperfusion stimulus after the index ischaemic event.
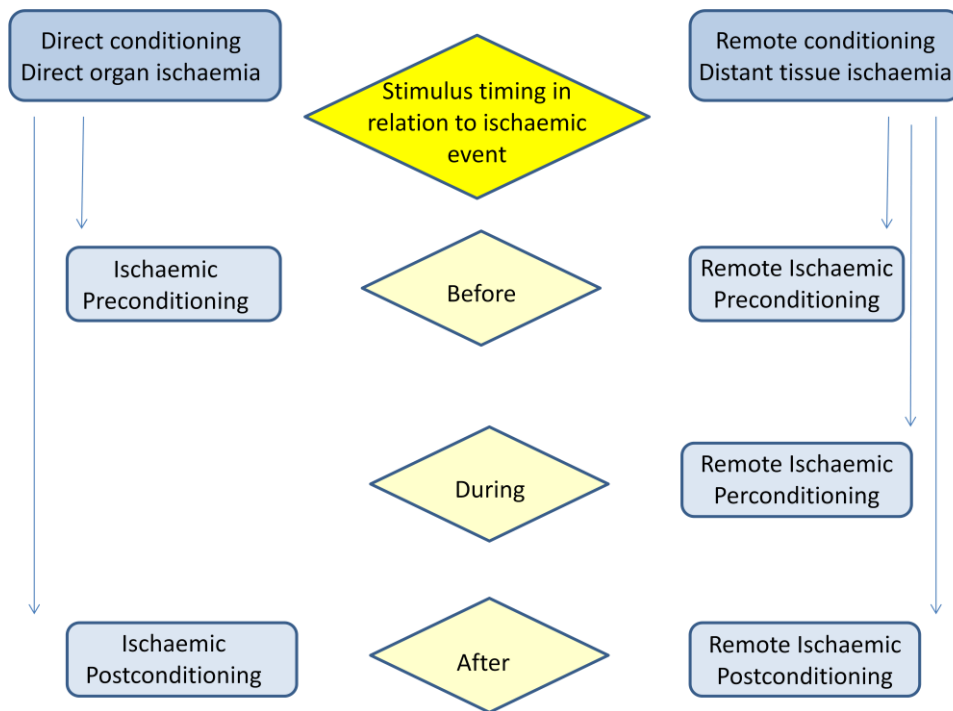
Table 1.6: Summary of the conditioning strategies

Type of conditioning strategy	Definition
Direct conditioning	
Ischaemic preconditioning	A phenomenon whereby brief periods of ischaemic and reperfusion in an organ or tissue can give the organ or tissue resistence to subsequent more sustained ischaemia
Ischaemic postconditioning	A phenomenon whereby brief periods of ischaemic and reperfusion in an organ or tissue can give that organ or tissue resistence to damage that is due to a prior ischaemic event
Remote conditioning	
Remote ischaemic preconditioning	A phenomenon whereby brief periods of ischaemic and reperfusion in an organ or tissue can give distant tissues or organs resistance to subsequent more sustained ischaemic events
Remote ischaemic perconditioning	A phenomenon whereby brief periods of ischaemia and reperfusion in an organ or tissue can give distant tissues or organs resistance to an ischaemic event that is occurring at the same time
Remote ischaemic postconditioning	A phenomenon whereby brief periods of ischaemic and reperfusion in an organ or tissue can give distant tissues or organs resistance to a prior ischaemic event

1.9: Origins of ischaemic preconditioning and remote ischaemic preconditioning

The counterintuitive idea of IPC was first demonstrated in 1986 by a research team in North Carolina [69]. Murry *et al.* performed two experiments on dogs, aiming to evaluate the effect of episodic coronary ischaemia on subsequent myocardial infarct size. The first of the experiments involved one group of seven dogs who underwent IPC induced by four five minute cycles of circumflex coronary artery occlusion with reperfusion between each cycle. This IPC stimulus was then followed by a sustained 40 minute circumflex occlusion and then myocardial infarct sizes were measured. The control group for this experiment was a group of 9 dogs who underwent a 40 minute circumflex occlusion only and then had infarct size measured. Murry *et al.* found that in the IPC group infarct size was reduced to 25% of that seen in the control group (p<0.001), which indicated that IPC conferred cardioprotection. There was also a preservation of high energy phosphates in the preconditioned myocardium. The analysis took into account the major baseline infarct size determinants of collateral blood flow and anatomic area at risk. In the second experiment, there were similar groups of dogs that received IPC via a similar protocol (9 dogs) and no IPC (7 dogs). These dogs then underwent a sustained 3 hour coronary occlusion followed by subsequent reperfusion for 3 days. At that point infarct size was measured. There was no difference in infarct size between the groups, indicating that the cardioprotection induced by IPC was not absolute and could be surmounted by a sustained ischaemic insult. The study and its conclusions were revolutionary.

A 1982 study by Geft *et al*. is also noteworthy because it addressed a similar topic and produced conclusions that both contradicted and affirmed Murry's later work [70]. Geft *et al.* performed an experiment where they subjected dogs to a series of 5 minute, 10 minute or 15 minute coronary occlusions. Occlusions were separated by 15 minute periods of reperfusion and creatinine kinase levels and infarct sizes were the outcomes. They found that some dogs developed myocardial injury and infarction after repeated ischaemic episodes and that these injuries would not have occurred if the number of ischaemic cycles had been fewer. This suggested that myocardial ischaemia may have a cumulative effect provided that the myocardium is not allowed to recover fully between ischaemic episodes. However, on the contrary, it also suggested that brief episodes of ischaemia and reperfusion conferred cardioprotection to some dogs – some dogs were able to survive

cumulative ischaemic times of over 3 hours without infarction. In this way the study highlighted interindividual variability.

Following the seminal work by Murry *et al*., other studies also showed that episodic circumflex ischaemia could induce cardioprotection in the circumflex distribution [71-73] and that left anterior descending coronary artery (LAD) occlusion could induce cardioprotection in the LAD distribution [74, 75]. A breakthrough came in 1993 when Przyklenk *et al*. showed that episodic circumflex ischaemia could induce a cardioprotective phenotype in the LAD distribution [76], thus giving rise to the concept of "preconditioning at a distance". This was not strictly RIPC as the stimulus was applied to the organ that was deriving protection; nonetheless it paved the way for further research. Although the mechanism was unknown at the time Przyklenk *et al*. postulated that the remote effect could have been initiated via a factor that was released as a result of the circumflex ischaemia.

Consequent studies found that animal skeletal muscle [77, 78], renal [79] and mesenteric [80] ischaemia had attenuating effects on induced myocardial infarct sizes and that tourniquet induced leg ischaemia reduced reperfusion arrhythmias [81]. Thus RIPC was born.

Regarding the clinical utility of IPC, it must be highlighted that IPC requires direct interference with an organ's blood supply. Thus in order to induce cardioprotection there must be episodic coronary ischaemia with the attendant risks. Similarly, the application of a remote preconditioning stimulus via limb arterial clamping, renal ischaemia or mesenteric ischaemia requires an invasive procedure with inherent risks. The potential of RIPC was obvious when Kharbanda *et al*. published a seminal manuscript detailing how they were able to show that tourniquet induced human forearm ischaemia could induce a protective phenotype and that tourniquet induced pig leg ischaemic could reduce pig myocardial infarct size [78]. As tourniquet induced limb ischaemia has an attractive risk profile and is simple to achieve, this method of inducing a preconditioning phenotype has dominated clinical research since. There have been multiple small trials in humans undergoing major cardiovascular surgery and PCI using cuff induced limb ischaemia as the preconditioning stimulus. Meta-analyses of these trials have consistently shown biochemical evidence of reduced myocardial injury although firm data on clinical outcomes are lacking [82-89]. Regarding IPC, there have been several proof of concept trials in human cardiothoracic surgery and meta-analysis has found evidence of benefits in terms of reductions in arrhythmia rates, inotrope

requirements and intensive care unit length of stay [90]. Evidence is lacking regarding the effects of IPC on harder clinical outcomes such as MI and mortality rates. Unfortunately, as IPC involves directly interfering with coronary blood flow (giving rise to ischaemia and the possibility of causing plaque rupture), the potential for widespread use is limited – its only practical role is likely to remain in elective cardiac surgery or elective percutaneous coronary intervention (PCI).

1.10: Underlying mechanisms of ischaemic preconditioning and remote preconditioning

Despite compelling evidence of benefits in animal models and in humans, the exact mechanisms underlying cardioprotection via IPC and RIPC remain unclear. Several theories exist although no theory is fully accepted – it is likely that no single mechanism is uniquely responsible but rather that several complementary pathways exist [91]. Proposed mechanistic components are initiation via a trigger at the site of the ischaemic stimulus, communication between the remote site and the myocardium and lastly the induction of cardioprotection at the heart or or other target organ (figure 1.3) [92]. Evidence suggests that IPC, RIPC and the postconditioning techniques share common mechanistic components [91, 92].

Proposed remote trigger molecules include adenosine, bradykinin, opioids, endocannabinoids and others while the final effect is thought to culminate in a strong cardioprotective and antiapoptotic response in the heart [91, 92]. Evidence implicates closure of the mitochondrial permeability transition pore (mPTP) in this final antiapoptotic step – opening of the mPTP during myocardial reperfusion is thought to initiate programmed cell death via cellular energy depletion [93]. Pharmacologically preventing mPTP opening has been shown to dramatically reduce infarct size in animal studies [93] and in humans mPTP opening inhibition with ciclosporin was shown to reduce infarct size in a small study [94].

Neural, humoral and systemic communication theories have been suggested [91] (figure 1.3). The neural hypothesis proposes that remote neurotransmitter release activates a neural link to the myocardium. Support for this comes from studies that found that the ganglion blocker hexamethonium attenuated the preconditioning effect [80, 95]. Another study found that trimetaphan, another autonomic ganglion blocker, also attenuated organ protection [96]. The humoral hypothesis suggests that circulating cardioprotective factors are released during remote site reperfusion and subsequently act on the myocardium – studies have shown that a preconditioning effect can be transferred via a blood transfusion to a non-preconditioned animal [97-99]. The final theory proposes that preconditioning can induce a systemic anti-inflammatory response with alteration of gene expression [91].

Figure 1.3: Diagram illustrating the theoretical mechanistic components implicated in ischaemic preconditioning and remote ischaemic preconditioning.
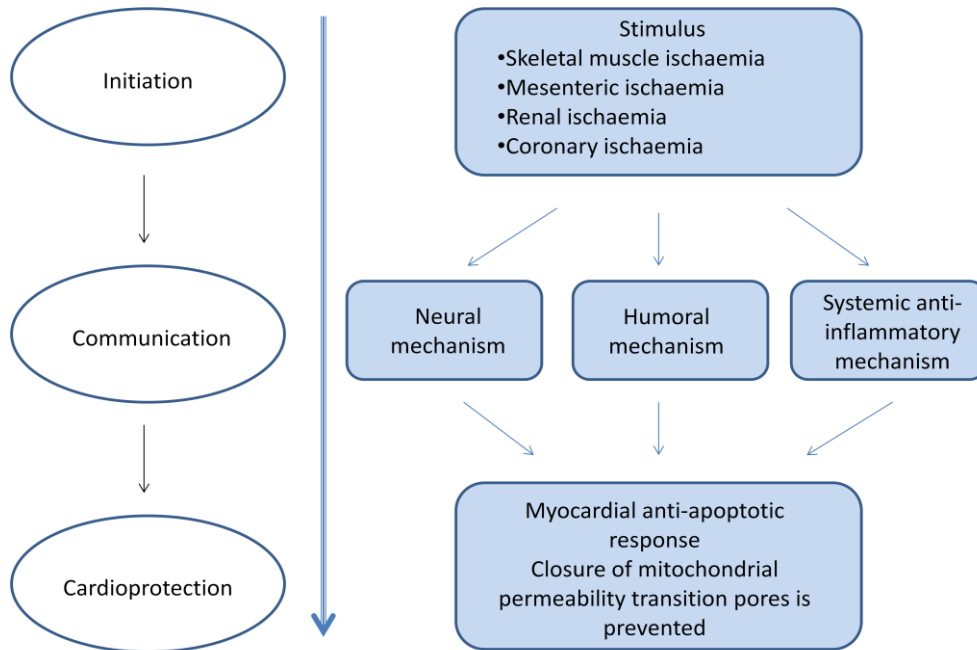
1.11: "Windows of protection" with ischaemic conditioning and remote ischaemic preconditioning

Three windows of IPC-induced cardioprotection have been identified [61]. These are distinguished from one another depending on the interval between the preconditioning stimulus and the injurious ischaemic episode. The first and most powerful window of cardioprotection occurs within the first two to three hours of the stimulus [61, 100] – therefore in order to maximise cardioprotective potential in clinical practice it is essential that the stimulus should take place no more than two hours before the likely ischaemic event. At 24 – 72 hours following the initial stimulus, there is a second and more sustained window of cardioprotection [61, 100]. The magnitude of cardioprotection that is present during this period is less than the magnitude of cardioprotection in the initial window although the second window involves resistance to myocardial stunning and myocardial infarction [61, 100]. The third distinct window has been identified at about six hours following coronary microembolisation [61, 101]. The mechanisms underlying each of the cardioprotective windows are different [61]. Initial protection is due to the activation of existing signalling molecules whereas the delayed forms of protection are thought to be due to increased expression of signalling molecules [100, 101].

RIPC in humans generates similar windows of protection. The first window of protection seems to have an immediate onset and lasts for about 2 to 3 hours. Evidence for this comes from the consistent findings of reduced myocardial injury (as assessed by cardiac enzyme release) following cardiac surgery when patients receive RIPC induced by limb ischaemia prior to surgery [102]. Similarly, a large study on acute ST-elevation MI patients found reduced infarct size when patients were preconditioned while in transit before undergoing emergency PCI [103]. Loukogeorgakis *et al.* performed a series of experiments on healthy volunteers in order to determine the time course and mechanisms of RIPC [96]. They caused forearm endothelial ischaemia-reperfusion injury through twenty minute periods of cuff-induced ischaemia and they assessed the ability of pre-injury RIPC to attenuate the ischaemia-reperfusion injury. RIPC was applied at varying time points –immediately before the injurious stimulus and at 4 hours, 24 hours and 48 hours before. Endothelial injury was assessed through ultrasound measurement of flow-mediated dilation in the brachial artery. RIPC attenuated endothelial injury when it was applied immediately before and at 24 and 48 hours before the ischaemia-reperfusion stimulus but not when it was applied 4 hours before the injury, providing strong evidence for distinct windows of protection.

1.12: Clinical applications of ischaemic preconditioning as a cardioprotective strategy in humans

Unfortunately, IPC involves directly interfering with coronary blood flow (giving rise to ischaemia and the possibility of causing plaque rupture). Furthermore, it can only be applied by invasive means at the time of a coronary intervention. Therefore the potential for widespread use is limited. Nonetheless, there have been several proof of concept trials of IPC in human cardiothoracic surgery and results from these have recently been pooled in a meta-analysis [90]. Twenty two studies (933 patients) were included in the review. The primary outcome was perioperative mortality and secondary outcomes were numbers of patients with postoperative ventricular arrhythmias requiring inotropic support, numbers of patients sustaining a myocardial infarction, numbers of patients sustaining a cerebrovascular accident (CVA) and duration of postoperative critical care unit stay. The study found that IPC conferred a significant reduction in ventricular arrhythmias (pooled odds ratio 0.11, 95% CI 0.04 – 0.29, p=0.001), inotrope requirements (pooled odds ratio 0.34, 95%CI 0.17 – 0.68, p=0.002) and critical care unit stay (weighted mean difference -3 hours, 95%CI -4.6 hours – -4.5 hours, p=0.0001). Evidence is lacking regarding the effects of IPC on harder clinical outcomes such as MI and mortality rates.

There have several proof of concept studies involving direct preconditioning on human myocardium although there is a lack of randomised controlled trials that used myocardial injury as a primary outcome. Surrogate evidence for a direct preconditioning effect comes from many studies that found that preinfarction angina reduces the size of subsequent MIs [104, 105]. Studies have examined the use of balloon induced coronary occlusion as a remote stimulus for the induction of renoprotection at the time of PCI. This has been explored in an observational study that found that balloon coronary occlusions induced renoprotection [106].

Despite exciting results in relation to both PCI and cardiovascular surgery the invasive nature of IPC combined with the counter-intuitive requirement for direct interference with coronary blood flow limit its attractiveness to clinicians. In contrast, RIPC represents an attractive cardioprotective strategy as it has a favourable risk profile and can be easily and cheaply applied.

1.13: Clinical applications of remote ischaemic preconditioning as a cardioprotective strategy in humans

At this stage, much of the experimentation in humans has been exploratory and has aimed to demonstrate "proof of concept" rather than practicality. In general, numbers of included patients have been small and there has been a focus on biochemical outcomes rather than patient important outcomes. There is uncertainty relating to many methodological issues and as further studies emerge it is likely that it will be possible to determine the optimal approaches, thereby allowing future multi-centre evaluation with a focus on clinical outcomes. With further research and increasing sample sizes, it is likely that a measure of the true effect of RIPC on clinical outcomes will emerge.

Clinical trials have evaluated RIPC in coronary artery bypass graft (CABG) surgery [88, 107-121], cardiac valvular surgery [109, 121-123] and congenital cardiac defect surgery in children [124-129]. Cardiac surgery has seen considerably more proof of concept studies than any other type of intervention; it is likely that several factors account for this. Firstly, it is probably a reflection of the fact that most cardiac surgery is performed electively and is therefore suitable for RIPC. Secondly, research on cardioprotection and initial IPC research were dominated by cardiothoracic surgery and this translated to interest in RIPC. A final reason for the dominance of cardiac surgery in RIPC research is that induced myocardial ischaemia is often an integral component of cardiac surgery and this makes cardioprotective strategies attractive.

Most of the cardiac surgery studies used cardiac injury biomarkers as primary outcomes and pooling these results via meta-analysis has confirmed a statistically significant benefit. This biomarker reduction is both consistent and plausible. As there is mounting evidence for the prognostic significance of isolated cardiac biomarker elevations [130], it is likely that RIPC may indeed have the ability to alter short and long term prognosis for patients undergoing cardiac surgery. However, at present the evidence for benefits in patient important outcomes is not convincing. Two meta-analyses that pooled cardiac surgery and PCI found statistical evidence of reduced MI rates with RIPC [85, 89] but another review that excluded the PCI studies did not find this significance [83]. It is likely that a more refined measure of the true effect of RIPC will emerge as international studies with larger sample sizes are completed. Interestingly, evidence has also emerged suggesting that RIPC may reduce the incidence of acute kidney injury in patients

undergoing cardiac surgery [113] – this further underlines the biological plausibility of achieving organ protection with RIPC.

Several studies have evaluated RIPC in emergency [103, 131] and elective PCI [132-136]. The results are variable – some studies [103, 131, 133, 135, 136] found RIPC to be beneficial in terms of myocardial injury biomarker levels but other studies did not find such a benefit [132, 134]. Surprisingly, one trial found RIPC to be associated with cardiac enzyme elevation although this trial had the limitation of a small sample size [134]. Two studies on RIPC in elective coronary angiography found that RIPC was able to reduce the incidence of contrast induced acute kidney injury [137, 138] – these studies did not aim to demonstrate cardioprotection. In relation to emergency PCI, a major challenge is timely administration of the RIPC stimulus in the setting of acute MI – one of the studies initiated RIPC during transit [103] and another initiated RIPC shortly before PCI commenced [131]. The trials have shown that this difficulty can be overcome and that benefits are likely to exist. However, as mentioned there is no consistently proven benefit in patient important outcomes for RIPC in the PCI group on its own and the challenge is to evaluate clinical outcomes in an adequately powered study. Long term clinical outcomes follow up data is available from the CRISP Stent trial [139] – interestingly, RIPC was associated with a lower major adverse cardiac and cerebral event rate at 6 years.

Trials have also evaluated RIPC in the setting of major vascular surgery: open abdominal aortic aneurysm (AAA) repair [140-142], endovascular aneurysm repair (EVAR) [143] and carotid endarterectomy [144]. There were two AAA repair trials where iliac artery cross-clamping served as the preconditioning stimulus [140, 141]. The larger of the two trials [140] (n=82) found a significant reduction in levels of cardiac troponin I, myocardial infarction rates and renal impairment rates with the RIPC intervention. The other AAA study (n=40) focused on biochemical markers of renal injury and could not confirm a benefit with RIPC [141]. 4 patients in the RIPC arm of this trial developed acute lower limb ischaemia requiring operative intervention – this has raised concerns about the suitability of iliac cross-clamping as the preconditioning stimulus. The final AAA RIPC study used the upper limb for the stimulus [142], considering the negative experiences with iliac cross clamping in the prior two studies. In this study (n=62), RIPC reduced markers of pulmonary and intestinal injury and it also reduced markers of systemic inflammatory response but there was no difference in clinical outcomes. The study on EVAR procedures found biochemical evidence of reduced renal injury with

RIPC but no difference in renal impairment or clinical outcomes [143]. Inflation of a cuff around the thigh served as the stimulus and there were no lower limb ischaemic events, which may suggest that non-invasive lower limb arterial occlusion is better than arterial clamping. RIPC in carotid endarterectomy was also evaluated using a thigh tourniquet (without lower limb adverse events) but without a demonstrable effect of RIPC on cardiac or neurological outcomes [144].

Overall, the trials in major cardiovascular surgery and PCI have had promising results. The feasibility of using RIPC in these groups has been established and the remaining challenge is to apply RIPC in larger studies with a focus on patient important outcomes. It appears as though upper limb tourniquet induced ischaemia might be the best approach for these patients given the likelihood of co-existing chronic occlusive lower limb arterial disease and the possibility for acute ischaemia when arteries are occluded via clamping.

1.14: Ischaemic preconditioning and remote ischaemic preconditioning as protective strategies in non-cardiovascular interventions

Animal models have confirmed a neuroprotective role for RIPC and IPC – studies found that preconditioning rodents with leg ischaemia reduced stroke size following middle cerebral artery occlusion [145, 146] and that direct rodent brain ischaemia was also protective [147, 148]. However, there are few studies on such neuroprotection in humans. A non-significant benefit in preservation of saccadic latency (a measure of neurologic function) was shown with RIPC in the carotid endarterectomy study mentioned earlier [144]. A study evaluating the effect of RIPC on spinal cord ischaemia-reperfusion injury on patients undergoing cervical decompression procedures found that RIPC reduced levels of neurological injury biomarkers [149]. There is also some evidence for a protective effect of direct brain ischaemia in humans – during berry aneurysm clipping, episodic and short-lived direct brain ischaemia was shown to have a beneficial effect on local pH and blood oxygen content [150]. A study on carotid stenting found that episodes of neurological dysfunction induced by angioplasty balloon inflation did not recur following repeated inflations [151], giving further support to the idea that neuroprotection can be achieved via conditioning.

RIPC has also been applied in plastic surgery. In reconstructive flap microsurgery, proof of concept studies confirmed that IPC and RIPC can reduce ischaemia-reperfusion injury and improve flap outcomes [152, 153]. IPC was first shown to be effective in this area in 1992 [154] and multiple in vivo animal studies followed [152]. Limb ischaemia was shown to be as effective as direct flap ischaemia in further animal work that followed [155, 156]. Although experimental data are promising, preconditioning has not achieved much clinical use in plastic surgery in humans to date; reasons for this are probably the increased operative time required and other practical difficulties. It follows that randomised clinical data are lacking and this is a target for the future.

In relation to liver surgery, IPC has been shown to reduce the severity of ischaemic injury in murine models of hepatic ischaemia [157, 158]. However, clinical benefits of IPC in human hepatectomy surgery have not materialised. A Cochrane review of IPC in elective liver resections found no benefit with IPC other than reduced blood transfusion requirements [159]. Another review found no clinical benefit but found that IPC reduced liver injury biomarker levels, a finding that is of uncertain significance [160]. A further review found no difference in a

variety of outcomes including clinical outcomes, transfusion requirements and aminotransferase levels [161]. Further high quality studies are needed in this area.

As mentioned in the preceding section, RIPC has been applied as a renoprotective strategy to counter the risk of contrast induced nephropathy (CIN) [137, 138]. These were two randomised controlled trials involving patients who were undergoing coronary angiography and both of them yielded results in favour of RIPC. The status and future potential of RIPC in the setting of CIN prevention will be discussed at a later point in this introduction.

1.15: Ongoing trials involving remote ischaemic preconditioning and cardiovascular surgery

There are several ongoing multi-centre trials investigating RIPC in major cardiovascular surgery. We are hopeful that definitive evidence of benefits in clinical outcomes will emerge with the completion of these trials.

The Remote Ischaemic Preconditioning for Heart Surgery study (RIPHeart-Study) is a multi-centre clinical trial in Germany that is currently recruiting patients who are undergoing surgery with a need for cardiopulmonary bypass [162]. It aims to recruit 2,070 adults including both high and low risk categories (high risk refers to Euroscore ≥ 5). The intervention comprises 4 cycles of 5 minutes of cuff-induced upper limb ischaemia with 5 minutes of reperfusion between each inflation. Another key design feature is robust blinding of surgical and anaesthetic teams, data collectors, analysis teams and the endpoint committee. This is achieved with a sham arm – only the person applying the intervention knows the treatment allocation. Furthermore, a total intravenous anaesthetic regimen is being used to eliminate the potential preconditioning effect of volatile anaesthetics [163]. Cardiopulmonary bypass management in the trial is standardised [162]. The primary outcome is a composite of all-cause mortality, non-fatal myocardial infarction, any new stroke, and/or acute renal failure until hospital discharge (up to a maximum of 14 days after surgery). The expected control group primary event rate for this is estimated at 12% and the investigators think that RIPC might reduce the event rate to 8%.

The Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Surgery (ERICCA) trial is a multi-centre trial in the United Kingdom that is currently recruiting high risk (Euroscore ≥ 5) patients who are undergoing coronary artery bypass graft surgery +/- valve surgery [164]. The trial aims to recruit 1,610 adults and it uses a similar RIPC intervention to the RIPHeart-Study. The blinding strategy in ERICCA is robust and uses an adjustable valve on the cuff rather than a sham arm. In contrast to the RIPHeart-Study, anaesthesia is not standardised which will probably increase external validity although it may hinder interpretation of the results. The primary outcome is a composite of cardiovascular death, non-fatal myocardial infarction, coronary revascularisation and stroke at one year. The control group event rate is predicted to be 20% and the RIPC group event rate is estimated to be 14.6%. The higher event rates reflect the exclusion of patients with Euroscore ≤ 5.

The Renal Protection Against Ischaemia Reperfusion in Transplantation (REPAIR) trial [165] is another multi-centre trial. It has completed recruitment (406 patients randomised) and published results are awaited. It aimed to determine the effect of RIPC on renal function after renal transplantation using estimated glomerular filtration rate (eGFR) at one year as the primary outcome. It also will report on some clinical outcomes at 2 – 5 years using registry follow up. There were 4 arms in the trial – control, early RIPC, late RIPC, combined early and late RIPC. Early RIPC was performed immediately pre-operatively and late RIPC was performed 24 hours before operations. Dual RIPC involved both. The stimulus was 3 cycles of 5 minutes of cuff induced arm ischaemia with 5 minutes reperfusion and routine anaesthetic practices were used.

1.16: Uncertainties and unresolved questions regarding remote ischaemic preconditioning

Though the potential of RIPC is great, there are many barriers that researchers must overcome. The unanswered questions largely fall into two categories: the mechanistic pathway issues and practical application issues.

We have examined theories on the biological basis for RIPC and IPC in a prior section of this introductory chapter and it is clear that sustained research efforts are needed. Knowledge of the involved pathways would enhance and focus future applications of preconditioning and might enable pharmacological initiation of preconditioning cardioprotective pathways.

There are many methodological uncertainties in RIPC research and it is important that efforts are made to elucidate these in order to increase research efficiency and facilitate comparisons between studies. Firstly, the optimal preconditioning stimulus has not been established. While undoubtedly skeletal muscle is the most attractive tissue to use for the stimulus, there is uncertainty regarding the optimal duration and number of ischaemia-reperfusion cycles. Furthermore, both upper and lower limbs are options. Theoretically, the increased muscle bulk in the lower limb is advantageous – one cardiac surgery study found that RIPC induced by both leg and arm ischaemia reduced myocardial injury compared to the control group but that RIPC induced by arm ischaemia only did not reduce myocardial injury [123]. However there were acute ischaemic complications with invasive lower limb arterial occlusion in one of the vascular trials mentioned earlier [141]. In contrast, there were no serious cuff-related lower limb or upper limb RIPC complications. Nonetheless, it is probably reasonable for researchers to use the upper limb only as it is rarely affected by peripheral vascular disease and has been successfully used in many clinical studies to date, establishing both feasibility and a beneficial effect on surrogate outcomes. In the absence of firm evidence, it is reasonable to propose that researchers use 3 or 4 cycles of 5 minutes ischaemia with 5 minutes reperfusion – there has been a tendency for negative results with a short stimulus time [119] and in studies that used 10 minutes ischaemic episodes [141, 143, 144]. Furthermore, there is no major downside to using 4 cycles – side effects from upper limb ischaemia are rare.

There is also uncertainty regarding the optimal target populations for intervention with RIPC. It is worth highlighting that RIPC induced protection is not absolute – prolonged ischaemia is always lethal and major insults are likely to surmount any

cardioprotection. The challenge is to focus efforts on heterogenous procedures with relatively high cardiac event rates as such patients will benefit maximally and such trials are likely to yield positive results at feasible levels of recruitment.

Lastly, it is important to reiterate that future studies on RIPC in major cardiovascular interventions should focus on patient important outcomes. In cardiovascular surgery, benefits in surrogate outcomes have been confirmed consistently by meta-analyses – the only way to advance is by shifting towards clinical outcomes where conclusions are less certain.

1.17: An introduction to remote ischaemic perconditioning

Remote ischaemic perconditioning (RIPerC) is the phenomenon whereby a protective phenotype can be induced in an organ or tissue through the distal application of a conditioning stimulus at the time of target organ ischaemia [166]. It was first proposed in 2007 by Schmidt *et al.* [167]. Schmidt *et al.* occluded the left anterior descending coronary arteries of a group of pigs and showed that limb ischemia applied after the start of coronary ischaemia and before reperfusion could induce a cardioprotective phenotype that led to reduced MI size [167].

As it is a relatively new concept, the mechanisms underlying RIPerC have yet to be elucidated. However, it is likely that there may be similarities with the mechanisms of RIPC [166] as described earlier in this introduction. Due to limited research on the topic of RIPerC the extent of the mechanistic overlap is unclear [166]. As studies evaluating the biology of RIPerC emerge, it is likely that understanding of all of the conditioning pathways will improve.

While the underlying theory of RIPerC is distinct from RIPC, RIPostC and the direct conditioning techniques, all of the conditioning techniques share many conceptual similarities (figure 1.2). The principle theoretical advantage of RIPerC is that it can be applied in any emergency or elective setting [166]. In contrast, regarding RIPC and IPC, target organ ischaemia must be anticipated and preceded by the administration of a preconditioning stimulus. A further advantage is that RIPerC does not prolong procedural time [166]. However, in practical terms, the distinction between RIPC and RIPerC is probably of little importance. Regarding many of the RIPC cardiovascular surgery trials mentioned earlier, although RIPC was initiated before potential target organ ischaemia, it was not always specified that RIPC finished before the ischaemic episode. Thus the conditioning strategies in many of the studies probably comprised a hybrid of RIPC and RIPerC. Furthermore, the stimulus in the trials mentioned earlier on RIPC in primary PCI could be thought of as either RIPC or RIPerC. If the primary ischaemic event is considered to be the initial MI, then RIPerC is the more accurate term whereas RIPC is more accurate if PCI is considered to be the event.

1.18: An introduction to remote ischaemic postconditioning

Remote ischaemic postconditioning (RIPostC) is the phenomenon whereby a protective phenotype can be induced in an organ or tissue through the distal application of a conditioning stimulus after reperfusion of the target organ has occurred [166]. The concept and the terminology of postconditioning were first introduced by Na *et al.* in 1996 [168]. Na *et al.* occluded the left anterior descending coronary artery in 29 cats for 20 minutes. 15/29 cats had subsequent uninterrupted reperfusion while 14/29 cats had intermittent reperfusion. 13/15 cats in the uninterrupted reperfusion group developed reperfusion induced ventricular fibrillation (VF) whereas only 1/14 cats in the intermittent reperfusion group developed reperfusion induced VF (p<0.001). Interestingly, the magnitude of cardioprotection was similar to that induced by IPC with one 10 minute occlusion with reperfusion before the 20 minute occlusion. A 2003 study by Zhao *et al.* [169] used similar methodology on three groups of dogs and found similar conclusions: both postconditioning and preconditioning were cardioprotective and the magnitude of cardioprotection with preconditioning was comparable to that from postconditioning.

Subsequently, Andreka *et al.* also demonstrated the concept of RIPostC [170]. They induced MIs in 24 pigs by the inflation of balloons in left anterior descending coronary arteries. 12 of the pigs had five four minute cycles of cuff induced limb ischaemia immediately after coronary balloon deflation (RIPostC). The post conditioned pigs had a 26% reduction in infarct size (p<0.05). Interestingly, a subsequent study evaluated RIPostC on humans undergoing PCI and did not find a benefit with RIPostC [171]. This was a trial that included 232 patients with angina who were undergoing PCI. The postconditioning stimulus was initiated five minutes after the last balloon deflation and comprised three five minute cycles of cuff induced limb ischaemia. There was no difference in the primary outcome of peak post-procedural troponin level but a subgroup analysis found that in the diabetic subgroup more patients who underwent RIPostC had a periprocedural MI. At present, there is a need for further research on the topic of RIPostC as it is difficult to draw definitive conclusions.

The mechanisms underlying postconditioning and remote postconditioning are uncertain but evidence suggests that there is some overlap with the mechanisms of RIPC [172]. There is also a suggestion that RIPostC may act via activation of the reperfusion salvage kinase pathway [173] in a similar way to the older concept of staged reperfusion [172].

From a clinical perspective, RIPostC may be applied in emergency and elective clinical settings and thus it is attractive as a theoretical risk reduction strategy – especially following mechanical reperfusion after acute MI [172]. The likely magnitude of cardioprotection appears to be similar or just slightly smaller than that from RIPC [172]. However at present the level of clinical research on humans and RIPostC lags far behind that of RIPC and this represents a major challenge.

1.19: Contrast induced kidney injury: another potential application for remote ischaemic preconditioning

As mentioned previously in this introduction, a novel potential role for RIPC is in the prevention of contrast induced kidney injury. Contrast induced kidney injury is a broad phrase that describes the toxic effect that intravenous radiographic contrast media can have on the kidneys. Contrast induced nephropathy (CIN) is a specific manifestation of contrast induced kidney injury and it is defined as an impairment in renal function with an absolute increase in serum creatinine of 44 μmol/L (0.5 mg/dl) or a relative increase in serum creatinine of 25% or more from baseline, occurring within 3 days of exposure to intravascular contrast medium in the absence of an alternative aetiology [174]. Contrast induced kidney injury and CIN represent spectrums of kidney injury – a key difference between the two concepts is that CIN has a strict threshold in its definition. Both terms reflect the same pathological process.

CIN is a leading cause of hospital acquired acute kidney injury (AKI) [175]. Unfortunately, exact data on its epidemiology do not exist – this is because the incidence varies dramatically depending on risk factors and reasons for contrast administration [176]. The principal CIN risk factors are listed in table 1.7. A further factor limiting the quality of the data on CIN epidemiology is the definition for CIN – studies often use creatinine levels as outcomes to define CIN but in reality CIN diagnosis requires the absence of an alternative cause of renal dysfunction. A large retrospective study of 32,161 patients who had serial creatinine measurements but no preceeding contrast exposure found that over half of patients had creatinine changes of over 25% [177]. This illustrates the strong potential for biased results in studies on CIN, particularly in retrospective studies.

Table 1.7: List of risk factors for contrast induced nephropathy [176].

Chronic kidney disease
Diabetic nephropathy
Causes of reduced renal perfusion such as heart failure, sepsis, hypovolaemia
High dose of contrast agent
Use of older first generation contrast agents
Percutaneous coronary intervention
Multiple myeloma

The occurrence of CIN has negative effects on both short term [178-181] and long term [178, 181] prognosis. Pharmacological CIN prevention interventions have been disappointing [182, 183] and currently the cornerstones of CIN prevention are the use of prehydration, low osmolar contrast agents and contrast dose minimisation [184]. As the incidence of CIN in set to increase along with rising prevalence of diabetes and chronic kidney disease and increased use of contrast based imaging [185], there is an urgent need for a simple and effective preventative strategy.

The mechanisms underlying contrast induced kidney injury are poorly understood but are thought to involve two components: renal vasoconstriction and a direct nephrotoxic effect [176]. A study showed that contrast led to a renal blood flow reduction of 30% at two hours following contrast administration [186]. Another study showed that contrast led to a blood flow reduction of about 40% throughout the first four hours after administration [187]. Many other studies based on animal models also suggest this vasoconstrictive effect [176]. The resultant medullary ischaemia leads to cellular oxidative stress [188, 189] and therefore RIPC appears theoretically attractive.

Two studies have evaluated the role of RIPC as a tool for contrast induced kidney injury prevention following coronary angiography [137, 138] and both studies provided encouraging results. Both of these studies were performed on patients at moderate to high risk for the development of CIN. The characteristics of these studies are provided in table 1.8.

Table 1.8: Characteristics of the clinical trials that evaluated RIPC for the prevention of contrast induced kidney injury.

Lead author and recruitment dates	Nature of PCI Number of participants RIPC:Control	Patients	Exclusion criteria	Nature of intervention	Outcomes reported	Results	Primary outcome result favours RIPC or control
Er 2012 January 2011 to June 2011	Elective coronary angiography 41:40	100 consecutive consenting patients with impaired renal function (eGFR<60ml/min/1.73m^2 or creatinine >1.4mg/dl) undergoing elective coronary angiography	End stage renal failure, age < 18 years	4x5 minute cycles of cuff induced upper limb ischaemia with 5 minutes reperfusion between cycle, started before coronary angiography with last cycle finishing, 45 minutes before coronary angiography	Primary outcome: Incidence of CIN (creatinine increase of ≥ 0.5mg/dl or 25% from baseline within 48 hours Secondary outcomes: maximal elevation of creatinine, cyctatin C, NGAL in 48 hours, composite cardiovascular outcome comprising death, rehospitalisaton, HD within 6 weeks	CIN incidence was 20/50 in controls and 6/50 in RIPC (p=0.002); Ppak creatinine, cystatin C and NGAL were also significantly lower in RIPC group Cardiovascular composite outcome occurred in 19/50 controls and 8/50 RIPC (p=0.018); this was driven by rehospitalisation rates (18/50 control versus 7/50 RIPC, p=0.016)	RIPC was significantly better than control

Lead author and recruitment dates	Nature of PCI Number of participants RIPC:Control	Patients	Exclusion criteria	Nature of intervention	Outcomes reported	Results	Primary outcome result favours RIPC or control
Igarashi 2013 February 2011 to October 2012	Elective coronary angiography 30:30	60 patients with eGFR of between 30 – 60 ml/min/1.73m^2 undergoing elective coronary angiography	Cardiogenic shock, acute renal failure; patients who had contrast doses of <40mls or >300mls were excluded	4x5 minute cycles of cuff induced upper limb ischaemia with 5 minutes reperfusion between cycles, started 2 hours before procedures	Primary outcome: Incidence of L-FABP based CIN (defined as L-FABP >17.4μg/gCr within 24 hours of contrast exposure or if baseline L-FABP was > 17.4μg/gCr and increase of 25% indicated CIN Secondary outcomes: several other inflammaroty and renal injury biomarkers	L-FABP based CIN developed in 8/30 controls and 2/30 in RIPC group (p=0.038)	RIPC was significantly better than control

CIN – contrast induced nephropathy; Cr – creatinine, eGFR – estimate glomerular filtration rate; HD – haemodialysis; L-FABP – liver-type fatty acid-binding protein; NGAL – neutrophil gelatinase-associated lipocalin; RIPC – remote ishaemic preconditioning.

1.20: Structure of the remaining chapters

The remaining chapters of this thesis explore aspects of RIPC in clinical practice. There are two systematic reviews: one on RIPC in percutaneous coronary intervention and one in major cardiovascular surgery. There are two randomised controlled clinical trials: one trial examines RIPC as a renoprotective strategy following radiographic contrast medium exposure during computed tomography (CT) scanning and the other trial evaluates the cardioprotective role of RIPC in the setting of major vascular surgery.

Chapter 2: A systematic review and meta-analysis of remote ischaemic preconditioning in percutaneous coronary intervention

2.1: Abstract

A body of evidence suggests that myocardial infarctions (MI) that are associated with percutaneous coronary intervention (PCI) have prognostic significance but it is uncertain whether remote ischaemic preconditioning (RIPC) offers periprocedural cardioprotection at the time of PCI.

Medline, Embase, the Cochrane Central Register of Controlled Trials and conference records were searched (January 1986 to August 2013) for randomised trials that evaluated the effect of RIPC induced by limb ischaemia-reperfusion versus no RIPC in patients who were undergoing PCI. All outcomes were considered for inclusion in the systematic review. Relevant data were extracted and summarised. Pooled odds ratios were used to determine the effect of RIPC compared to control on three prespecified outcomes: troponin positive events in elective PCI, periprocedural MI incidence in elective PCI and acute kidney injury (AKI) incidence in emergency or elective PCI.

Eight trials (1119 patients) were found of which six (983 patients) had primary outcomes that were significantly in favour of RIPC. There was no difference in troponin positive events between RIPC and control groups (pooled OR 0.529, 95%CI 0.206 – 1.358, p=0.185) (three studies, 377 patients). There was a significant reduction in periprocedural MI incidence with RIPC (pooled OR = 0.577, 95%CI 0.400 – 0.833, p=0.003) (four studies, 636 patients). There was no difference in AKI incidence (pooled OR = 0.672, 95%CI 0.252 – 1.787, p=0.425) (two studies, 407 patients).

Primary outcomes favoured RIPC in most of the studies. RIPC significantly reduced the incidence of periprocedural MI. Included studies were heterogeneous in methodology and quality.

2.2: Rationale for the use of remote ischaemic preconditioning in percutaneous coronary intervention

Percutaneous coronary intervention (PCI) is a key component of the management of coronary artery disease [190]. About 1.5 million people undergo PCI in the United States each year and about 5 to 30% of these patients develop periprocedural myocardial infarction (MI), depending on the definitions used [191]. Though there is controversy regarding the prognostic significance of most small periprocedural MIs, a body of evidence suggests that periprocedural MIs are associated with adverse outcomes [191]. Therefore, there is a need for a simple and cheap intervention that might offer periprocedural cardioprotection. Remote ischaemic preconditioning (RIPC) may be suitable for this role.

Ischaemic preconditioning (IPC) is a phenomenon whereby brief periods of ischaemia in an organ or tissue can confer resistance against subsequent more sustained ischaemic insults [91]. In trials on cardiac surgery, IPC has been shown to reduce arrhythmia rates, inotrope requirements and intensive care unit length of stay [90] but the need for direct interference with coronary blood flow limits its utility. Subsequently, it was shown that episodic ischaemia in distant organs or tissues can induce cardioprotection [76, 78] – this concept was termed RIPC. Multiple exploratory studies have evaluated the role of RIPC in a wide range of cardiovascular interventions (including four PCI trials) and meta-analysis has shown reductions in biomarkers of myocardial injury with RIPC [89]. However, to date there has been no systematic review that examined the effect of RIPC in PCI solely. Performing such a PCI review is worthwhile as vascular surgery, cardiac surgery and PCI have different risk profiles – conclusions derived from pooled data may not apply to the individual subgroups. Therefore a systematic review and meta-analysis of RIPC in PCI was performed.

2.3: Methodology for the systematic review and meta-analysis

This systematic review was performed in accordance with the PRISMA guidelines [192].

Medline, EMBASE and the Cochrane Central Register of Controlled Trials were searched from 1986 to 12th August 2013 using the search (ischaemic preconditioning OR ischemic preconditioning OR remote preconditioning OR remote ischaemic preconditioning OR remote ischemic preconditioning). Conference proceedings from the American College of Cardiology (2002 – 2013), American Heart Association (2002 – 2012), British Society of Cardiology (2002 – 2013) and European Society of Cardiology (2002-2012) were searched manually. Eligible studies provided any clinical or biochemical outcomes from randomised controlled trials that compared RIPC induced by transient limb ischaemia with no RIPC in patients who were undergoing emergency or elective PCI. There was no language restriction and there were no exclusion criteria.

Two reviewers (DA Healy and P Carroll) independently screened titles and abstracts for eligibility. Manuscripts of potentially relevant articles were retrieved and examined by the same two reviewers to finalise eligibility. Uncertainties were resolved by discussion with another reviewer (SR Walsh). The citation lists of included articles were examined for further relevant publications. For each included study, the following data were extracted by two reviewers (DA Healy and P Carroll) independently: publication year, recruitment period, whether the study involved emergency or elective PCI, details on trial participants, exclusion criteria, details on the nature of the RIPC stimulus, outcomes included and results. An attempt was made to contact authors via email if study manuscripts did not provide details on the numbers of patients who had troponin positive events, periprocedural MIs and acute kidney injury (AKI). Data were entered into a computerised spreadsheet for analysis. Outcomes considered for meta-analysis were incidence of troponin positive events within 24 hours of elective PCI, incidence of periprocedural MI following elective PCI and incidence of AKI in elective or emergency PCI. The definitions for outcomes were the definitions specified in the manuscripts of included studies. Study quality was assessed by one author (DA Healy) by applying the Cochrane Collaboration's tool for assessing risk of bias [143] to each study and by reviewing protocols if they were available.

Statistical analyses were performed using Statsdirect 2.5.7 (Statsdirect Ltd., Altrincham, UK). Pooled odds ratios (OR) were used to calculate the effect of

RIPC on troponin positive events, periprocedural MI and AKI. These were determined using random effects modelling as described by DerSimonian *et al.* [193]. Statistical heterogeneity was assessed by Cochran's Q statistic, a hypothesis test in which a p value below 0.05 is taken to indicate the presence of significant heterogeneity.

2.4: Results of the systematic review and meta-analysis

The literature search identified 7,331 sources. Figure 2.1 summarises the result of
the search. In total, 7,308 citations were excluded based on titles and abstracts. 23
full text articles were screened. When ineligible studies were excluded, 8 fulfilled
the criteria for inclusion in the systematic review. No additional studies were found
from the grey literature or from included article reference lists.

The eight [103, 131-136, 194] included studies (1,119 patients) are summarised in
table 2.1. Six studies involved elective PCI [132-136, 194] (772 patients) and two
studies [103, 131] (347 patients) involved emergency PCI for ST elevation MI
(STEMI). All studies were parallel group trials – seven trials had 1:1 allocation
ratios and one study [131] involved a 1:1:1 ratio: RIPC, RIPC with morphine and
control groups. For the purposes of this review, RIPC and RIPC with morphine
were combined and compared with the control arm. Six of the studies used cuff
induced ischaemia-reperfusion of one upper limb to induce the RIPC stimulus
[103, 131-133, 135, 194], one study applied the stimulus to both upper limbs [134]
and one study used the lower limb for the stimulus [136]. The duration of
ischaemia reperfusion cycles also varied between studies. Six studies [132-136,
194] used cardiac enzyme levels as primary outcomes, one trial [131] used the
number of patients with ST segment resolution as the primary outcome and one
trial [103] used myocardial salvage index as the primary outcome. Overall, six
trials had primary outcome results that significantly favoured RIPC [103, 131, 133,
135, 136, 194], one trial found no difference between RIPC and control [132] and
one trial found a significant result in favour of the control group. Table 2.2
summarises the study quality assessment.

Chapter 2: Systematic review and meta-analysis of remote ischaemic preconditioning in percutaneous coronary intervention

Figure 2.1: Summary of the search results for the systematic review and meta-analysis of remote ischaemic preconditioning in percutaneous coronary intervention

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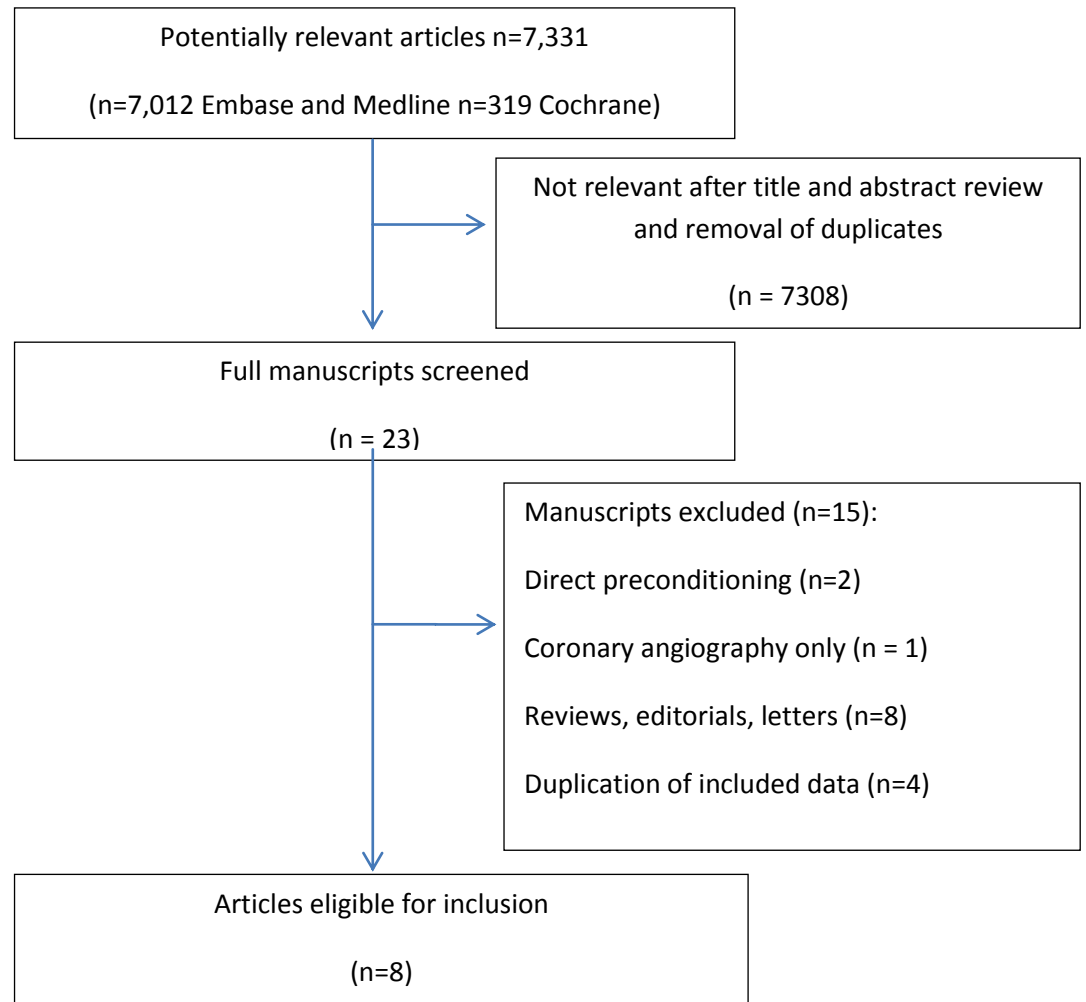
Table 2.1: Included study characteristics.

Lead author and recruitment dates	Nature of PCI Number of participants RIPC:Control	Patients	Exclusion criteria	Nature of intervention	Outcomes reported	Results	Primary outcome result favours RIPC or control
Ahmed 2013 [135] March to November 2010	Elective PCI 77:72	149 consecutive patients with undetectable preprocedural cTnT undergoing elective PCI. Mean age was 53.5 in controls, and 54.59 in RIPC group. 88 had DES inserted.	Elevated cTnT at baseline, renal dysfunction, arteriovenous fistula, lymphoedema, severe endocrine or hepatic disease.	3x5 minute cycles of cuff induced upper limb ischaemia with 5 minutes reperfusion between cycles, immediately before arrival in catherisation room.	Primary outcome: cTnT at 16 hours. Secondary outcomes: MI rates, CKMB levels at 16 hours, CRP level at 16 hours.	Mean cTnT was 0.020ng/ml in RIPC group versus 0.047ng/ml in controls (p=0.047). Secondary outcomes were not different.	RIPC was significantly better
Ghaemian 2012 [136] 2008 to 2009	Elective PCI 40:40	80 consecutive patients undergoing elective PCI with DES insertion. Mean age in controls was 61, mean age in RIPC group was 58.8. A mildly elevated cTnT was not an exclusion.	Emergency PCI, angina in 48 hours before procedure, previous MI within 6 weeks, nicorandil or suplhonylurea, PVD.	2x5 minute cycles of cuff induced lower limb ischaemia with 5 minutes reperfusion between cycles, one hour before the procedure.	Primary outcomes: Number of patients with elevated cTnT at 24 hours. Secondary outcomes: arrythmias, postprocedural chest pain, ST segment deviation, death within 28 days, MI rates, emergency	Numbers of patients with cTnT elevations at 24 hours were higher in control group (16/40 control, 5/40 RIPC, p=0.01). However, mean cTnT at 24 hours was 0.063 ng/ml in RIPC versus 0.016 in controls (p=0.009).	RIPC was significantly better

					revascularisation rates, long term MACE (hospitalisation for ACS, MI or death due to MI).	RIPC reduced ST segment deviation time (p=0.02). No other secondary outcome difference.	
Luo 2012 [194] March to August 2012	Elective PCI 101:104	205 patients undergoing elective PCI with DES insertion. Mean age in controls was 59.3 years; mean age in RIPC group was 59.2 years.	Emergency PCI, baseline cTnI>0.04ng/ml, nicorandil or glibenclamide medication, second PCI of a staged procedure.	3x5 minute cycles of cuff induced upper limb ischaemia with 5 minutes reperfusion between cycles, less than 2 hours before PCI procedure.	Primary outcome: hsTnI at 16 hours, MI at 16 hours. Secondary outcomes: creatinine, eGFR and AKI at 16 hours.	Median hsTnI was 0.11ng/ml in RIPC group versus 0.21ng/ml in controls (p<0.01). MI rates were lower with RIPC also (39/101 with RIPC versus 57/104 with control, p=0.029). Secondary outcomes not different.	RIPC was significantly better
Rentoukas 2010 [131] Recruitment period was not specified	Emergency PCI 66:30	96 patients undergoing emergency PCI for STEMI. There were three arms: RIPC, RIPC with morphine, control. Mean age in controls was 61.2 (n=30). Mean age across the two RIPC groups was 63.4 (n=66).	Shock, moderate/severe renal impairment (creatinine > 1.5mg/dl).	3x4 minute cycles of cuff induced upper limb ischaemia with 4 minutes reperfusion between cycles, starting 10 minutes before estimated time for first balloon inflation.	Primary outcome: number of patients with ST segment resolution 30 minutes after PCI. Secondary outcomes: percentage reduction in ST segment deviation score between presentation and 2 hours post procedure,	More people who had RIPC had full ST segment resolution (51/66 compared with 16/30; p=0.03*). Significant improvements in ST segment resolution score with RIPC. Peak cTnI levels with RIPC were 134.7ng/ml	RIPC was significantly better

					peak cTnI during hospitalisation.	SEM15.9 versus control 255.5ng/ml SEM 35.5, p=0.003.	
Iliodromitis 2006 [134] Not specified	Elective PCI 20:21	41 consecutive patients undergoing elective PCI. Mean age in controls was 62 and mean age in RIPC group was 61.	ACS, complex lesions, additional cardiac disease, renal/hepatic disease, malignancy, rheumatoid arthritis, active infection.	3x5 minute cycles of cuff induced ischaemia of both upper limbs, with 5 minutes reperfusion between cycles. Angioplasty was carried out immediately after.	The primary outcome was not specified. Outcomes were CK, CKMB, cTnI, CRP at 12,24,48 hours.	AUC for CKMB was significantly greater in RIPC group (83 SEM 24 versus 21 SEM 8; p<0.05). AUC for TnI was also greater in RIPC group (24 SEM 7 versus 8 SEM 1.7; p<0.05). No difference in CRP or CK between groups.	Control was significantly better
Prasad 2013 [132] November 2006 to November 2008	Elective PCI 47:48	95 patients undergoing non-emergency PCI for stable or unstable angina. Mean age in controls was 65.1, mean age in RIPC group was 67.2.	Preprocedural cTnT≥0.03ng/dl, emergency PCI, hypotension (<90mmHg systolic), shock, arteriovenous fistula or lymphoedema, pregnancy or lactation, severe comorbidity.	3x3minute cycles of cuff induced upper limb ischaemia with 3 minutes reperfusion between cycles immediately preceding PCI	Primary outcome: Number of patients with peak cTnT ≥0.003ng/dl, checked at 6, 12, 24 hours. Secondary outcomes: CKMB (at 6, 16, and 24 hours) and CRP levels (16hrs) and endothelial progenitor cell counts.	No difference in primary or secondary outcomes with the exception of CKMB levels which were higher in RIPC group at 24 hours only.	No difference was found
Hoole 2009 [133]	Elective PCI	202 consecutive	Emergency PCI,	3x5 minute cycles of	Primary outcome: cTnI	Median cTnI at 24	RIPC was significantly

July 2006 to November 2009	104:98	patients with undetectable preprocedural cTnI undergoing elective PCI and stent insertion. Mean age in controls was 61.8 and in the RIPC group it was 63.2.	women of child bearing age, nicorandlil or glibenclamide use, life expectancy < 6 months.	cuff induced upper limb ischaemia with 5 minutes reperfusion between cycles, starting about an hour before the procedure.	at 24 hours. Secondary outcomes: ischaemic symptoms, ECG evidence of ischaemia during balloon inflation, renal dysfunction, adverse cardiac and cerebral events at 6 months.	hours was 0.06ng/ml IQR 0.02 - 0.56) in RIPC group versus 0.16ng/ml IQR (0.04-1.04) in controls, (p=0.04). There was less chest discomfort (p=0.0006) and ST segment deviation (p=0.005) with RIPC also. Major adverse cardiac and cerebral events at 6 months were lower with RIPC (4 versus 13, p=0.018).	better
Botker 2010 [103] February 2007 to November 2008	Emergency PCI for STEMI 126:125 However, 73:69 had primary outcome assessment at 30 days.	251 patients undergoing emergency PCI for STEMI. Mean age in controls was 63 years and mean age in RIPC was 62 years.	Left bundle branch block, previous MI, fibrinolytic therapy within 30 days, previous CABG, left main stem disease requiring CABG, severe heart failure requiring mechanical ventilation or a balloon pump.	4x5 minute cycles of cuff induced upper limb ischaemia with 5 minutes reperfusion between cycles, starting during transit to the hospital.	Primary outcome: myocardial salvage index at 30 days. Secondary outcomes: final infarct size at 30 days, cTnT levels, markers of reperfusion, death, reinfarction, admission with heart failure within 30 days, LVEF, NYHA class at	Higher median salvage index with RIPC in the per protocol analysis - RIPC (n=73) 0.75 IQR 0.5 -0.93 versus control (n=69) 0.55 IQR-0.35-0.88. Median difference 0.10 95%CI 0.01 - 0.22, p=0.033.) No difference in other outcomes.	RIPC was significantly better

					30 days.		

*This hypothesis test was performed by using Fisher's exact test on data extracted from the Rentoukas *et al.* manuscript.

ACS – acute coronary syndrome; AUC – area under the curve; CABG – coronary artery bypass graft; CK – creatine kinase; CKMB – creatine kinase MB isoenzyme; CRP – C reactive protein; cTnI – cardiac troponin I; cTnT – cardiac troponin T; DES – drug eluting stent; ECG – electrocardiograph; eGFR – estimated glomerular filtration rate; IQR – interquartile range; LVEF – left ventricular ejection fraction; MI – myocardial infarction; NYHA – New York Heart Association; PCI – percutaneous coronary intervention; RIPC – remote ischaemic preconditioning; SEM – standard error of the mean.

Table 2.2: Results of the study quality assessment

Included study	Domain	Support for judgement	Judgement
Ahmed 2013 [135]	Random sequence generation	No description of random sequence generation.	Unclear
	Allocation concealment	No description of methods for maintaining allocation concealment	Unclear
	Blinding of participants and personnel	No sham procedure. No mention of blinding patients and personnel.	High risk of bias
	Blinding of outcome assessment	No mention of blinding of assessors.	High risk of bias
	Incomplete outcome data	Number of excluded patients and attrition rates were not specified. Amount of missing data was not specified.	Unclear
	Selective reporting	No link to trial protocol was given. All outcomes specified in manuscript were reported.	Unclear
	Other sources of bias	None	Not available
Ghaemian 2012 [136]	Random sequence generation	Computer randomisation was specified.	Low risk of bias
	Allocation concealment	No description of methods for maintaining allocation concealment.	Unclear
	Blinding of participants and personnel	Controls had placement of a non inflated cuff. An independent team member applied RIPC and finished 45 minutes before procedures began.	High risk of bias
	Blinding of outcome assessment	No mention of blinding of assessors.	Unclear
	Incomplete outcome data	Attritions and exclusions are described. No mention of amount of missing data.	Unclear
	Selective reporting	The protocol says that	Unclear

		cTnT levels are the primary outcome. It does not specify how levels are interpreted. The manuscript dichotomises these results into >0.03ng/ml and <0.03ng/ml. RIPC is favoured using the dichotomy but not when results are analysed continuously using mean values.	
	Other sources of bias	None	Not available
Luo 2013 [194]	Random sequence generation	The method of generating the random sequence was not described.	Unclear
	Allocation concealment	No description of methods for maintaining allocation concealment.	Unclear
	Blinding of participants and personnel	No sham procedure was employed. RIPC started 2 hours before PCI.	High risk of bias
	Blinding of outcome assessment	Outcomes were biochemical and assessors did not know allocation.	Low risk of bias
	Incomplete outcome data	Attrition rates and exclusions are not described. No mention of amount of missing data.	Unclear
	Selective reporting	No link to trial protocol was given. All outcomes specified in manuscript were reported	Unclear
	Other sources of bias	None	Not available
Rentoukas 2009 [131]	Random sequence generation	The method of generating the random sequence was not described.	Unclear
	Allocation concealment	No description of methods for maintaining allocation concealment.	Unclear
	Blinding of participants and personnel	Controls had inflation of an arm cuff to	High risk of bias

		20mmHg.	
	Blinding of outcome assessment	Physicians assessing the primary outcome were blinded with no mention of blinding of other assessors	Low risk of bias
	Incomplete outcome data	Number of excluded patients and attrition rates were not specified. Amount of missing data was not specified.	Unclear
	Selective reporting	No link to trial protocol was given. All outcomes specified in manuscript were reported. Mortality was not reported but is relevant in a primary PCI study.	Unclear
	Other sources of bias	None	Not available
Iliodromitis 2006 [134]	Random sequence generation	The method of generating the random sequence was not described.	Unclear
	Allocation concealment	No description of methods for maintaining allocation concealment.	Unclear
	Blinding of participants and personnel	Controls had placement of non inflated cuffs. No blinding of personnel.	High risk of bias
	Blinding of outcome assessment	Blinding of outcome assessors was not mentioned.	High risk of bias
	Incomplete outcome data	Number of excluded patients and attrition rates were not specified. Amount of missing data was not specified.	Unclear
	Selective reporting	No link to trial protocol was given. All outcomes specified in manuscript were reported. MI rates are not reported.	Unclear
	Other sources of bias	None	Not available
Prasad 2013 [132]	Random sequence	The method of	Unclear

	generation	generating the random sequence was not described.	
	Allocation concealment	No description of methods for maintaining allocation concealment.	Unclear
	Blinding of participants and personnel	Controls had inflation of a sham cuff to 10mmHg. No mention of blinding of personnel.	High risk of bias
	Blinding of outcome assessment	Blinding of outcome assessors was not mentioned	High risk of bias
	Incomplete outcome data	Number of excluded patients and attrition rates were not specified. Amount of missing data not specified.	Unclear
	Selective reporting	Protocol specifies that primary outcome measurement should take place at 16 hours but peak levels were reported in the manuscript. Protocol mentions non reported secondary outcomes – coronary perfusion measurement and procedural ST segment elevation. These omissions are unlikely to change conclusions. MI rates were not reported.	Low risk of bias.
	Other sources of bias	None	Not available
Hoole 2009 [133]	Random sequence generation	A computer generated sequence was used.	Low risk of bias.
	Allocation concealment	Allocation was concealed using sealed envelopes that were stored in a separate unit and opened only by independent research staff.	Low risk of bias
	Blinding of participants and personnel	Controls had placement of a non inflated cuff.	Low risk of bias

		Interventions were completed before procedures. Cardiologists did not know allocation.	
	Blinding of outcome assessment	Biochemical measurements were made without knowledge of groups.	Low risk of bias
	Incomplete outcome data	There were 21 post randomisation exclusions in the RIPC group and 19 in the control group. Reasons were similar. It is unlikely that final result would be impacted. Only 1 patient was lost to 6 month follow up.	Low risk of bias.
	Selective reporting	No detailed protocol was available.	
	Other sources of bias	None	Not available
Botker 2010 [103]	Random sequence generation	A computer generated random sequence was used.	Low risk of bias.
	Allocation concealment	Hospital on call doctors opened sequential sealed opaque envelopes and communicated result to paramedic team.	Low risk of bias.
	Blinding of participants and personnel	No sham procedure. Paramedics and cardiologists were not blinded.	High risk of bias.
	Blinding of outcome assessment	Data analysts were blinded.	Low risk of bias
	Incomplete outcome data	Exclusions and attritions are documented. 82 patients were excluded post randomisation. RIPC and control exclusions were similar and unlikely to introduce selection bias.	Low risk of bias.
	Selective reporting	The protocol mentions some secondary outcomes that are not reported in the	Low risk of bias

	manuscript.	
Other sources of bias	None	Not available

cTnT – cardiac troponin T; MI – myocardial infarction; PCI – percutaneous coronary intervention; RIPC – remote ischaemic preconditioning.

Table 2.3 summarises the available data on the three prespecified outcomes for
meta-analysis.

Table 2.3: Summary of the data used in the meta-analysis.

Troponin positive events					
Study	Definition used for troponin positive events	RIPC participant number	Number of troponin positive events in RIPC group	Control participant number	Number of troponin positive events in control group
Ghaemian	cTnT > 0.03ng/ml at 24 hours	40	5*	40	16*
Prasad	cTnT ≥ 0.03ng/dl at 24 hours	47	22	48	19
Hoole	cTnI ≥ 0.04ng/ml at 24 hours	104	60	98	74
Myocardial infarction					
Study	Definition used for MI	RIPC participant number	Number of MIs in RIPC group	Control participant number	Number of MIs in control group
Ahmed	2007 type 4a MI definition [195], specified as cTnT increase of greater than 3 times the 99th percentile URL	77	6	72	12
Ghaemian	no definition was specified	40	0	40	0
Luo	2012 type 4a MI definition [21], specified as HscTnI increase of greater than 5 times 99th percentile URL corresponding to HscTnI>0.2ng/ml	101	39	104	57
Hoole	2007 type 4a MI definition [195], specified as cTnI increase of greater than 3 times the 99th percentile URL corresponding to cTnI>0.12ng/ml	104	47	98	53
Acute kidney injury					
Study	Definition used for AKI	RIPC participant number	Incidence o f AKI in RIPC group	Control participant number	Incidence of AKI in control group
Luo	>25% increase in serum creatinine from baseline level, checked at 16 hours	101	2	104	1
Hoole	>25% increase in serum creatinine from baseline level, checked at 24 hours	104	6	98	10

*At baseline, 2/24 in RIPC group and 6/24 in control group were troponin positive - these patients are included in
the post procedure troponin positive group. This study did not exclude participants on the basis of preprocedural
troponin levels.

AKI – acute kidney injury; cTnI – cardiac troponin I; cTnT – cardiac troponin T; HscTnI – highly sensitive
cardiac troponin I; MI – myocardial infarction; RIPC – remote ischaemic preconditioning; URL – upper reference
limit.

Three studies (377 patients) [132, 133, 136] provided data on the incidence of troponin positive events. Troponin positive events occurred in 87/191 RIPC patients and in 109/186 controls. There was evidence of heterogeneity between studies (Cochran Q = 7.79, p=0.02). The random effects model demonstrated no significant difference in troponin positive events between groups (pooled OR = 0.529, 95%CI 0.206 – 1.358, p=0.185) (figure 2.2).

Figure 2.2: Forest plot showing the pooled effect of RIPC on troponin positive events
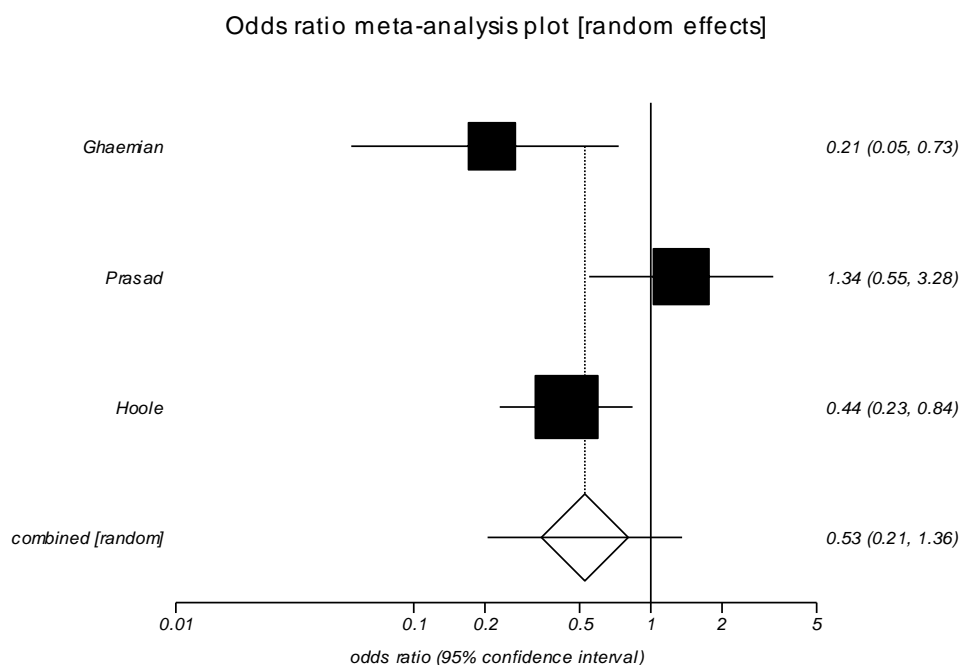
Odds ratio meta-analysis plot [random effects]

Ghaemian	0.21 (0.05, 0.73)
Prasad	1.34 (0.55, 3.28)
Hoole	0.44 (0.23, 0.84)
combined [random]	0.53 (0.21, 1.36)

odds ratio (95% confidence interval)

Four studies (636 patients) [133, 135, 136, 194] provided data on periprocedural MI. Periprocedural MI occurred in 92/322 RIPC patients and in 122/314 controls. There was no evidence of heterogeneity between studies (Cochran Q = 0.95, p=0.62). The random effects model demonstrated a significant reduction in periprocedural MI with RIPC (pooled OR = 0.577, 95%CI 0.400 – 0.833, p=0.003) (figure 2.3).

Figure 2.3: Forest plot showing the pooled effect of RIPC on periprocedural myocardial infarction.
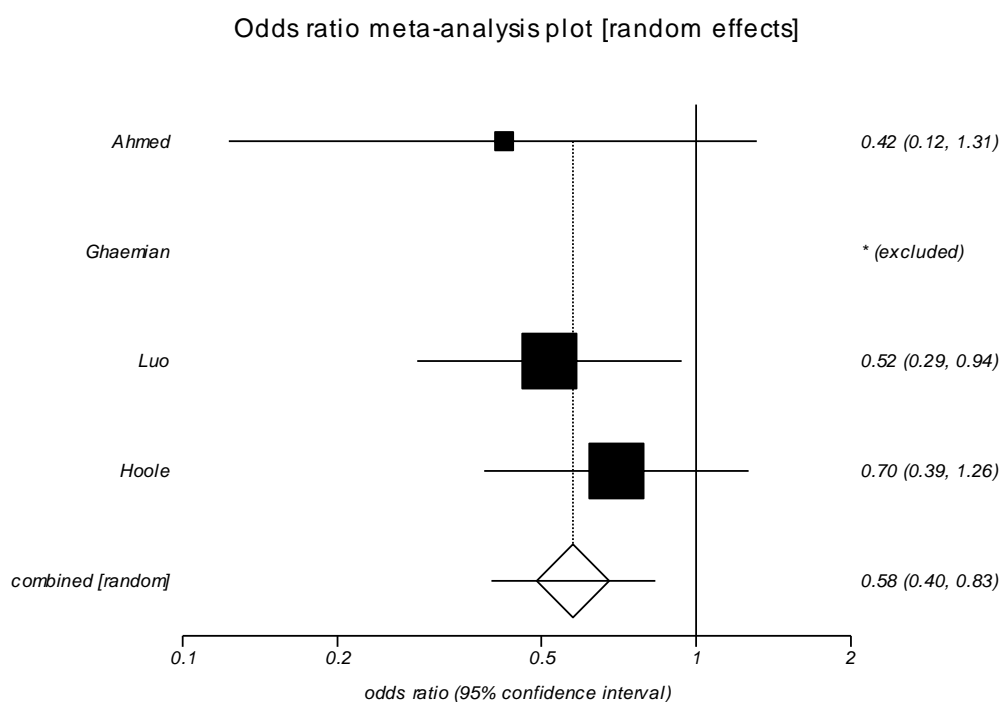
Chapter 2: Systematic review and meta-analysis of remote ischaemic preconditioning in percutaneous coronary intervention

Two studies (407 patients) [133, 194] provided data on AKI. AKI occurred in 8/205 RIPC patients and in 11/ 202 controls. There was no evidence of heterogeneity between studies (Cochran Q = 1.01, p=0.31). The random effects model demonstrated no significant difference in AKI (pooled OR = 0.672, 95%CI 0.252 – 1.787, p=0.425) (figure 2.4).

Figure 2.4: Forest plot showing the pooled effect of RIPC on incidence of acute kidney injury

Odds ratio meta-analysis plot [random effects]

		odds ratio (95% confidence interval)
Luo		2.08 (0.11, 123.93)
Hoole		0.54 (0.15, 1.72)
combined [random]		0.67 (0.25, 1.79)
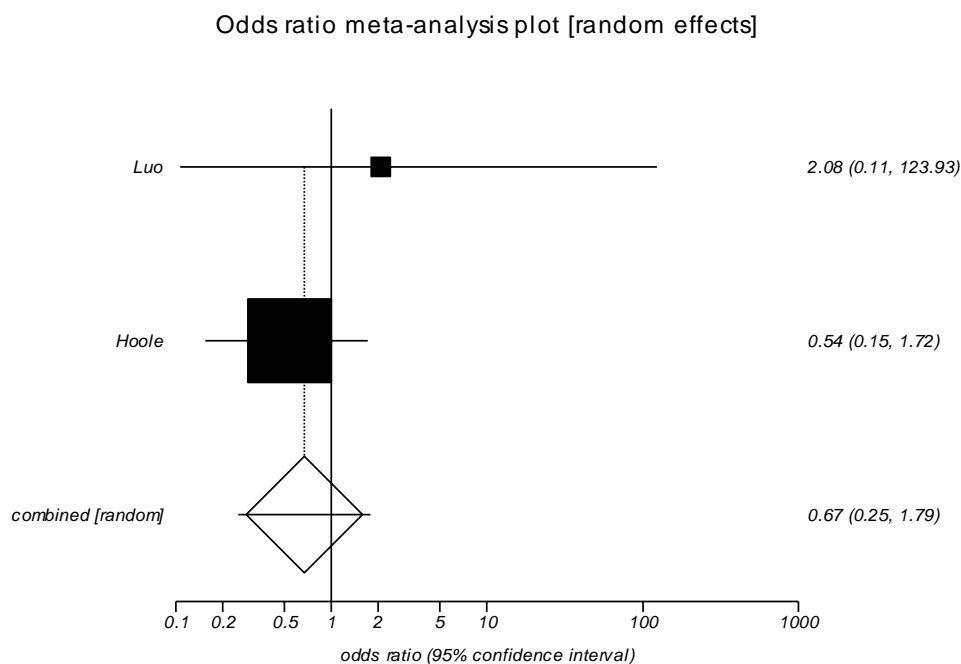
2.5: Discussion

This systematic review identified eight studies [103, 131-136, 194] (1119 patients) that examined the cardioprotective potential of RIPC in PCI. Six of these studies [103, 131, 133, 135, 136, 194] (983 patients) had primary outcomes that significantly favoured RIPC. Meta-analyses of pooled data relating to three pre-specified outcomes were performed: number of troponin positive events following elective PCI (377 patients) [132, 133, 136], periprocedural myocardial infarction incidence in elective PCI (636 patients) [133, 135, 136, 194] and acute kidney injury incidence in elective or emergency PCI (407 patients) [133, 194]. No difference was found between RIPC and control groups in relation to the incidence of troponin positive events or AKI – this is unsurprising given that these data were not available for many of the included studies despite an effort to contact individual study authors. However, our results show a significant benefit with RIPC in terms of reduction in the incidence of periprocedural MI (OR = 0.577, 95%CI 0.400 – 0.833, p=0.003). Although we were unsuccessful in attempts to acquire unpublished data on MI rates in two elective PCI studies [132, 134], the missing data relate to a small proportion (137/772) of elective PCI patients. Overall, this systematic review provides an up-to-date summary of the current status of RIPC in PCI and it represents a valuable opportunity to critically review prior studies and to consider future goals. In relation to the meta-analysis, the finding of reduced periprocedural MI incidence with RIPC highlights the importance of performing meta-analysis – though meta-analysis is not a flawless technique, the result compliments the proof of concept studies and may focus future research on RIPC.

Conclusions from a systematic review can only be as strong as the individual studies – in this review the included studies were heterogeneous in methodology (table 2.1) and in quality (table 2.2). Studies included patients with varying demographics undergoing both emergency and elective PCI with varying RIPC stimuli. Furthermore a range of primary outcome measures were used that included biochemical, electrocardiographic and radiographic endpoints. The method of reporting cardiac biomarker endpoints also differed between studies – some studies used biomarker positive events and others used mean and median biomarker levels. The hazards of this approach and the importance of having a published trial protocol with predefined outcomes are illustrated in the results of the Ghaemian *et al.* trial [136] – mean troponin T was significantly higher at 24 hours in the RIPC group compared to control (0.063ng/ml versus 0.016ng/ml, p=0.009) but the

number of troponin positive events at 24 hours was significantly lower in the RIPC group compared to control (5/40 versus 16/40, p=0.01). Among the included studies, secondary outcomes were also diverse although some overlap existed. Despite the large amount of clinical heterogeneity, the finding that six of the eight studies were in favour of RIPC represents a promising result. If one adopts an optimistic viewpoint, it could be argued that the study by Iliodromitis *et al.* [134] (it found a significantly worse result with RIPC) was limited by a small sample size and that the Prasad *et al.* study [132] (it found no difference between RIPC and control) may have experienced a type two error. A more conservative viewpoint would be to consider individual study weaknesses and biases (table 2.2) and await large studies that address these problems before drawing conclusions. Regarding the meta-analysis, it is important to reiterate that data were extracted based on definitions specified in the relevant manuscripts (table 3.3) and that these definitions differed between studies, most notably for periprocedural MI.

The strengths of this review relate to the thorough search strategy (including a detailed grey literature search), the predefined outcomes for meta-analysis and the large number of included patients. Furthermore, the application of the Cochrane bias assessment tool has added transparency and highlights areas that researchers could consider addressing. The principle limitation of this review is that heterogeneity of the included studies may limit extrapolation. However, highlighting this may help to guide future studies. Furthermore, the reported results represent published data only as we were unsuccessful in attempts to acquire unpublished data. As a result, the pooled outcomes described in this article correspond to a subset of the total number of relevant patients. As mentioned, the principle limitation of the meta-analyses, particularly the MI analysis, is that definitions differed between studies.

Many studies have confirmed that RIPC attenuates myocardial injury in a variety of cardiovascular interventions [89] and this review strongly suggests that a cardioprotective role also exists in PCI specifically. Furthermore, the potential for a long term benefit with RIPC has been suggested by long term follow up of the Hoole *et al.* study [196]. In order for the benefits of RIPC to be realised, we advocate a shift from surrogate outcomes and single centre studies to large scale studies with a focus on patient important outcomes – RIPC's proof of concept foundation is convincing. If large individual studies can confirm a reduction in periprocedural MI rates, RIPC use should become widespread before PCI.

Chapter 3: A systematic review and meta-analysis of the effect of remote ischaemic preconditioning on major clinical complications following major cardiovascular surgery

3.1: Abstract

A number of 'proof-of-concept' trials suggest that remote ischaemic
preconditioning (RIPC) reduces surrogate markers of end-organ injury in patients
undergoing major cardiovascular surgery. To date, few studies have involved hard
clinical outcomes as primary endpoints.

Randomised clinical trials of RIPC in major adult cardiovascular surgery were
identified by a systematic review of electronic abstract databases, conference
proceedings and article reference lists. Clinical end-points were extracted from
trial reports. In addition, trial principal investigators provided unpublished clinical
outcome data.

In total, 23 trials of RIPC in 2,200 patients undergoing major adult cardiovascular
surgery were identified. RIPC did not have a significant effect on clinical end-
points (death, perioperative myocardial infarction (MI), renal failure, stroke,
mesenteric ischaemia, hospital or critical care length of stay).

Pooled data from pilot trials cannot confirm that RIPC has any significant effect on
clinically relevant end-points. Heterogeneity in study inclusion and exclusion
criteria and in the type of preconditioning stimulus limits the potential for
extrapolation at present. An effort must be made to clarify the optimal
preconditioning stimulus. Following this, large-scale trials in a range of patient
populations are required to ascertain the role of this simple, cost-effective
intervention in routine practice.

3.2: Rationale for the use of remote ischaemic preconditioning in major cardiovascular surgery

Since the first suggestion that ischaemic preconditioning of one vascular bed could confer protection on a distant vascular bed [197], numerous laboratory studies have confirmed the existence of this 'remote preconditioning'. Virtually any organ or tissue may provide the remote stimulus [91]. Clinically, the most significant observation has been that brief periods of peripheral limb ischaemia followed by reperfusion confer protection to at-risk critical organs such as the heart, kidney and brain [198]. Such peripheral limb ischaemia has been applied non-invasively via blood pressure cuff induced occlusion of limb arteries and invasively by cross-clamping limb arteries during procedures. Interest in remote ischaemic preconditioning (RIPC) is increasing and there has been some progress in translating it from an experimental observation to a clinical intervention [61, 92]. It has the potential to provide a potent and cost-effective clinical intervention which can reduce the deleterious consequences of ischaemia-reperfusion injury in various organs, regardless of the initiating insult. The technique's simplicity and low adverse event profile renders it attractive to clinicians and healthcare providers.

Most 'proof-of-concept' studies in human patients have evaluated the ability of RIPC to protect against myocardial injury as determined by serum cardiac biomarkers. The majority of trials have been conducted in adult patients undergoing major cardiovascular surgery. These small trials have relied upon comparisons of tissue injury biomarkers such as troponins or neutrophil gelatin-associated lipocalin to determine whether RIPC has induced any protection. A number of them report some clinical end-points, such as perioperative death or hospital length of stay. The need to move away from such 'first-flail' surrogate markers and towards robust clinical evidence requiring large-scale clinical trials has been highlighted recently [199, 200]. All of the published meta-analyses confirm a significant biomarker reduction (table 3.1) [61, 82-89, 201, 202] and two of these reviews found significant reductions in MI rates [85, 89] – notably these reviews also contained patients undergoing percutaneous coronary intervention (PCI). A systematic review focusing solely on PCI studies also found a significant reduction in MI rates [203] and a recent large cardiac surgery trial reported improved all-cause mortality and reduced major adverse cardiac and cerebrovascular events (MACCE) with RIPC but these were secondary endpoints [204]. Unsurprisingly for an intervention that is at the "proof of concept" stage benefits in patient important outcome have yet to be definitively established.

Table 3.1: Details of prior systematic reviews and meta-analyses on RIPC and cardiovascular surgery

Author Year	Procedure types	Number of included CVS trials	Number of included CVS patients	Outcomes reported	Results
Yetgin [84] 2012	PCI and CABG	13 – also included 4 PCI trials	891– not including PCI	Biomarkers	Significant reduction in cardiac biomarkers
Takagi [83] 2011	CVS	9	482	Biomarkers, mortality and perioperative MI	Significant reduction in cardiac biomarkers but no clinical benefit
Takagi [82] 2008	CVS	4	184	Biomarkers	Significant reduction in cardiac biomarkers
Alreja [85] 2012	CVS and PCI	13 – also included 4 PCI trials	814 – not including PCI	Biomarkers and MI, CVA, AF, ventricular arrhythmia, CHF, inotrope usage, HD need, mortality	Significant reduction in biomarkers and MI rate across CVS and PCI studies
Pilcher [88] 2012	Cardiac surgery	10	693	Biomarkers and mortality	Significant biomarker reduction only
Zhou [87] 2012	Cardiac surgery	15	1155	Biomarkers and mortality, MV duration, ICU LOS, hospital LOS	Significant biomarker reduction only
Brevoord [89] 2012	CVS and PCI	19 – also included 4 PCI trials	1166 – not including PCI	Biomarkers and mortality, CVA, MI, AF, kidney injury, hospital LOS, ICU LOS	Significant reduction in biomarkers and MI rate.
D'Ascenzo [86] 2012	CABG	9	704	Biomarkers and length of hospital stay	Significant cardiac biomarker reduction.
Yang [201]	Cardiac	19	1234	Biomarkers,	Significant

2013	surgery			mortality, complications, hospital and ICU LOS.	cardiac biomarker reduction.
Yasin [202] 2014	Cardiac surgery	25	1762	Biomarkers, kidney injury, lung injury, mortality, ICU and hospital LOS, ventilation time.	Significant cardiac biomarker reduction.

AF – atrial fibrillation; CABG – coronary artery bypass graft; CHF – congestive heart failure; CVA – cerebrovascular accident; CVS – cardiovascular surgery; HD- haemodialysis; ICU – intensive care unit; LOS – length of stay; MI – myocardial infarction; MV – mechanical ventilation; PCI – percutaneous coronary intervention; RIPC – remote ischaemic preconditioning.

A collaborative group was formed, all of whom have undertaken RIPC 'proof-of-concept' studies. This group aimed to undertake a systematic review of RIPC in major adult cardiovascular surgery. The aim was to evaluate the effect of RIPC on clinical end-points using a combination of published and unpublished clinical outcome data from the 'proof-of-concept' trials. Though several prior systematic reviews and meta-analyses have been published [82-89, 201, 202] (table 3.1), a large quantity of clinical outcome data has remained unpublished.

3.3: Methodology for the systematic review and meta-analysis

The systematic review was conducted in accordance with the Preferred Reporting In Systematic Reviews and Meta-Analyses (PRISMA) guidelines [192].

Studies were eligible for inclusion provided that the following criteria were fulfilled: randomised clinical trial; adult patients aged 18 years or older; patients undergoing major elective or emergency cardiac or vascular surgery; patients randomised to standard management or to standard management with RIPC; RIPC involving brief periods of upper or lower limb ischaemia followed by reperfusion. Studies conducted in children or healthy volunteers were excluded in order to minimise clinical heterogeneity – these groups have a lower prevalence of atherosclerosis and therefore would likely have lower incidences of the relevant clinical outcomes.

In order to identify eligible studies, the Medline and Embase electronic databases were searched in January 2011 and supplementary searches were undertaken in August 2012, January 2013 and January 2014. The last search was performed on 2nd January 2014. The search was performed using the following combinations of free text: ([remote isch(a)emic preconditioning] OR [remote preconditioning] OR [isch(a)emic preconditioning]). Titles and abstracts were screened initially and full manuscripts were retrieved to finalise eligibility. Eligible article reference lists were scrutinised for further relevant studies. One reviewer (DA Healy) performed the search and identified potentially eligible manuscripts and two reviewers (DA Healy & SR Walsh) determined eligibility and extracted data. In addition, conference abstracts from a number of major cardiovascular conferences were reviewed for potentially relevant trials (Society of Cardiothoracic Surgery in Great Britain and Ireland (2004-2013), European Association for Cardio-thoracic Surgery (2004-2013), European Society of Thoracic Surgeons (2004-2013), European Society for Cardiovascular and Endovascular Surgery (2004-2011) and the Society of Cardiovascular Anesthesiologists (2004-2013). The American Heart Association (AHA) online abstract archive containing abstracts from all major AHA-sponsored meetings was also searched. Individual trial investigators were asked to provide details of any unpublished trials of which they were aware. The United States National Institutes of Health Clinical Trials database and the ISRCTN Register were searched for completed but as yet unreported trials.

The outcome measures recorded for the meta-analysis were: perioperative death, myocardial infarction (MI), new-onset cardiac arrhythmia requiring treatment,

cerebrovascular accident (CVA), renal failure requiring renal replacement therapy, mesenteric ischaemia, hospital stay and intensive care unit (ICU) stay. Perioperative was defined as within 30 days of surgery. These outcomes were predefined and are summarised in table 3.2). Where outcomes were not reported in trial manuscripts, unpublished data regarding the outcomes of interest were requested from the relevant trial principal investigators.

Statistical analyses were performed using RevMan 5 (The Cochrane Collaboration, Copenhagen, Denmark). Risk ratios (with 95% confidence intervals) were calculated for dichotomous outcomes. Pooled effects of RIPC on continuous outcomes were estimated using mean differences (with 95% confidence intervals). Meta-analyses were performed with Mantel-Haenszel random effects models. The potential influence of bias was estimated by visual inspection of funnel plots for each outcome. Statistical heterogeneity was evaluated using I^2 statistic ($I^2 \geq 50\%$ is considered to indicate heterogeneity). The 5% level was taken as significant throughout.

Chapter 3: Systematic review and meta-analysis of remote ischaemic preconditioning in major cardiovascular surgery

Table 3.2: Predefined 30-day outcomes

Outcome	Definition
Death	Death from any cause within 30 days of surgery
Myocardial infarction	The presence of at least 2 of the following: i) Characteristic ischaemic symptoms lasting at least 20 minutes ii) Electrocardiographic changes including acute ST elevation followed by the appearance of Q waves or the loss of R waves, the development of new left bundle branch block, new persistent T wave inversion lasting at least 24 hours or new ST segment depression persisting over 24 hours iii) Positive cTnT (>0.1 ng/ml) or cTn I (>0.1mg/ml) levels with a characteristic rise and fall in levels or CKMB greater than institutional limits with a characteristic rise and fall
New arrhythmia requiring treatment	1.Ventricular fibrillation requiring counter-shock 2. Ventricular tachycardia requiring counter-shock or medication 3.Atrial fibrillation of greater than 15 minutes duration requiring counter-shock or medication
Cerebrovascular accident	New onset neurological deficit, accompanied by evidence of cerebral infarction or intra-cerebral haemorrhage on CT scan, or confirmed at autopsy
Renal failure requiring renal replacement therapy	Haemodialysis, haemofiltration or peritoneal dialysis commenced post-operatively, within 30 days of surgery.
Mesenteric ischaemia	Small or large bowel ischaemia requiring laparotomy or found at autopsy or proven on colonic biopsy

cTnI - cardiac troponin I; cTnT – cardiac troponin T; CKMB – creatine kinase MB isozyme; CT – computed tomography.

3.4: Results of the systematic review and meta-analysis

The results of the search are summarised in figure 3.1. 9,512 potentially relevant citations were initially identified. 9,463 citations were excluded based on titles and abstracts and 49 full manuscripts were retrieved. 23 studies were finally eligible for inclusion. This number comprised 22 trial reports in full manuscript format [107-109, 111-116, 118, 121-123, 140-144, 204-207] and 1 trial report in abstract format only [208]. Characteristics of the included cardiac surgery trials are summarised in table 3.3 and of the included vascular surgery trials are summarised in table 3.4. The majority of trial reports did not include the clinical outcome data specified in table 3.2. These previously unpublished data were provided post-hoc by trial principal investigators. Data from two trials conducted in the same unit were provided in pooled form and are treated as a single trial in the meta-analysis [107, 108]. One trial [123] had three arms (control, upper limb RIPC, dual upper and lower limb RIPC). As summary outcome data only were provided, we chose to use only the control and upper limb RIPC groups for the purposes of this review.

Chapter 3: Systematic review and meta-analysis of remote ischaemic preconditioning in major cardiovascular surgery

Figure 3.1: Summary of the search results for the systematic review and meta-analysis of remote ischaemic preconditioning in major cardiovascular surgery

Potentially relevant articles n=9,512

(Medline and Embase n=9,510; grey literature n=2)

Not relevant after title and abstract review

(n = 9,463)

Manuscripts screened

(n = 49)

Manuscripts excluded (n=26):

Duplication of data or substudies (n=3)

Letters or reviews (n=10)

Studies involving children (n=5)

Not limb RIPC in cardiovascular surgery (n=4)

No clinical outcomes data available despite efforts to contact author (n=4)

Studies eligible for inclusion n=22

(22 full manuscripts and 1 abstract)
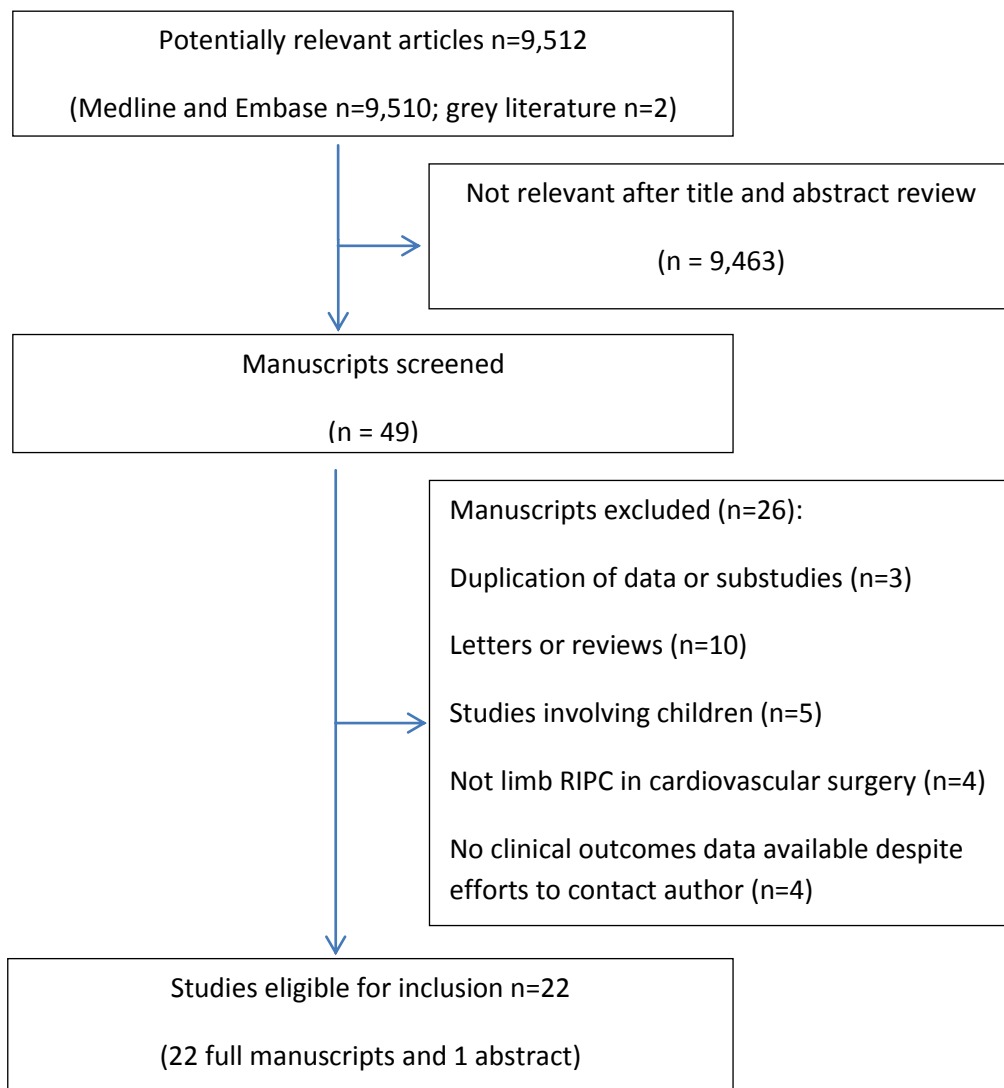
Table 3.3: Characteristics of included trials on cardiac surgery

Author Year Participant number (RIPC:Control)	Significant primary outcome result in favour of RIPC	Type of surgery	Site and method of applying of the ischaemia/reperfusion stimulus	Timing of the stimulus in relation to anaesthetic and surgery	Use of volatile anaesthetic agents	Age profile of participants	Specified exclusion criteria	Blinding	Original outcomes reported	Result
Hausenloy [107] 2007 27/30	Yes	Elective CABG on CPB	Upper limb 3x5min cycles	After induction of anaesthesia and before surgery.	No volatile agents.	Mean age of 67 in both groups.	Unstable angina; left main stem disease; age > 80 years; hepatic, pulmonary or renal disease; upper limb PVD; glibenclamide use	Controls had a deflated cuff placed on the arm to bind surgeons.	AUC for serum cTnT levels for 72 hours post-operatively	Significant reduction in post operative cTnT.
Venugopal [108] 2009 23/22	Yes	Elective CABG on CPB	Upper limb 3x5 min cycles	After induction of anaesthesia and before surgery.	17/22 in control group and 17/23 in RIPC group had inhalational anaesthesia.	Mean age was 64 in control group and 62 in RIPC group.	Unstable angina; age > 80 years; hepatic, pulmonary or renal disease; upper limb PVD; diabetes mellitus	Controls had a deflated cuff placed on the arm to blind surgeons. Anaesthetists and investigators were not blinded.	AUC for serum cTnT levels for 72 hours post-operatively	Significant reduction in post operative cTnT.
Ali N [109] 2010 50/50	Yes	Elective CABG on CPB	Upper limb 3x5 min cycles.	After induction of anaesthesia and before	General anaesthesia. No further details	Mean age 51.6 in control arm and 56 in RIPC	Significant renal or hepatic disease;	Controls had deflated cuff placement to	Post-operative serum CKMB levels; post-	Significant reduction in post operative

				extracorporeal bypass.	stated.	arm.	haemodynamic instability; ECG or cardiac enzyme levels indicating ongoing ischaemia and infarction; MI in previous 4 weeks; significant PVD	blind surgeons.	operative MI; major neurological complication; inotrope requirements	CKMB. No difference in other outcomes.
Li Cardiac [205] 26/27 2010	No	Valve replacement on CPB	Lower limb 3x4 min cycles	After induction of anaesthesia. Time in relation to surgery commencement not stated.	Isoflurane used for maintenance	Mean age 42.3 in control arm and 45.8 in RIPC arm	Infective endocarditis; previous cardiac surgery; complications with coronary artery disease, hypertension, diabetes mellitus or lower limb PVD; aspirin, statin, corticosteroid or ACEI user; patients < 18 years or > 65 years of age	Controls had placement of a deflated cuff to blind surgeons. Data collectors and analysers were kept blind to allocation group.	Intensive care stay; hospital stay; inotrope requirement; AUC for serum cTnI levels for 72 hours post-operatively	No difference in AUC for cTnI for 72 hours. There was a significant reduction at 5 and 30 minutes however. No difference for other outcomes.
Rahman [111] 2010 80/82	No	Elective and emergency CABG on CPB	Upper limb 3x5 min cycles	After induction of anaesthesia. Time in relation	Enflurane or sevoflurane maintenance	Median age 65 in control arm and 63 in RIPC	MI in previous 30 days; angina pain < 48 hours	Controls had cuff inflation of a cuff on a	AUC for serum cTnT levels for 48 hours post-	No difference in post operative cTnT levels. No

				to surgery commencement not stated.	while on CPB.	arm	pre-procedure; diabetes mellitus; pregnancy; pre-operative dialysis; radial artery usage; additional non-CABG surgery	dummy arm. Blinding of all staff and patients was achieved.	operatively; myocardial injury; intensive care unit stay; hospital stay; reperfusion arrhythmias; death	difference in other outcomes.
Hong [112] 2010 65/65	No	Elective CABG without CPB	Upper limb 4x5 min cycles	After induction of anaesthesia with surgery proceeding.	Maintenance with sevoflurane.	Mean age 65.1 in control arm and 65.7 in RIPC arm.	Age > 80 years; unstable angina; pre-operative inotrope or mechanical cardiac assistance; LVEF < 30%; major concomitant procedures; severe renal, pulmonary or hepatic disease; MI in previous 7 days; systemic infection or sepsis in previous 7 days; upper limb amputation; nicorandil use	Controls had cuff placement with simulated inflation to blind surgeon (air escaped via three-way tap). Blinding of all staff and patients was achieved.	AUC for serum cTnI levels for 72 hours post-operatively; death; MI; stroke; hospital stay; intensive care unit stay	No difference in AUC for cTnI. No difference in other outcomes.
Choi [122]	No	Elective valve	Lower limb	After induction	Maintenance	Mean age 60 in	Age > 80years;	Controls had	Post-operative	No difference in

2011 38/38		surgery under CPB	3x10 min cycles	of anaesthesia and at least 10 minutes before CPB.	with sevoflurane.	control group and 57 in RIPC.	left main coronary disease greater than 50%; hepatic, renal or pulmonary disease; infective endocarditis; LVEF < 30%; MI within 3 weeks; lower limb PVD; tricuspid valve surgeries; hypothermic circulatory arrest; patients taking sulphonyureas or nicorandil.	placement of a deflated leg cuff to blind surgeon and anaesthetist. No further details on blinding methods.	serum creatinine, cystatin C, NGAL; acute kidney injury; myocardial injury	renal injury. CKMB levels significantly lower in RIPC group at 24 hours. Intensive care unit stay was significantly shorter in RIPC group.
Zimmerman [113] 2011 60/60	Yes	Elective CABG +/- aortic valve surgery with CPB	Lower limb 3x5 min cycles	After induction of anaesthesia and at least 10 minutes before CPB.	Maintenance with isoflurane.	Mean age 65 in control group and 62 in RIPC group.	Age < 18 or > 80 years; lower limb PVD with tissue loss; end-stage renal disease; planned off-pump surgery; inability to provide informed	No sham procedure for controls. Only patients were blinded.	AKI defined as any rise in serum creatinine $\geq$0.3ng/ml above baseline or $\geq$ 50% above baseline; hospital stay; death; MI	Significant reduction in AKI in RIPC group, no difference in other outcomes.

							consent			
Karupassamy [114] 2011 27/27	No	Elective CABG surgery with CPB	Upper limb 3x5 min cycles	After induction of anaesthesia. Time in relation to surgery commencement not stated.	Maintenance with isoflurane until CPB.	Mean age 67.3 in control group and 66.9 in RIPC group.	Unstable angina; age > 85 years; hepatic, pulmonary or renal disease; upper limb PVD; use of sulphonylureas or other RIPC-inhibiting or activating drugs	Controls had a deflated cuff placed on the arm to blind surgeons. No further details regarding blinding stated.	AUC for serum cTnI levels for 48 hours post-operatively; inotrope usage; hospital stay; intensive care stay	No difference in CTnI or other outcomes.
Wu [123] 2011 25/25	No	Mitral valve replacement with CPB.	Upper limb 3x5 min cycles. As summary outcome only were stated, we excluded lower limb RIPC group.	After induction of anaesthesia and before surgery.	No volatile agents	Mean age 43.6 in control group and 46.2 in RIPC group.	Age > 60 or <18; other cardiac abnormalities; NYHA Class IV; history of respiratory infection, asthma, cardiac surgery; peripheral vascular disease affecting relevant limbs; glibenclamide use	Controls had a deflated cuff placed on the arm. No further details on blinding.	Serial cTnI levels over 72 hours post-operatively	No difference between groups.
Young [206] 2012 48/48	No	Elective CABG +/- valve surgery	Upper limb 3x5 min cycles	After induction and beginning at first incision.	All had maintenance with propofol and isoflurane.	Mean age 64.4 in control group and 65.5 in RIPC group.	Age<18; upper limb PVD, requirement for deep	Controls had inflation of a cuff around a dummy arm to	High sensitivity cTnT 6 hours and 12 hours after crossclamp	Increased cTnT in the RIPC group. No other difference in

							hypothermic circulatory arrest, radial artery harvest being considered.	blind surgeon. Blinding of all staff and patients was achieved	removal, duration of noradrenaline use in ICU survivors, worst post op renal injury plus secondary outcomes.	outcomes.
Lucchinetti [115] 2012 27/28	No	Elective CABG with CPB	Lower limb 4x5 min cycles	After induction of anaesthesia and before CPB.	Maintenance with isoflurane.	Mean age 62 in control arm and 59 in RIPC arm.	Emergency surgery; MI within 48 hours before surgery as defined by raised serum cardiac enzymes; DM; BMI > 35; concomitant non cardiac surgery, severe peripheral vascular disease	Controls had placement of a deflated cuff to blind surgeons. Data collectors and analysers were blinded.	Peak and AUC for high sensitivity cTnT levels for 72 hours post-operatively; secondary outcomes plasma NT-proBNP, high sensitivity CRP, S100 and short and long term clinical outcomes	No difference between groups.
Xie [116] 2012 38/35	No	Elective CABG with CPB	Upper limb 3x5 min cycles	After induction of anaesthesia and completed before surgery.	A few patients needed sevoflurane for maintenance.	Mean age 50.4 in control arm and 51.1 in RIPC arm.	Detection of limb ischaemia.	Controls had placement of a deflated cuff. Patents, outcome assessors and analysts were blinded.	AUC for serum cTnI levels for 72 hours post-operatively; B mode ultrasound measurement of cardiac	Reduced cTnI levels in RIPC group. Improved cardiac function in RIPC group.

										function, mortality, NYHA class	
Lomivorotov [118] 2012 40/40	No	Elective CABG with CPB	Upper limb 3x5 min cycles	After induction of anaesthesia and not more than 20 minutes until aortic crossclamp	Maintenance with isoflurane.	Mean age 56.5 in RIPC arm and 58.1 in control arm.	LVEF<50% Renal Failure Hepatic/Pulmonary disease Diabetes MI in last 4weeks	Controls had placement of a deflated cuff on the arm. No further blinding details.	Serial cTnI & CKMB Haemodynamic outcomes Duration of ventilation ICU stay Complications Blood loss Mortality	No difference in biomarker levels. Increased cardiac index immediately after RIPC.
Thielmann [204] 2013 162/167	Yes	CABG with CPB	Upper limb 3x5 minute cycles	After induction of anaesthesia and before skin incision	Anaesthesia was maintained with isoflurane in 250/329 participants	Mean age was 69.1 years on control group and 68.2 in RIPC group	Baseline renal disease (creatinine > 200µmol/l, upper limb PVD, ACS within 4 weeks, need for inotropic or mechanical support before anaesthesia, disorders that would increase cTnI, surgery without CPB, emergency or repeat or	A non inflated cuff was used on controls. Patients, surgeons and intensivists were unaware of allocation.	AUC for cTnI, mortality, MI, CVA, need for coronary revascularisation.	Significant reduction in cTnI with RIPC. All cause mortality at 1 year was lower with RIPC.

							comcommitant surgery.			
Meybohm [121] 2013 90/90	No	Elective cardiac surgery on CPB	Upper limb 4x5 minute cycles	After induction of anaesthesia and before surgery	No volatile agents	Mean age was 68 in control group and 70 in RIPC group.	Concommitant carotid surgery, minimally invasive surgery, MI within 7 days, LVEF<30%, atrial fibrillation within 6 months, previous stroke, renal failure or respirator disease, pacemaker or defibrillator in situ, antiarrythmic, sulfonamide or nocorandil medication.	Controls had cuff inflation to 20mmHg to blind surgeons and anaesthetists. Patients and outcome assessors were blinded.	Postoperative neurocognitive dysfunction, duration of ventilator support, reintubation rate, LOS, AKI, incidence of new atrial fibrillation, troponin T at 12, 24 and 48 hours.	No significant difference in any outcome
Candilio [208] 2013 90:90	Yes	CABG +/- valve surgery	Upper and lower limb simultaneously. 2x5 minute cycles.	Stimulus began prior to induction of anaesthesia	Not specified	Not specified	Not specified	Upper and lower limb non0inflated cuffs were placed on controls.	AUC for cTnT, AKI, new AF, ICU stay, hospital stay, inotrope score, mortality, MI, stroke, revascularisation.	Significant results favouring RIPC regarding cTnT, AKI, new AF and ICU stay.

Joung [207] 2013 35/35	No	Elective off pump CABG	Upper limb 3x5min cycles	After induction of anaesthesia and before coronary anastamosis	No	Mean age of 61.1 years in RIPC group and 59 in controls.	Emergency surgery, age≤40 or ≥80 years, mechanical assistance device, preoperative inotrope use, LVEF<30%, neuropsychiatric disease.	Controls had a deflated cuff placed on the arm.	Cognitive dysfunction, extubation time, ICU length of stay, maximal cardiovascular component of SOFAc score.	No difference between groups.

ACEI – angiotensin converting enzyme inhibitor; AF – atrial fibrillation; AKI – acute kidney injury; AUC – area under curve; BMI – body mass index; CABG – coronary artery bypass graft; CKMB – creatine kinase MB isozyme; CPB – cardiopulmonary bypass; CRP – C reactive protein; cTnI – cardiac troponin I; cTnT – cardiac troponin T; DM – diabetes mellitus; ECG – electrocardiogram; eGFR – estimated glomerular filtration rate; ICU – intensive care unit; LVEF – left ventricular ejection fraction; MI – myocardial infarction; NGAL – neutrophil gelatinase-associated lipocalin; NT pro BNP – N-terminal pro b-type natriuretic peptide; NYHA – New York Heart Association; PVD – peripheral vascular disease; RIPC – remote ischaemic preconditioning; SOFAc – sequential organ failure assessment score.

Table 3.4: Characteristics of included trials on vascular surgery

Author Year Participant number (RIPC:Control)	Significant primary outcome result in favour of RIPC	Type of surgery	Site and method of applying the ischaemia/reperfusion stimulus	Timing of the stimulus	Use of volatile anaesthetic agents	Age profile of participants	Specified exclusion criteria	Blinding	Original outcomes reported	Overall result
Ali ZA [140] 2007 41/41	Yes	Elective open AAA repair	Lower limb 1x10 min cycle on each leg sequentially by cross clamping iliac artery	After laparotomy	Maintenance with desflurane.	Mean age 74 in RIPC group and 75 in control group	Age > 90 years; concomitant procedure required; ACS or MI in previous 3 months; inability to provide informed consent; nicorandil or sulphonylurea user	Patients and data collectors were blinded but not surgeons or anaesthetists.	Myocardial injury defined as rise in serum cTnI >0.4ng/ml; MI; renal impairment 9peak serum creatinine > 177 μmols/ml); death	RIPC reduced cTnI levels. MI and renal impairment rates were reduced in RIPC group.
Walsh EVAR [143] 2009 18/22	No	Elective endovascular repair of AAA	Lower limb 1x10 min cycle on each leg sequentially by cuff inflation	After induction of anaesthesia	Maintenance with desflurane.	Mean age 74 in RIPC group and 76 in control group	Previous history of renal disease, renal replacement therapy or renal transplant; baseline serum creatinine > 150 mmols/l; baseline serum urea >	No blinding.	Urinary RBP and ACR levels; serum creatinine and eGFR; post-operative serum cTnI > 0.15 ng/ml; major adverse cardiac events; death.	No significant difference in outcomes.

							20mmols/l; adjunctive procedures; previous lower limb amputation			
Walsh Carotid [144] 2010 34/36	No	Elective carotid endarterectomy	Lower limb 1x10 min cycle on each leg sequentially by cuff inflation	After induction of anaesthesia	Maintenance with desflurane or isoflurane.	Mean age 69.5 in RIPC group and 68.4 in control group	ABPI < 0.7; previous lower limb amputation; visual loss in one or both eyes;	.No blinding	Significant post-operative deterioration in saccadic latency; serum cTnI. 0.15ng/ml; major adverse cardiac events; death	No difference between groups.
Walsh Open AAA [141] 2010 22/18	No	Elective open repair of AAA.	Lower limb 1x10 min cycle on each leg sequentially by cross clamping iliac artery	After initial laparotomy	Maintenance with desflurane.	Mean age 75 in RIPC group and 72 in control group	Previous history of renal disease, renal replacement therapy or renal transplant; baseline serum creatinine > 150 mmols/l; baseline serum urea > 20mmols/l; adjunctive procedures; previous lower limb amputation;	No blinding.	Urinary RBP and ACR levels; serum creatinine and eGFR; major adverse cardiac events; death.	No significant differences between groups.

							suprarenal clamp required; previous EVAR.			
Li [142] 2013 31/31	Yes	Elective open infrarenalAAA repair	Upper limb 3x5 minute cycles	After induction of anaesthesia and before surgery	No volatile agents were used.	Mean age was 67 in control group and 62 in RIPC group	Age>80, Mi within 3 months, angina within 48 hours, EF<40%, respiratory disease including COPD, chronic intestinal disease, recent diarrhoea	Surgeons were blinded by using a non-inflated cuff placed on the arm of control group patients. Data collectors and outcome assessors were blinded too.	a/A ratio, other pulmonary injury makers, intestinal injury biomarkers, intestinal and pulmonary injury scores, inflammatory markers, ventilator time, ICU and hospital free days, new arrhythmias, MI, CHF, neurologic events, dialysis use, upper limb ischaemia.	Pulmonary and intestinal injury were reduced with RIPC as was inflammatory response. No difference in other outcomes.

AAA – abdominal aortic aneurysm; a/A – arterial alveolar oxygen tension; ABPI – ankle brachial pressure index; ACR – albumin creatinine ratio; ACS – acute coronary syndrome; AKI – acute kidney injury; cTnI – cardiac troponin I; CHF – congestive heart failure; COPD – chronic obstructive pulmonary disease; eGFR – estimated glomerular filtration rate; EVAR – endovascular aneurysm repair; LOS – length of stay; LVEF – left ventricular ejection fraction; MI – myocardial infarction; RBP – retinal binding protein; RIPC – remote ischaemic preconditioning.

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Perioperative death

Perioperative mortality data were available from 21 trials [107, 108, 111-113, 115, 116, 118, 121-123, 140-144, 204-208]. There were 14 deaths among 1,019 RIPC patients compared to 14 among 1,027 control patients. Overall, RIPC had no effect on perioperative mortality (pooled risk ratio = 0.91; 95% CI = 0.43 to 1.95). There was no evidence of heterogeneity ($I^2 = 0\%$). The funnel plot was symmetrical (figure 3.3).

Figure 3.2: Forest plot for the outcome of perioperative death

Study or Subgroup	RIPC Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Ali ZA	2	41	3	41	19.0%	0.67 [0.12, 3.78]
Candilio	1	90	1	90	7.5%	1.00 [0.06, 15.74]
Choi	0	38	0	38		Not estimable
Hausenloy, Venugopal	0	50	0	50		Not estimable
Hong	0	65	0	65		Not estimable
Joung	0	35	0	35		Not estimable
Li AAA	0	31	0	31		Not estimable
Li Cardiac	0	26	0	27		Not estimable
Lomivorotov	0	40	0	40		Not estimable
Lucchinetti	0	27	0	28		Not estimable
Meybohm	3	90	1	90	11.4%	3.00 [0.32, 28.30]
Rahman	0	80	0	82		Not estimable
Thielmann	3	162	6	167	30.5%	0.52 [0.13, 2.03]
Walsh Carotid	0	34	0	36		Not estimable
Walsh EVAR	1	18	0	22	5.8%	3.63 [0.16, 84.11]
Walsh Open	3	22	0	18	6.8%	5.78 [0.32, 105.12]
Wu	0	25	0	25		Not estimable
Xie	0	38	1	35	5.7%	0.31 [0.01, 7.31]
Young	1	48	1	48	7.6%	1.00 [0.06, 15.53]
Zimmerman	0	59	1	59	5.7%	0.33 [0.01, 8.02]
Total (95% CI)		1019		1027	100.0%	0.91 [0.43, 1.95]
Total events	14		14			

Heterogeneity: Tau² = 0.00; Chi² = 5.06, df = 8 (P = 0.75); I² = 0%
Test for overall effect: Z = 0.23 (P = 0.82)

Figure 3.3: Funnel plot for outcome of perioperative death

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Myocardial infarction

These data were available from 17 trials [107-109, 111-113, 115, 121, 122, 140-144, 204, 205, 208] (1,777 patients) (figure 3.4). Remote preconditioning had no significant effect on the risk of perioperative MI (25/883 RIPC group versus 44/894 controls; pooled risk ratio 0.69; 95% CI 0.34 to 1.40) (figure 3.4). There was no evidence of heterogeneity ($I^2 = 31\%$) and the funnel plot was symmetrical (figure 3.5).

Figure 3.4: Forest plot for the outcome of perioperative myocardial infarction

Study or Subgroup	RIPC Events	RIPC Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Ali N	0	50	0	50		Not estimable
Ali ZA	2	41	11	41	15.5%	0.18 [0.04, 0.77]
Candilio	0	90	0	90		Not estimable
Choi	0	38	0	38		Not estimable
Hausenloy, Venugopal	0	50	0	50		Not estimable
Hong	0	65	0	65		Not estimable
Li AAA	2	31	1	31	7.5%	2.00 [0.19, 20.93]
Li Cardiac	0	26	0	27		Not estimable
Lucchinetti	3	27	1	28	8.4%	3.11 [0.34, 28.09]
Meybohm	4	90	1	90	8.6%	4.00 [0.46, 35.09]
Rahman	4	80	7	82	19.6%	0.59 [0.18, 1.92]
Thielmann	8	162	21	167	28.5%	0.39 [0.18, 0.86]
Walsh Carotid	0	34	0	36		Not estimable
Walsh EVAR	1	18	1	22	5.9%	1.22 [0.08, 18.20]
Walsh Open	1	22	1	18	5.9%	0.82 [0.05, 12.19]
Zimmerman	0	59	0	59		Not estimable
Total (95% CI)		883		894	100.0%	0.69 [0.34, 1.40]
Total events	25		44			

Heterogeneity: Tau² = 0.30; Chi² = 10.13, df = 7 (P = 0.18); I² = 31%
Test for overall effect: Z = 1.02 (P = 0.31)

CI – confidence interval; M-H – Mantel–Haenszel random effects model; RIPC remote ischaemic preconditioning.

Figure 3.5: Funnel plot for the outcome of perioperative myocardial infarction
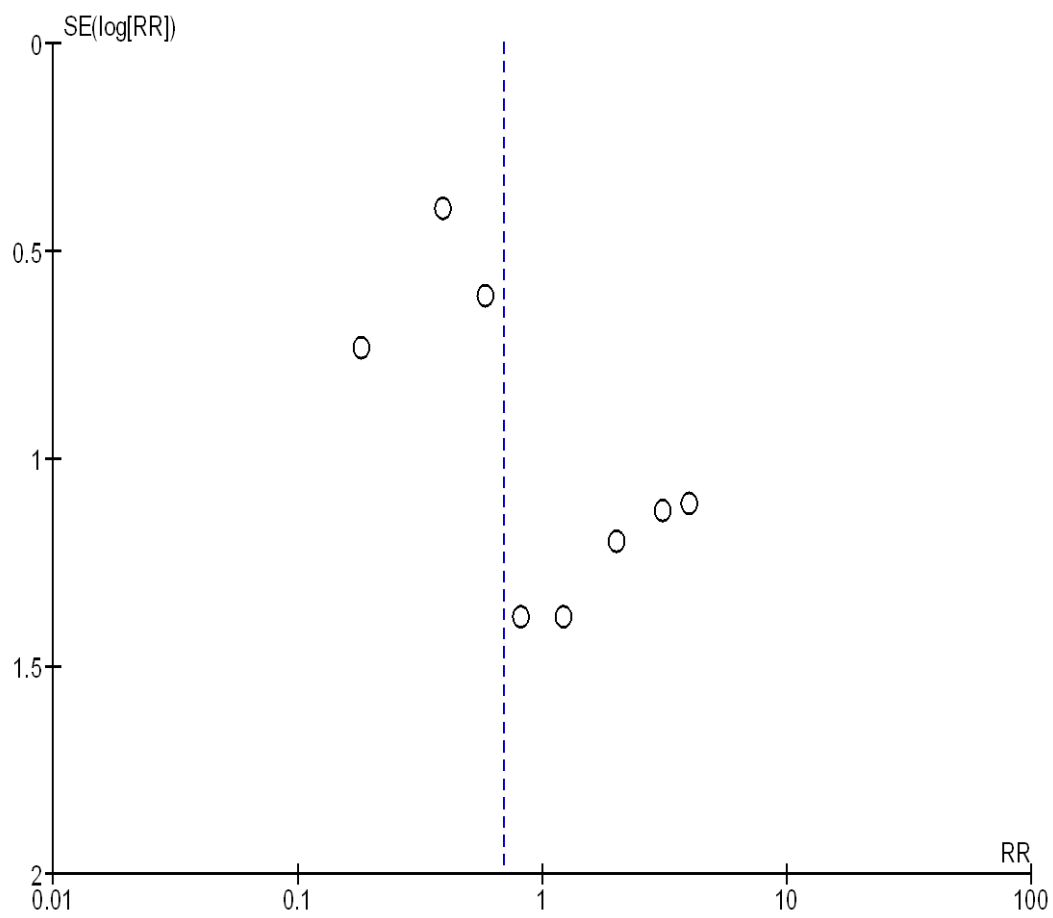
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Cerebrovascular accident

CVA rates were obtained from 18 trials [107-109, 112, 113, 115, 118, 121, 122, 140-144, 204, 205, 207, 208] (1,765 patients). There were 7 strokes among 878 RIPC patients compared to 7 of 887 controls, a non-significant difference (pooled risk ratio 1.02; 95% CI 0.35 to 3.02) (figure 3.6). There was no statistical heterogeneity ($I^2 = 0\%$) and the funnel plot was symmetrical (figure 3.7).

Figure 3.6: Forest plot for the outcome of cerebrovascular accident

Study or Subgroup	RIPC Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Ali N	0	50	0	50		Not estimable
Ali ZA	0	41	0	41		Not estimable
Candilio	2	90	0	90	12.8%	5.00 [0.24, 102.71]
Choi	0	38	1	38	11.7%	0.33 [0.01, 7.93]
Hausenloy, Venugopal	1	50	2	50	20.9%	0.50 [0.05, 5.34]
Hong	0	65	0	65		Not estimable
Joung	0	35	0	35		Not estimable
Li AAA	0	31	0	31		Not estimable
Li Cardiac	0	26	0	27		Not estimable
Lomivorotov	0	40	0	40		Not estimable
Lucchinetti	0	27	0	28		Not estimable
Meybohm	4	90	2	90	41.9%	2.00 [0.38, 10.65]
Thielmann	0	162	2	167	12.8%	0.21 [0.01, 4.26]
Walsh Carotid	0	34	0	36		Not estimable
Walsh EVAR	0	18	0	22		Not estimable
Walsh Open	0	22	0	18		Not estimable
Zimmerman	0	59	0	59		Not estimable
Total (95% CI)		878		887	100.0%	1.02 [0.35, 3.02]
Total events	7		7			

Heterogeneity: Tau² = 0.00; Chi² = 3.58, df = 4 (P = 0.47); I² = 0%
Test for overall effect: Z = 0.04 (P = 0.97)

98

Figure 3.7: Funnel plot for the outcome of cerebrovascular accident
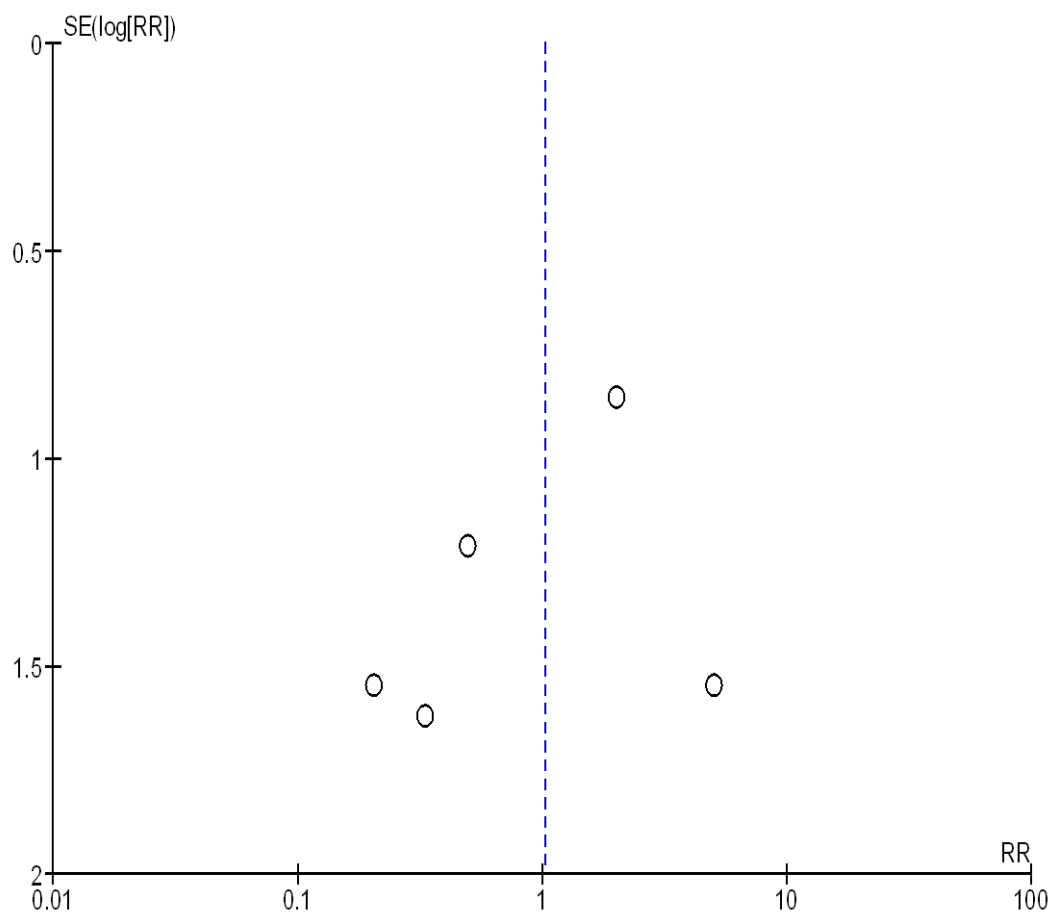
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Renal failure requiring renal replacement therapy

Data on the incidence of renal failure requiring renal replacement therapy were available for 17 trials [107, 108, 111-113, 115, 118, 121, 122, 140-144, 204-206] (1,673 patients). Renal replacement therapy was required in 15 of 831 RIPC patients compared to 9 of 842 control patients. The difference was not statistically significant (pooled risk ratio 1.55; 95% CI 0.67 to 3.58) (figure 3.8). There was no evidence of heterogeneity ($I^2 = 0\%$) nor of publication bias (figure 3.9).

Figure 3.8: Forest plot for the outcome of renal failure requiring renal replacement therapy

Study or Subgroup	RIPC Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Ali ZA	0	41	0	41		Not estimable
Choi	0	38	0	38		Not estimable
Hausenloy, Venugopal	2	50	1	50	12.4%	2.00 [0.19, 21.36]
Hong	1	65	0	65	6.9%	3.00 [0.12, 72.31]
Li AAA	0	31	0	31		Not estimable
Li Cardiac	0	26	0	27		Not estimable
Lomivorotov	0	40	0	40		Not estimable
Lucchinetti	0	27	0	28		Not estimable
Meybohm	4	90	2	90	25.0%	2.00 [0.38, 10.65]
Rahman	3	80	1	82	13.9%	3.08 [0.33, 28.95]
Thielmann	2	162	4	167	24.6%	0.52 [0.10, 2.78]
Walsh Carotid	0	34	0	36		Not estimable
Walsh EVAR	0	18	0	22		Not estimable
Walsh Open	2	22	0	18	7.9%	4.13 [0.21, 80.91]
Young	1	48	1	48	9.3%	1.00 [0.06, 15.53]
Zimmerman	0	59	0	59		Not estimable
Total (95% CI)		831		842	100.0%	1.55 [0.67, 3.58]
Total events	15		9			

Heterogeneity: Tau² = 0.00; Chi² = 2.82, df = 6 (P = 0.83); I² = 0%
Test for overall effect: Z = 1.03 (P = 0.30)

Figure 3.9: Funnel plot for the outcome of renal failure requiring renal replacement therapy

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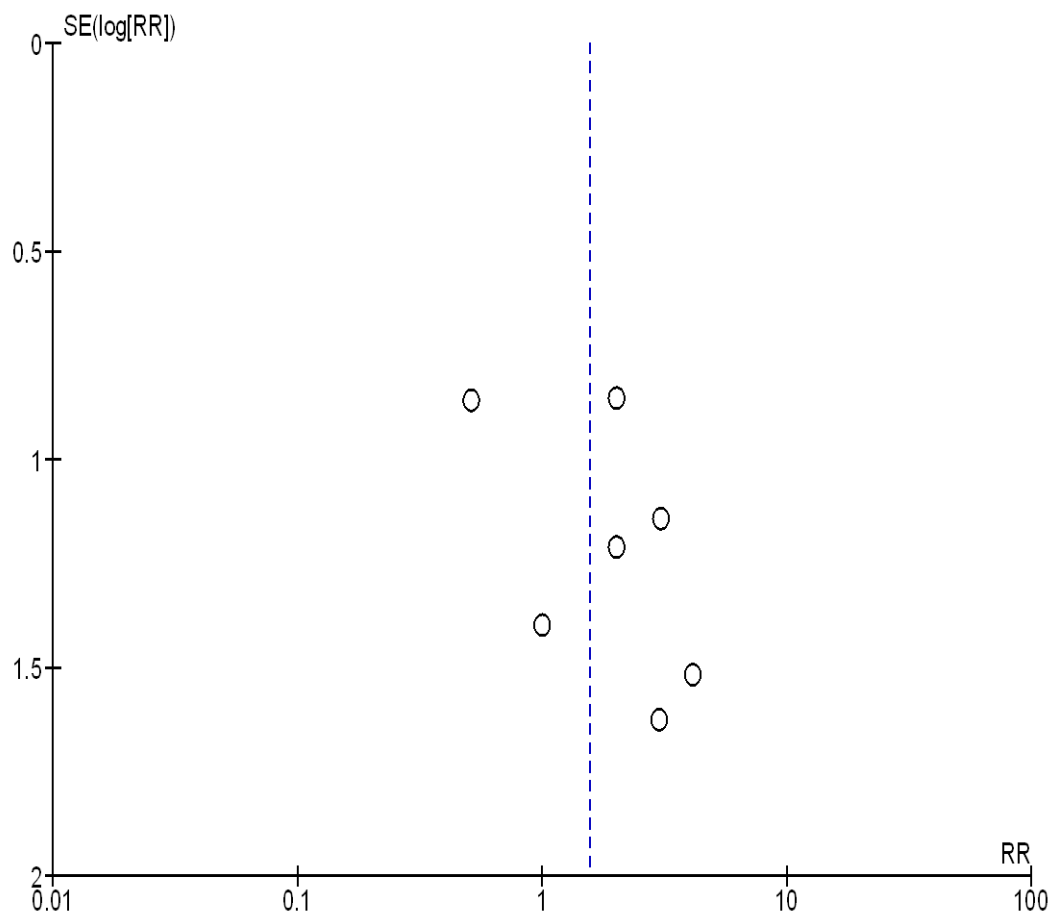
New-onset arrhythmias

There was no evidence that RIPC reduced new-onset arrhythmias requiring treatment. These data were available from 16 trials [107, 108, 111, 112, 115, 118, 121, 122, 140-144, 204, 205, 208] (814 RIPC patients; 825 controls). Arrhythmias occurred in 152 RIPC patients, compared to 167 controls (pooled risk ratio 0.92; 95% CI 0.76 to 1.12) (figure 3.10). There was no evidence of heterogeneity ($I^2 = 1\%$) and the funnel plot was symmetrical (figure 3.11).
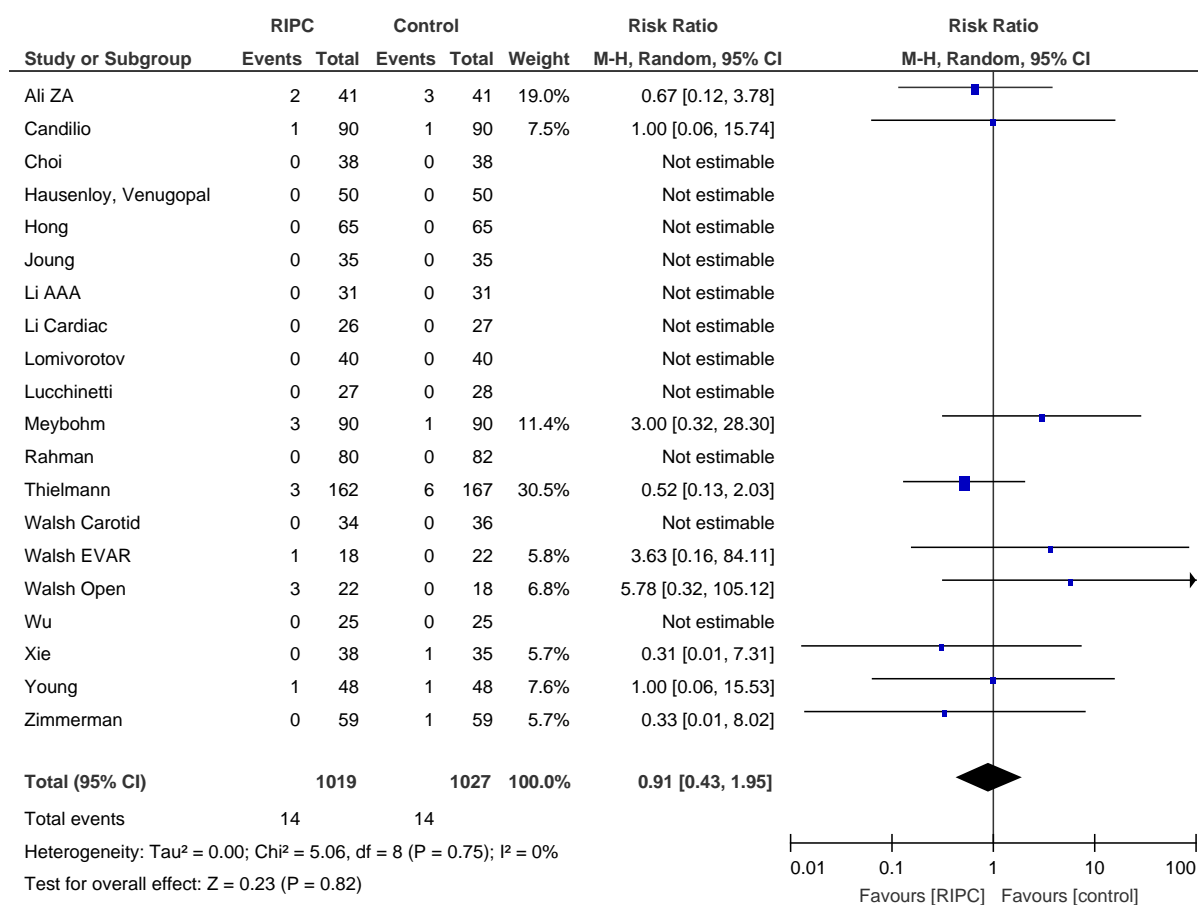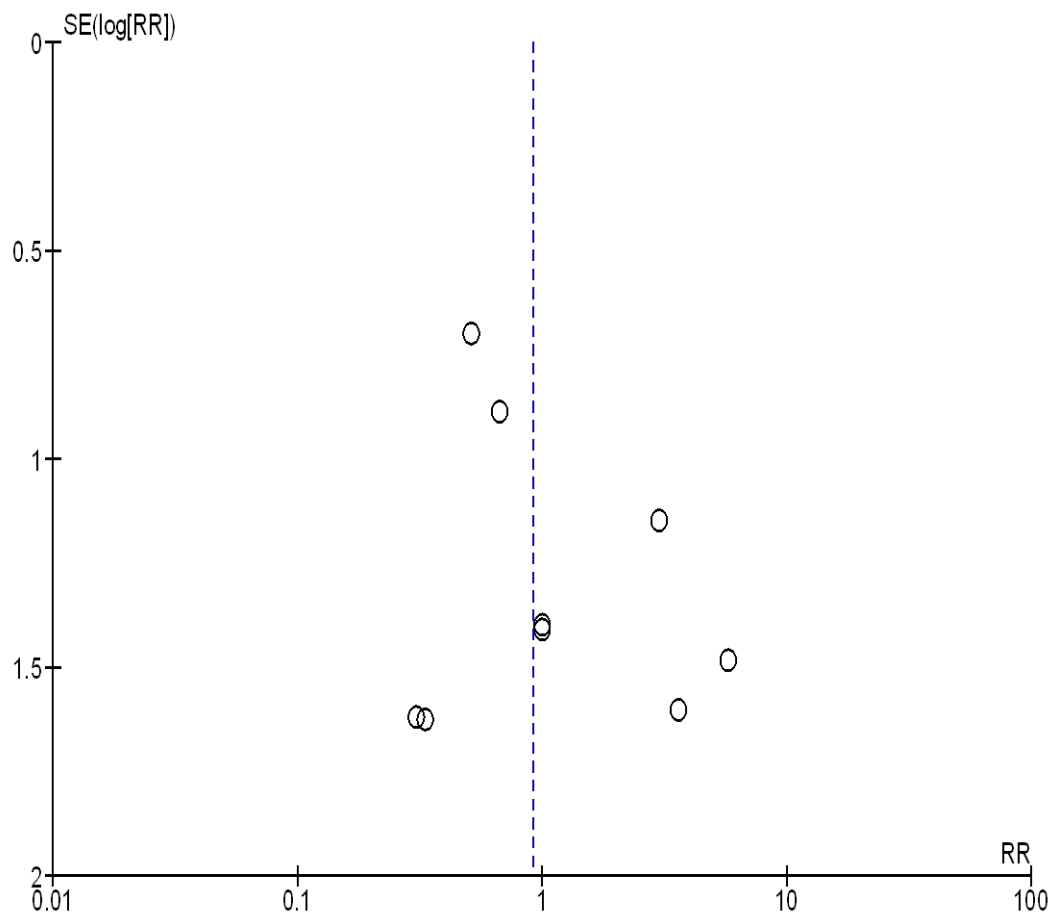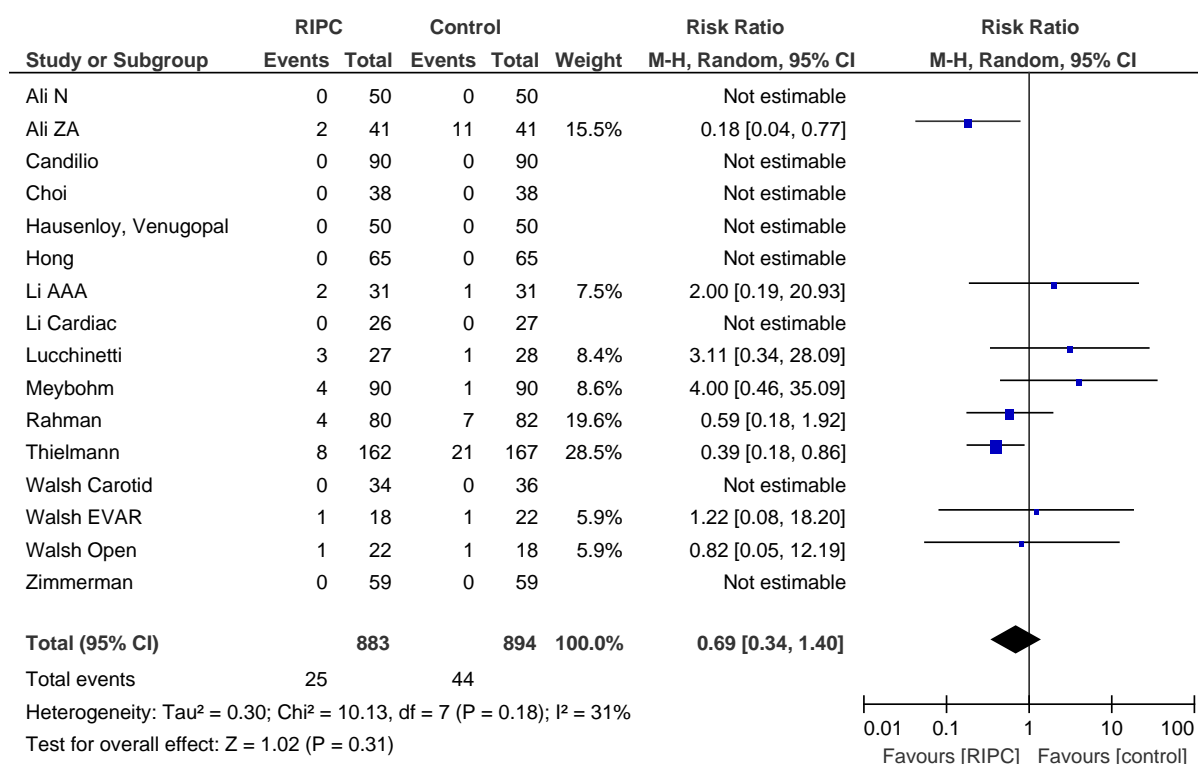
Figure 3.10: Forest plot for the outcome of new-onset arrhythmias

Study or Subgroup	RIPC Events	RIPC Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Ali ZA	2	41	2	41	1.0%	1.00 [0.15, 6.76]
Candilio	10	90	22	90	7.6%	0.45 [0.23, 0.90]
Choi	4	38	3	38	1.8%	1.33 [0.32, 5.56]
Hausenloy, Venugopal	12	50	16	50	8.9%	0.75 [0.40, 1.42]
Hong	14	65	12	65	7.6%	1.17 [0.59, 2.33]
Li AAA	4	31	3	31	1.8%	1.33 [0.32, 5.47]
Li Cardiac	0	26	0	27		Not estimable
Lomivorotov	1	40	0	40	0.4%	3.00 [0.13, 71.51]
Lucchinetti	10	27	5	28	4.2%	2.07 [0.81, 5.28]
Meybohm	35	90	35	90	26.5%	1.00 [0.69, 1.44]
Rahman	28	80	30	82	21.0%	0.96 [0.63, 1.45]
Thielmann	27	162	36	167	17.7%	0.77 [0.49, 1.21]
Walsh Carotid	0	34	2	36	0.4%	0.21 [0.01, 4.25]
Walsh EVAR	3	18	0	22	0.4%	8.47 [0.47, 154.04]
Walsh Open	2	22	1	18	0.7%	1.64 [0.16, 16.62]
Total (95% CI)		814		825	100.0%	0.92 [0.76, 1.12]
Total events	152		167			

Heterogeneity: Tau² = 0.00; Chi² = 13.07, df = 13 (P = 0.44); I² = 1%
Test for overall effect: Z = 0.80 (P = 0.42)

Figure 3.11: Funnel plot for the outcome of new-onset arrhythmias

Mesenteric ischaemia

Data regarding mesenteric ischaemia were available from 13 trials [107, 108, 112, 113, 118, 121, 122, 140, 141, 143, 144, 204, 205] (645 RIPC patients, 653 control patients). 2 RIPC group patients and 1 control group patient developed mesenteric ischaemia (pooled risk ratio 1.51; 95% CI 0.19 to 12.04) (figure 3.12). There was no evidence of heterogeneity ($I^2 = 0\%$) and the funnel plot was symmetrical (figure 3.13).

Figure 3.12: Forest plot for the outcome of mesenteric ischaemia

Figure 3.13: Funnel plot for the outcome of mesenteric ischaemia
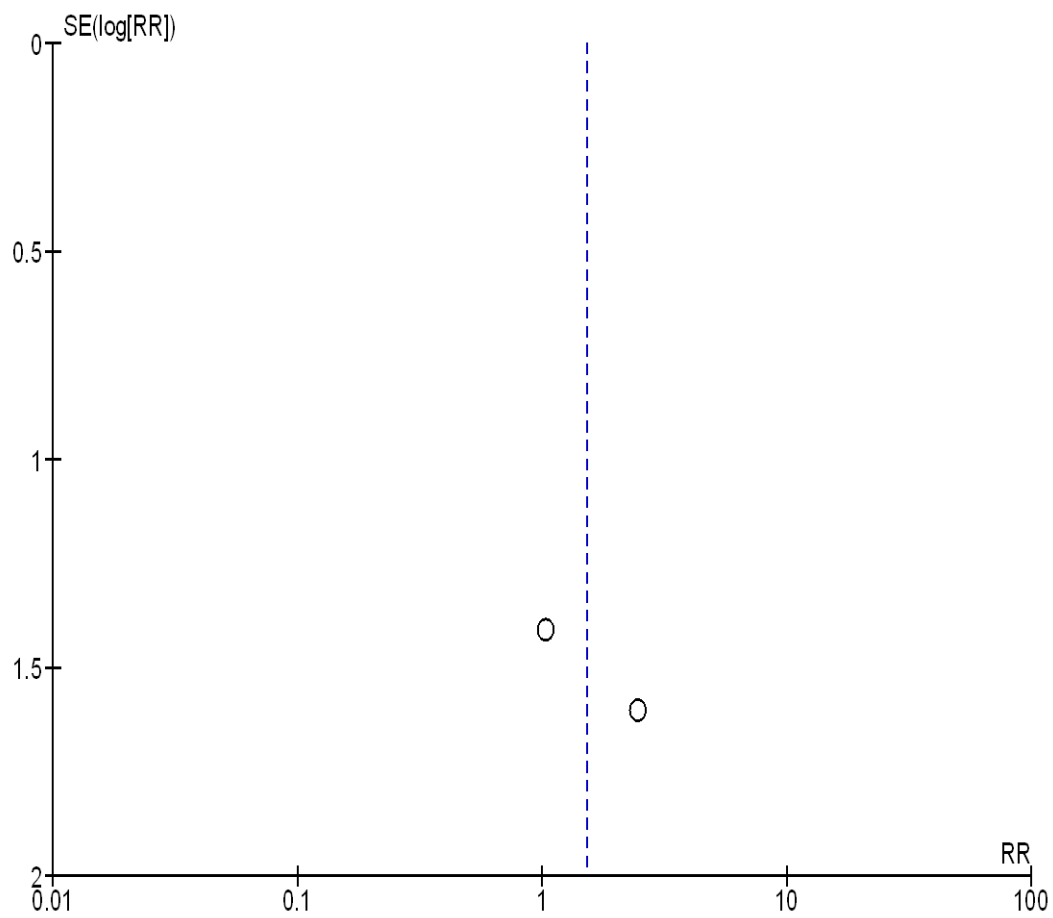
Intensive care unit stay

Duration of intensive care unit stay was available from 15 trials [107, 108, 112-114, 118, 122, 123, 140, 141, 143, 204-207] (656 RIPC patients, 662 controls). There was no significant difference between the groups (weighted mean difference -0.05 days; 95% CI -0.21 to 0.11 days) (figure 3.14). There was evidence of significant heterogeneity ($I^2 = 35\%$) although the funnel plot was symmetrical (figure 3.15).

Figure 3.14: Forest plot for the outcome of duration of intensive care unit stay

Study or Subgroup	RIPC Mean	RIPC SD	RIPC Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Ali ZA	3.17	4.44	41	4.05	3.13	41	0.9%	-0.88 [-2.54, 0.78]
Choi	2.7	0.7	38	3.4	1.4	38	7.4%	-0.70 [-1.20, -0.20]
Hausenloy, Venugopal	3.86	16.32	50	1.52	1.25	50	0.1%	2.34 [-2.20, 6.88]
Hong	2	1.6	65	1.9	1.2	65	7.7%	0.10 [-0.39, 0.59]
Joung	2.304	0.817	35	2.338	1.117	35	8.3%	-0.03 [-0.49, 0.42]
Karuppasamy	1.0375	0.4583	27	0.971	0.313	27	18.3%	0.07 [-0.14, 0.28]
Li Cardiac	0.983	0.308	26	1.133	0.571	27	16.3%	-0.15 [-0.40, 0.10]
Lomivorotov	1.9	0.5	40	1.9	0.5	40	17.8%	0.00 [-0.22, 0.22]
Thielmann	3.5	2.7	162	3.9	3.9	167	4.1%	-0.40 [-1.12, 0.32]
Walsh EVAR	1.39	1.51	18	1.5	1.09	22	3.2%	-0.11 [-0.94, 0.72]
Walsh Open	3.6	3.86	22	3.83	6.3	18	0.2%	-0.23 [-3.56, 3.10]
Wu	2.975	0.683	25	2.854	0.342	25	13.8%	0.12 [-0.18, 0.42]
Young	3.25	5.82	48	1.08	0.64	48	0.9%	2.17 [0.51, 3.83]
Zimmerman	2.54	3.27	59	2.74	5.15	59	1.0%	-0.20 [-1.76, 1.36]
Total (95% CI)			656			662	100.0%	-0.05 [-0.21, 0.11]

Heterogeneity: Tau² = 0.02; Chi² = 19.95, df = 13 (P = 0.10); I² = 35%

Test for overall effect: Z = 0.62 (P = 0.53)

-100   -50   0   50   100

Favours [RIPC]   Favours [control]

Figure 3.15: Funnel plot for the outcome of duration of intensive care unit stay

Chapter 3: Systematic review and meta-analysis of remote ischaemic preconditioning in major cardiovascular surgery

Hospital stay

Data regarding hospital stay were available from 12 trials [107, 108, 112, 114, 122, 123, 140, 141, 143, 144, 204, 205] (508 RIPC, 516 controls). There was no significant difference between the groups (weighted mean difference 0.26 days; 95% CI -0.06 to 0.59 days) (figure 3.16). There was no evidence of heterogeneity ($I^2 = 0\%$) and the funnel plot was symmetrical (figure 3.17).
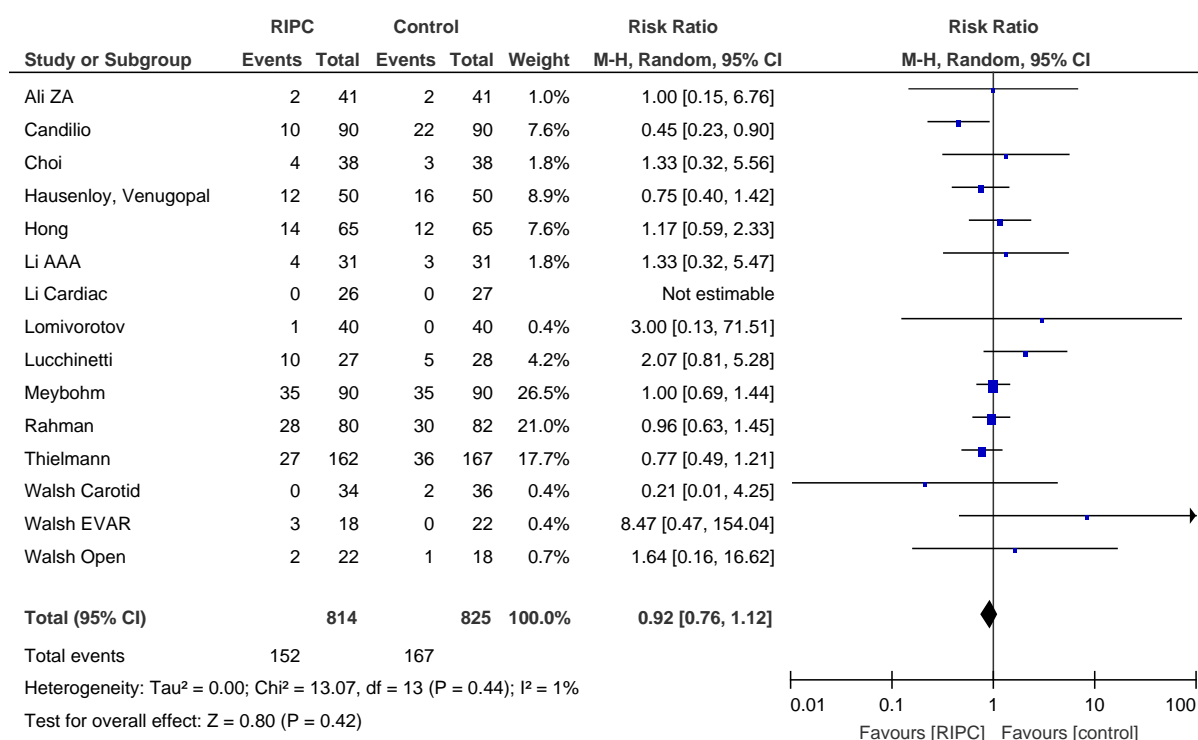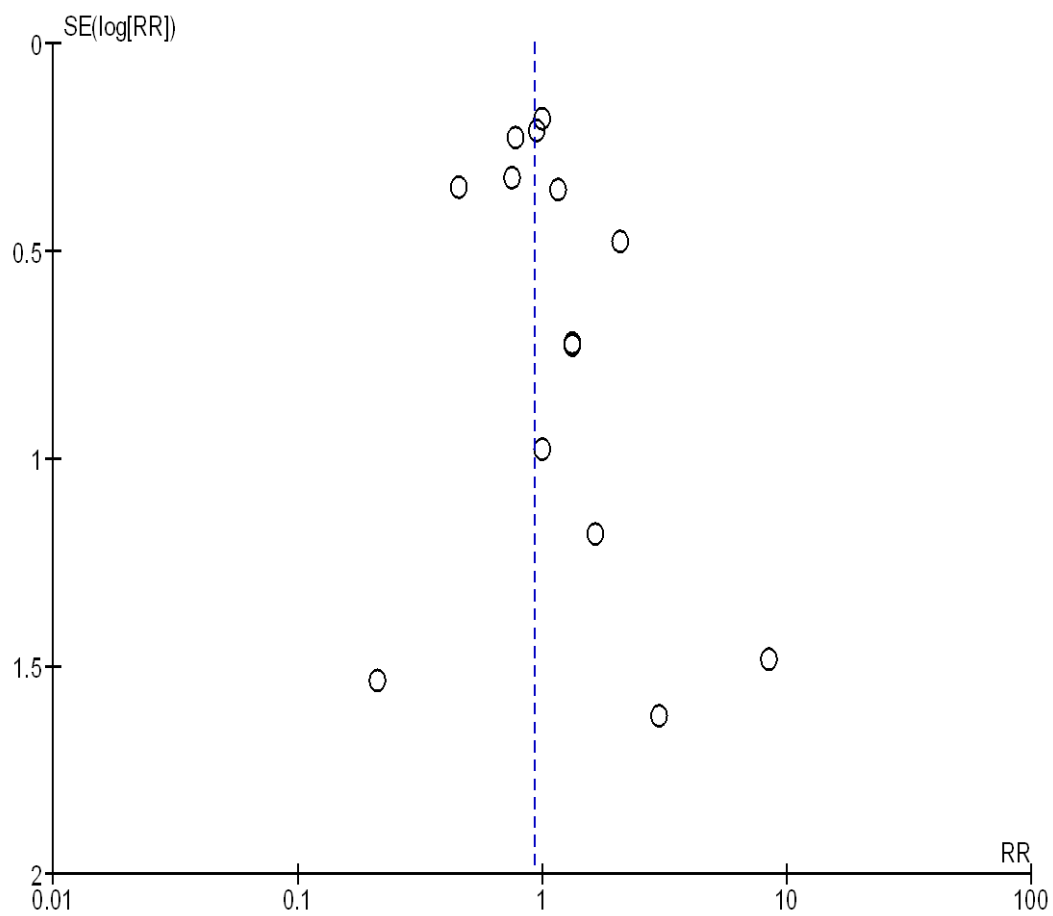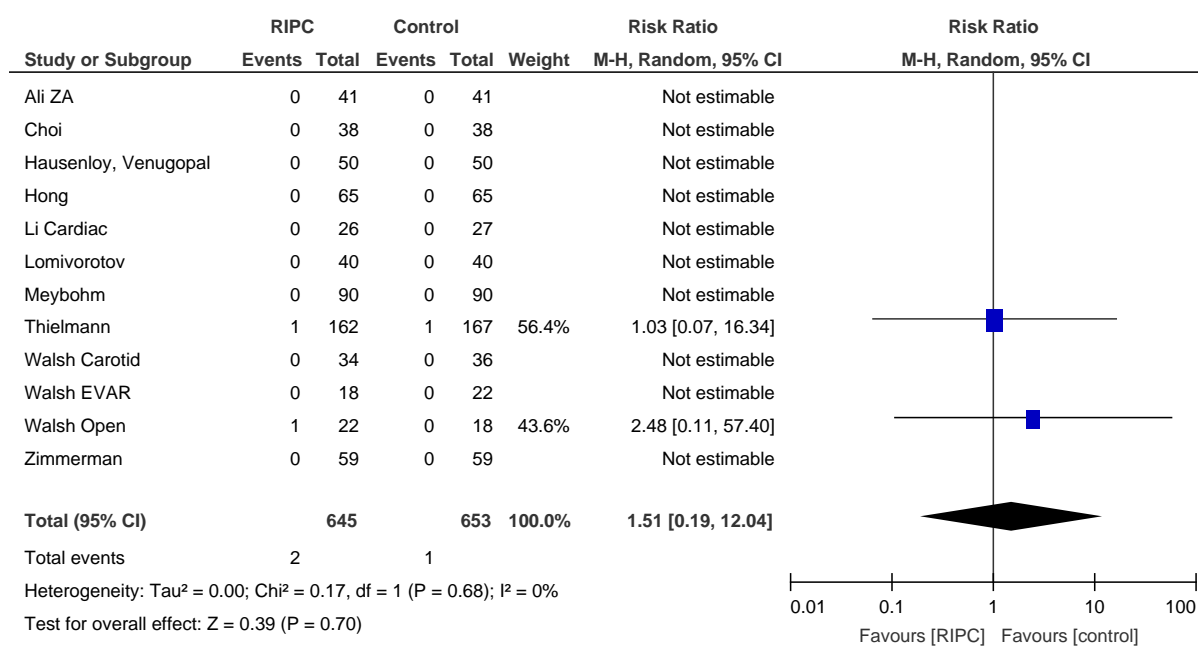
Figure 3.16: Forest plot for the outcome of duration of hospital stay

Study or Subgroup	RIPC Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ali ZA	11.75	6.62	41	12.77	10.01	41	0.8%	-1.02 [-4.69, 2.65]
Choi	11.1	3.4	38	12	6.1	38	2.1%	-0.90 [-3.12, 1.32]
Hausenloy, Venugopal	11.58	19.29	50	9.35	5.31	50	0.3%	2.23 [-3.32, 7.78]
Hong	8.5	3.2	65	7.8	2.9	65	9.3%	0.70 [-0.35, 1.75]
Karuppasamy	6.9	3.2	27	7.5	5.4	27	1.8%	-0.60 [-2.97, 1.77]
Li Cardiac	9.5	0.9	26	9	1.1	27	35.2%	0.50 [-0.04, 1.04]
Thielmann	10.7	2.6	162	10.3	2.4	167	35.1%	0.40 [-0.14, 0.94]
Walsh Carotid	2.9	0.72	34	3.67	3.01	36	10.0%	-0.77 [-1.78, 0.24]
Walsh EVAR	7.1	6.7	18	6	5.98	22	0.6%	1.10 [-2.88, 5.08]
Walsh Open	13.65	11.6	22	17.7	22.8	18	0.1%	-4.05 [-15.64, 7.54]
Wu	12.1	2.6	25	12.4	2.8	25	4.6%	-0.30 [-1.80, 1.20]
Total (95% CI)			508			516	100.0%	0.26 [-0.06, 0.59]

Heterogeneity: Tau² = 0.00; Chi² = 9.41, df = 10 (P = 0.49); I² = 0%
Test for overall effect: Z = 1.62 (P = 0.11)

108
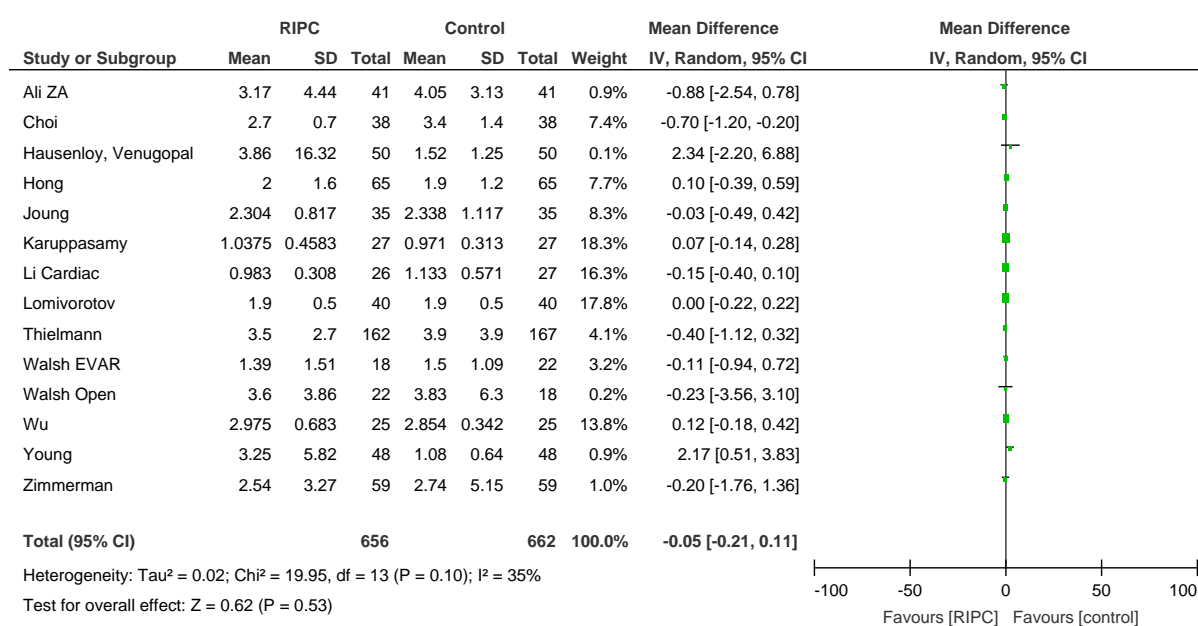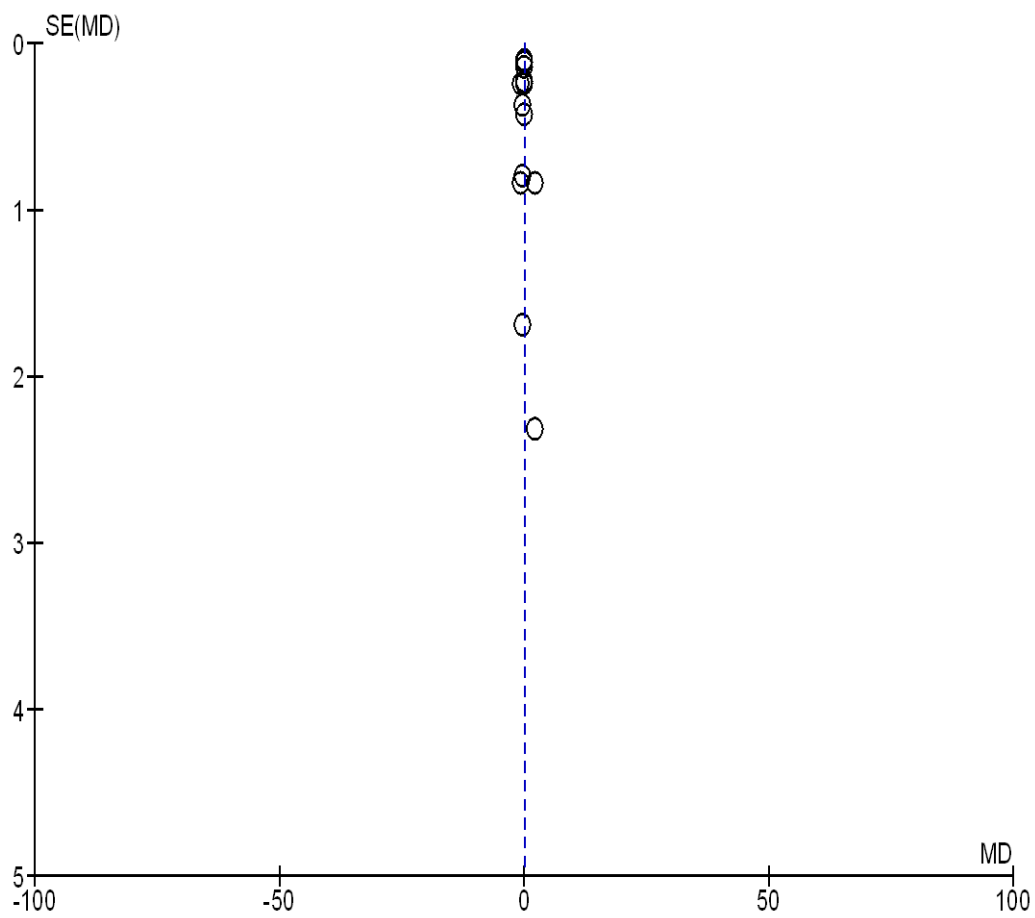
Figure 3.17: Funnel plot for the outcome of duration of hospital stay
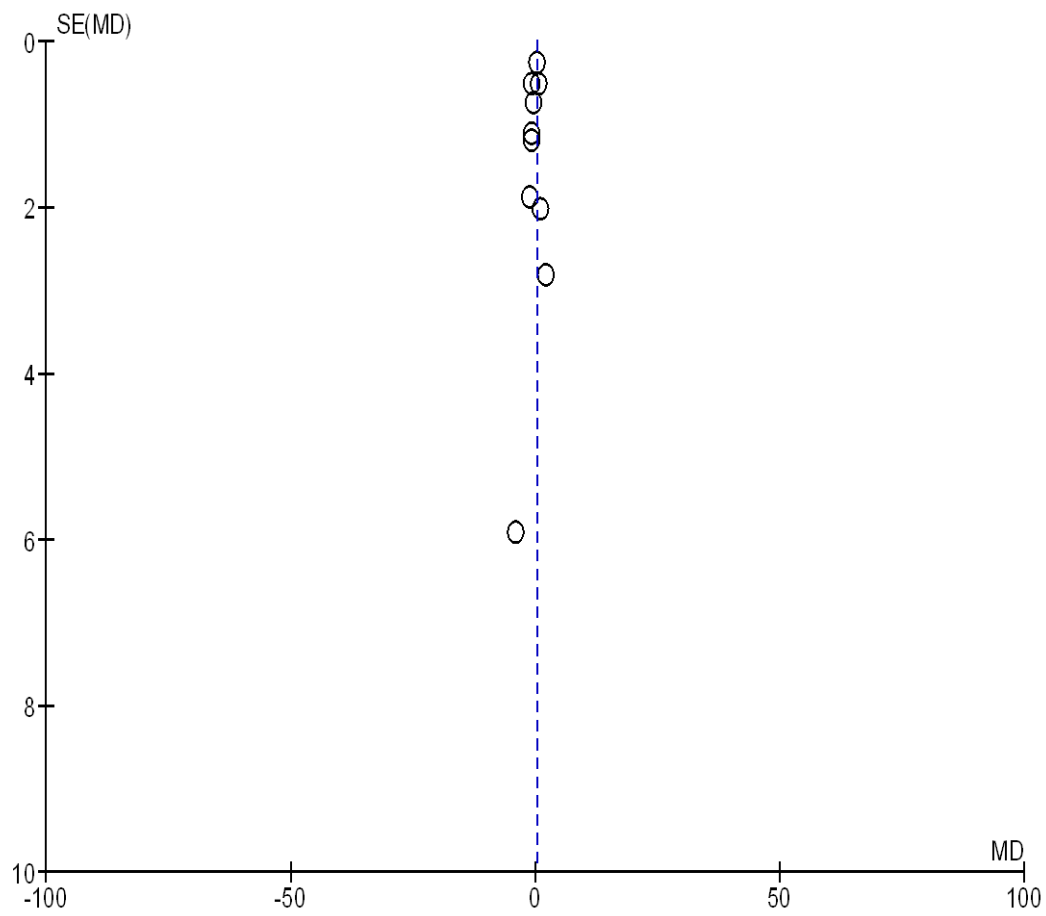
Chapter 3: Systematic review and meta-analysis of remote ischaemic preconditioning in major cardiovascular surgery

Missing data

Each included study did not provide data in relation to all outcomes. Table 3.5 summarises the numbers of included and excluded trials and participants for each outcome due to unavailability of data.

Table 3.5: Details on the numbers of studies that provided data on each outcome and the number of studies that did not provide data on each outcome.

Outcome	Number of studies that provided data and the number of participants		Number of studies with outcome data missing and the number of participants	
	Number of studies	Number of participants	Number of studies	Number of participants
Perioperative mortality	21	2046	2	154
Myocardial infarction	17	1777	6	423
Cerebrovascular accident	18	1765	5	435
Renal failure	17	1673	6	527
New-onset arrhythmia	16	1639	7	561
Mesenteric ischaemia	13	1298	10	902
Intensive care unit stay	15	1318	8	882
Hospital stay	12	1024	11	1176

3.5: Discussion

The current review adds considerably to the evidence on RIPC and patient important outcomes in cardiovascular surgery. Published and unpublished data from 23 cardiovascular surgery trials (2,200 patients) were pooled and the focus was solely on major outcomes – thus surrogate outcomes and minor complications were omitted. This contrasts with other meta-analyses as these largely focused on biochemical outcomes. Interestingly, although every previous review found biochemical results that favoured RIPC, none of the analysed clinical outcomes in this review were significantly affected by RIPC. Undoubtedly, "proof of concept" exists at a biochemical level– the challenge is to determine clinically important effects in specific groups of patients in order to justify the use of RIPC in clinical practice. The results from this review suggest that larger trials are needed and it is likely that the lack of significant results reflects sample size. However, there are grounds for optimism - notably, the incidence of perioperative MI in the RIPC arm was almost half that of the control arm (2.8% versus 4.9%). As perioperative MI in cardiac surgery occurs with a frequency of 4-5% [209, 210], a reduction by half would be clinically important. About 1,200 patients would be required in each arm of a trial to confirm that RIPC reduces perioperative MI rates from 4% to 2% with 80% power at the 5% significance level. While there was some heterogeneity in the cohort (a combination of cardiac and vascular surgery patients), there was no evidence of statistical heterogeneity in relation to the MI analysis and the funnel plot did not suggest bias. Therefore, the results of the MI analysis provide an encouraging indication that remote preconditioning may affect clinical end-points in cardiovascular surgery patients.

It is feasible that RIPC has the potential to reduce the incidence of all of the predefined outcomes in this review with one notable exception: mesenteric ischaemia. Organ protection derived from RIPC is relative and is not absolute –prolonged ischaemia is always lethal. As bowel ischaemia in the setting of major cardiovascular surgery is usually caused by large atheroemboli, inadequate collaterals (in the setting of sacrifice of superior mesenteric artery e.g. in abdominal aortic aneurysm surgery) or systemic vasoconstriction, relative resistance to ischaemia reperfusion injury is unlikely to prevent anything other than mucosal ischaemia. Nonetheless, including mesenteric ischaemia as an outcome in this review is important for a few reasons. Firstly it is a well-documented complication in vascular surgery and secondly its incidence should be similar both with and without RIPC and thus it could thus serve to gauge validity of findings in relation to other outcomes. Nonetheless, as the numbers of vascular procedures in this review are relatively low, it is not surprising that there were only 3 incidences of mesenteric ischaemia.

The main strength of this review is the inclusion of a large quantity of previously unpublished clinical outcome data, provided according to predefined criteria by trial principal investigators. This has allowed a thorough evaluation of the clinical utility of RIPC, rather than relying on surrogate markers or limited clinical data. As all of the studies to date involved small patient numbers, meta-analysis is

an important tool for interpretation. The emergence of only a suggestion of a reduced MI rate despite inclusion of 2,200 patients reinforces the importance of meta-analysis. However, the use of meta-analysis in the setting of RIPC requires a note of caution. By using meta-analysis, one makes the assumption that all of the applications of RIPC are similarly effective. In reality, this is unlikely to be the case as the studies used different RIPC stimuli and different application times in relation to anaesthesia and surgery. Similarly, some studies excluded diabetic patients, older patients and patients with certain other co-morbidities while other studies did not exclude such patients (tables 3.3 and 3.4). The anaesthetic method is a further potentially important consideration as volatile anaesthetic agents [211] and opiates [212] are thought to have preconditioning effects. Some data suggest that propofol may abrogate the RIPC stimulus [117, 213]. Future trials should consider the use of an adaptive design which switches trial patients to a propofol-free anaesthesia protocol should interim data suggest that RIPC confers no benefit. Another concern with the present study relates to missing data – even though the numbers of included participants in this study are considerable, much data were still unavailable for each outcome (table 3.5). Finally it is important to highlight that minor common complications such as delirium, mild cognitive dysfunction, mild renal injury and mild intestinal ischaemia were not evaluated in the current review. Although these data would be invaluable, they were infrequently reported and thus we focused solely on major complications. Despite these inherent flaws, an effort to pool all available clinical outcome data from the proof of concept trials was necessary in order to determine whether sufficient equipoise exists to justify further research. It is also important to note that subgroup analysis was not performed due to the weaknesses described.

This review gives individual researchers the opportunity to weigh up considerably more evidence for guidance on methodological issues – there are many areas where uncertainties exist. For example, the optimal preconditioning stimulus has not been demonstrated [92]. It is unknown whether upper or lower limb ischaemia is superior, although upper limb cuff-induced ischaemia has a more attractive risk profile as ischaemic lower limb complications have been described with cross-clamping of lower limb arteries [141]. Furthermore, the most favourable timing/duration of the stimulus is unclear. Most cardiovascular surgery studies used either 3 or 4 cycles comprising 5 minutes of ischaemia and 5 minutes of reperfusion. There is scant evidence supporting this, though some negative trials used 10 minute ischaemic episodes [141, 143, 144]. Furthermore the most practical time for stimulus initiation is unclear although it should be applied as close as possible to potential ischaemic events – evidence confirms that a short window for acute protection exists and that the interval between preconditioning and the ischaemic event should not exceed 2 hours [61].

The study highlights the need for large scale clinical trials in a variety of settings (e.g. cardiac and vascular surgery). Several such trials are currently recruiting. The RIPHeart-Study is a multi-centre trial investigating clinical outcomes with cardiopulmonary bypass procedures [162]. The aim is to recruit over 2,000 patients and recruitment is currently at over 1,000. There are two arms: control and

RIPC (via blood pressure cuff induced upper limb ischaemia). Primary outcomes are all-cause mortality, non-fatal MI, any new stroke and/or acute renal failure. The ERICCA Trial is currently recruiting and aims to recruit over 1,600 patients undergoing coronary artery bypass graft (CABG) operations [164]. The primary outcomes are cardiovascular death, non-fatal MI, coronary revascularization and stroke at one year. Again, there are two arms: control and RIPC (via blood pressure cuff induced upper limb ischaemia). Finally, the REPAIR Trial [165] aimed to examine the effect of RIPC on renal function after renal transplantation. It has completed recruitment at 406 patients and a report is awaited. Though the main outcome was biochemical (glomerular filtration rate at one year), clinical outcomes at two to five years will be reported. In an effort to elucidate the optimal time for the ischemia/reperfusion stimulus, it had four arms: control, early RIPC, late RIPC, dual RIPC.

Definitive evidence of the clinical benefits of RIPC may emerge with the completion of large trials. If these trials do not demonstrate a clinical benefit, perhaps combining lessons from these and prior works would allow elucidation of the optimal preconditioning stimulus and appropriate inclusion/exclusion criteria in order to streamline future research.

Chapter 4: Remote ischaemic conditioning and renal function after contrast-enhanced CT scan: A randomised trial

4.1: Abstract

Remote ischemic conditioning has been shown to protect against kidney injury in animal and human studies of ischemia-reperfusion. Recent evidence suggests that conditioning may also provide protection against kidney injury caused by contrast medium. Therefore, the aim of this study was to determine if conditioning protected against increases in serum creatinine (SCr) after contrast-enhanced computed tomography (CECT).

This randomised controlled trial (NCT 01741896) was performed with institutional review board approval and informed patient consent. Adult in-patients undergoing abdomino-pelvic CECT were allocated to conditioned or control groups. Conditioning consisted of four cycles of five minutes of cuff-induced arm ischemia with three minutes of reperfusion applied ~40 minutes before CECT. The primary outcome was SCr change after CECT.

Baseline characteristics were similar in both groups. For all patients, conditioning reduced the risk ratio (RR) of increased SCr; RR 0.65 (95% confidence intervals 0.41 to 1.04). The protective effect was greater and the evidence for protection stronger when analysis was restricted to patients with pre-scan reduced renal function (eGFR <90 mL/min/1.73 m^2); RR 0.40 (95% confidence intervals 0.17 to 0.95). Logistic regression revealed that conditioning was the only model variable that predicted decreased SCr; odds ratio 0.24 (95% confidence intervals 0.07 to 0.84) in patients with reduced baseline eGFR.

Remote conditioning decreased the risk of CECT-associated increases in serum creatinine by 60% in patients with reduced baseline eGFR. In future studies stratification of analysis based on baseline eGFR is warranted because benefit from conditioning will occur only when there is risk of injury.

4.2: Rationale for the use of remote ischaemic preconditioning for renoprotection in the setting of contrast media administration

Remote conditioning, the phenomenon whereby brief periods of ischaemia-reperfusion in one tissue or organ protect against later ischaemic insult in a distant tissue, was first proposed [197] and confirmed in the heart [76]. Subsequently, its application was extended to protect against kidney injury and consequent loss of function. Renal protection has been demonstrated in animal models of ischaemia-reperfusion [214, 215] and in human surgical practice; after abdominal aortic aneurysm repair [140, 143], coronary artery bypass grafts [113, 216], and renal transplantation [217, 218]. The concept of remote conditioning was recently further extended to reduce the incidence of contrast agent-mediated damage [106]. Contrast agents enhance diagnostic imaging; but, because they are eliminated through the kidneys, can also cause injury. The mechanism of such renal injury is multifaceted and complex [219]; but, because ischaemia-reperfusion plays a role [220], remote conditioning might limit damage. Most studies designed to investigate this protection focus on severe kidney injury, so-called contrast-induced nephropathy (CIN), in patients with acute myocardial infarction [137, 221, 222]. Nonetheless, emerging evidence indicates that even small decreases in renal function are important because they are associated with later adverse outcomes [223]. It is possible that remote conditioning would reduce the incidence of such decreases. Therefore, the aim of this randomized trial was to determine if remote conditioning protected against increases in serum creatinine (SCr) after non-emergent contrast-enhanced computed tomography (CECT) scans. Under these circumstances, it was anticipated any change in SCr would be modest, but nevertheless responsive to remote conditioning.

4.3: Methodology for the randomised controlled trial

Institutional review board approval was obtained and this single-centre trial was registered (NCT01741896; http://clinicaltrials.gov/); no changes made after initial registration. No interim analyses were undertaken and the trial was stopped when 100 patients were recruited.

Eligible participants were hospital in-patients aged over 17 years scheduled for abdomino-pelvic CECT-scans who were likely to remain in hospital for at least two days after the scan. Exclusion criteria were: allergy or hypersensitivity to iodinated contrast, hospital admission SCr >150 µmol/dL (a contraindication to iodinated contrast), prior renal transplant, history of acute renal failure that required management by a nephrologist, and current use of either sulphonlyurea or nicorandil.

Written informed consent was obtained. Patients were randomised to remote conditioning or no intervention (1:1) using a block design (block sizes of 4, 6, and 8 were used) stratified by the presence of diabetes mellitus and chronic kidney disease (CKD; defined as baseline eGFR <60 mL/min/1.73 m^2). The sequence was computer generated by a third party not involved in the trial. Nobody else had access to the randomization sequence. Allocation concealment was achieved using sequential sealed envelopes. The envelopes were opened approximately 40 minutes before the anticipated scanning time. Three investigators (D Healy, I Feeley, C Keogh) performed all recruitment, randomization, remote conditioning, and data collection. A single investigator was available each day and so if two eligible patients were scheduled for scans in close succession, only one was recruited. Hence, all consecutive eligible patients were not recruited.

Patients underwent conditioning approximately 40 minutes before contrast was given; the procedure took 32 minutes. The conditioning stimulus comprised four, five-minute cycles of arm ischaemia with three minutes of reperfusion between each cycle. Ischaemia was induced by repeated inflation and deflation of a blood pressure cuff positioned on the patient's arm. Ischaemia was achieved by inflating the cuff to a pressure of 200 mmHg or 15 mmHg above systolic pressure if that was >200 mmHg. Control group patients received no sham intervention.

All patients received an intravenous bolus of iohexol (Omnipaque, GE Healthcare, Oslo, Norway), iopamidol (Niopam 300, Bracco Ltd, Buckinghamshire, UK), or iodixanol (Visipaque, GE Healthcare, Oslo, Norway). At University Hospital Limerick, most patients receive a dose of 90 mL, but patients heavier than 110 kg may receive 120 mL. All patients with eGFR <60 mL/min/1.73m^2 receive iodixanol. Any use of hydration prior to the procedure was at the discretion of the physician who ordered the scan.

The primary outcome was the change in SCr after the CECT-scan. Serum creatinine was measured using kinetic alkaline picrate methodology (Architect c Systems, Abbott Laboratories, Illinois, USA). Samples were obtained at three times; before the scan, and at 24 and 48 hours after. Secondary outcomes were serum urea at 24 and 48 hours after the CT-scan, incidence of reduced urine output (defined as <30 mL/hour for five consecutive hours) within 48 hours of the scan, and length of hospital stay from the scan until discharge date.

There was no prespecified subgroup analysis. However, because contrast-related effects on kidney function are more likely to occur in patients with already reduced renal function, an analysis was performed on participants with decreased eGFR (<90 mL/min/1.73 m^2), as defined by the National Kidney Foundation Clinical Practice Guidelines [224].

Continuous variables were reported as means and 95% confidence intervals (CI) or medians with interquartile range (IQR) as appropriate. Intergroup comparisons were made using Student's t-test or Mann-Whitney U test. Categorical parameters were presented as proportions with their corresponding 95% CI and were compared using the Chi square test. Risk ratios (RR) were calculated for the reduction in incidence of CECT-scan-associated increases in SCr. Logistic regression was used to determine parameters associated with decreased kidney function. Formal power analysis calculations were not performed because there was no comparable study to provide guidance. However, it was determined that with the proposed enrollment a reduction of $>33\%$ in the incidence of increased SCr would be required. Analyses were performed using Stata version 12.1 (StataCorp, College Station, Texas, USA).

4.4: Results of the trial

Patients were recruited from November 2012 to March 2013. 202 patients were assessed; 102

excluded (figure 4.1) and 100 randomized equally between groups. The primary reasons for the

CECT-scans were abdominal pain (control 65%, conditioned 54%; P=0.40) and suspected malignancy

(control 31%, conditioned 43%; P=0.29).

Figure 4.1: Trial flow diagram
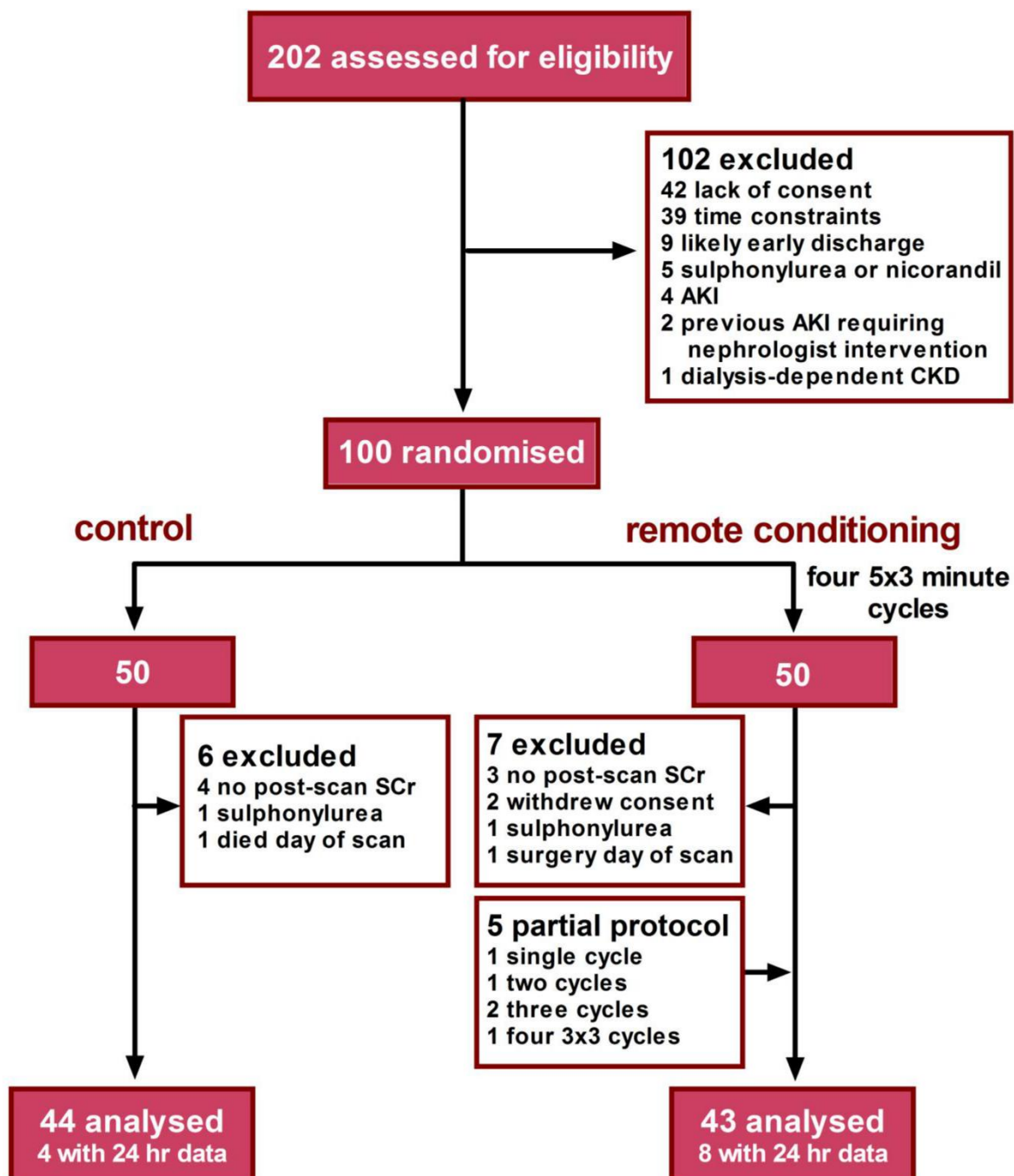
No adverse events were associated with conditioning. However, three patients failed to complete the conditioning protocol because of discomfort associated with inflation of the pressure cuff. One patient completed two cycles of ischemia-reperfusion, one completed three cycles, and one completed four cycles of three-minute inflations (because the patient was unable to tolerate five minute cycles). Time constraints prevented two additional patients receiving the complete conditioning protocol; one completed three cycles and the other one cycle. Intention-to-treat analysis was employed and these patients were included.

There were no differences in patient characteristics, medications, and baseline clinical parameters most likely to influence outcomes (table 4.1). Similarly, the incidence of other comorbidities (smoking history, previous cardiac procedure, chronic obstructive pulmonary disease, and benign prostatic hyperplasia) and medication use (beta blocker, angiotensin receptor blocker, angiotensin converting enzyme inhibitor, calcium antagonist, and warfarin) did not differ. Ninety patients received 90 mL of contrast, three received 100 mL (two conditioned), and one control received 120 mL. There were no difference in nonsteroidal anti-inflammatory drug use between groups; although, on day two post scan, there was weak evidence that conditioned patients had greater use (13% vs. 2%; P=0.06). However, given the number of comparisons made, it is possible that this difference could have occurred by chance alone.

Table 4.1: Patient characteristics, comorbidities, medications, and baseline parameters

	Control (n = 44)	Conditioned (n = 43)	P value
Male	59 (44, 74)	51 (36, 66)	0.16
Age (years)	62 (57, 67)	63 (57, 69)	0.82
Body mass (Kg)	83 (74, 91)	79 (73, 85)	0.44
Current smoker	16 (5, 27)	21 (8, 33)	0.55
IDDM	7 (0, 14)	5 (0, 11)	0.66
NIDDM	7 (0, 14)	12 (2, 21)	0.44
Angina pectoris	2 (0, 7)	5 (0, 11)	0.54
Hypertension	41 (26, 56)	44 (29, 59)	0.76
Antiplatelet agent use	27 (14, 41)	23 (10, 36)	0.67
Statin use	32 (18, 46)	33 (18, 47)	0.94
Diuretic use	18 (6, 30)	23 (10, 36)	0.56
Pre-hydration treatment	32 (18, 46)	42 (27, 57)	0.33
Pre-scan SCr (µmol/L)	75 (62, 85)	73 (59, 85)	0.50
Pre-scan serum urea (mmol/L)	4.3 (3.1, 5.4)	4.7 (3.8, 5.9)	0.44

Values are expressed as proportions (%) together with their 95% confidence intervals. However, when units are given and the distribution of the values was normal (age and body mass), the values represent means and 95% confidence intervals. When the distribution was not normal (SCr and serum urea), the values represent medians and the interquartile range.

IDDM – insulin-dependent diabetes mellitus; NIDDM – noninsulin-dependent diabetes mellitus; SCr – serum creatinine

Seven patients (three conditioned), discharged before any post-scan SCr measurement, were excluded from risk ratio and logistic regression analysis. However, twelve patients (eight conditioned) discharged after one post-scan SCr measurement were included. Four of these (two per group) had reduced eGFR. Because the direction of most (79%) patients' one-day SCr change corresponded to their two-day change, therefore it is unlikely that this inclusion produced misclassification and bias.

Conditioning reduced the risk of increased SCr after CECT-scan. For all patients, evidence in support of a difference was weak. However, the evidence strengthened when patients with reduced baseline eGFR were assessed (table 4.2). Furthermore, there was also evidence for a difference amongst conditioned patients when divided on the basis of baseline eGFR; reduced eGFR versus normal – RR = 0.40 (0.17 to 0.94; P=0.02).

Table 4.2: Risk of increased serum creatinine after contrast-enhanced CT-scan

patients	N	Conditioned risk	Control risk	RR (95% CI)	P value
all	87	0.37 (16/43)	0.57 (25/44)	0.65 (0.41-1.04)	0.07
normal eGFR	40	0.55 (11/20)	0.60 (12/20)	0.92 (0.54-1.56)	0.75
reduced eGFR	47	0.22 (5/23)	0.54 (13/24)	0.40 (0.17-0.95)	0.02

Normal defined as eGFR >90 mL/min/1.73 m^2, reduced eGFR defined as <90 mL/min/1.73 m^2 [224]

CI – confidence interval; N – number of patients; RR – risk ratio

Logistic regression analysis was conducted with increased SCr as the binary outcome. When conditioning was included as a variable, there was weak evidence for a reduction in odds ratio (OR); OR = 0.45 (0.19 to 1.06; P=0.07). The model was not improved by addition of any other variables (evaluated using likelihood ratio tests). When analysis was restricted to patients with reduced baseline eGFR, the evidence for a protective effect of conditioning was again strengthened (OR 0.24 (0.07 to 0.84); P=0.02). Use of likelihood ratio tests indicated the model was not improved by addition of any other variables.

No inter-group differences were found in any of the secondary endpoints. None of the patients had reduced urine output. There were no differences in serum urea after CECT-scan at either day-one (control 3.9 [IQR 3.2, 5.5] mmol/L; conditioned 4.2 [IQR 3.2, 5.7] mmol/L; P=0.23) or day-two (control 4.1 [IQR 3.3, 5.6] mmol/L; conditioned 4.2 [IQR 3.1, 6.0] mmol/L; P=0.76). Hospital length-of-stay was also similar (control 5 [IQR 3, 9] days, conditioned 4 [IQR 2, 9] days; P=0.59). If analysis was restricted to patients with reduced baseline eGFR, still no differences were found.

4.5: Discussion

This study found that remote conditioning decreased the risk of CECT-scan-associated increases in serum creatinine by 60% versus no intervention in patients with reduced baseline eGFR (<90 mL/min/1.73m^2).

In future studies analysis stratification based on renal function is warranted because conditioning-mediated benefit occurs only when there is risk of injury. Therefore, patients with normal kidney function (i.e., large functional reserve) exposed to small volumes of contrast medium would not show ill-effects. Additional support for such stratification in this trial comes from the finding of a 60% risk reduction for serum creatinine increases in conditioned patients with reduced eGFR versus conditioned patients with normal eGFR; identical to that found in conditioned patients with reduced eGFR versus controls with reduced eGFR (Table 4.2).

It is proposed that stratification be considered in all conditioning analysis. The association between minimal risk and minimal benefit was demonstrated in cardiac studies. For example, when the area at risk of infarction was less than 25% of the left ventricle, no difference in infarct size was observed between remote conditioned and control patients with ST-segment elevation myocardial infarction (STEMI) [225]. Infarct size reduction in conditioned hearts was apparent only when risk area exceeded 25% of the left ventricle. Similar results were found by Ovize and colleagues in data from four post-conditioning studies in patients with STEMI [226]. The degree of protection depended upon the size of the risk region (assessed as a percent of ventricular circumference). Little evidence of protection was seen when risk regions were less than 35%. However, for large risk areas, infarcts in conditioned hearts were appreciably smaller than controls. Some renal conditioning studies avoided the minimal risk issue by enrolling only patients with CKD. Other studies, including the current trial, did not. Crimi and colleagues examined renal function (assessed by maximum post-procedure SCr) in remote conditioned and control patients after percutaneous intervention for STEMI and found no overall group difference [227]. Nevertheless, when they divided patients according to baseline eGFR, conditioning was associated with reduced maximum SCr for the lowest tertile (<77 mL/min/1.73 m^2),

but not the others (77-95 and >95 mL/min/1.73 m^2). This finding is consistent with the results of the current study and emphasizes the importance of risk stratification.

Lack of stratification may explain equivocal results derived in some meta-analyses of conditioning [102, 228]. For instance, meta-analysis of remote conditioning's effect on acute kidney injury in patients undergoing vascular and cardiac procedures found only weak evidence of benefit. The combined risk ratio was 0.70 (95% CI 0.48 to 1.02; P=0.06) [228]. Not all included studies reported eGFR. Nonetheless, mean baseline eGFR in the conditioned group of the four most negative contributors was high and probably constituted minimal risk (85±34 mL/min/1.73 m^2 [143], 115 (range 62 to 152) mL/min/1.73 m^2 [229], 82±20 mL/min/1.73 m^2 [122], and 101±20 mL/min/1.73 m^2 [230]). In contrast, eGFR in the most positive study was consistent with CKD and thereby provided opportunity for protection (41±9 mL/min/1.73 m^2 [137]). Numerous factors influence outcomes after conditioning; however, we propose risk stratification in renal studies should be routine.

In the current study, analysis focused on the direction of SCr change. Still, the specific amount and potential clinical significance must be considered. When SCr increases occurred in patients with reduced baseline eGFR, both groups exhibited similar changes; control 10 (95% CI 5 to 15) μmol/L and conditioned 13 (95% CI 8 to 19) μmol/L. Such SCr increases corresponded to eGFR changes of; control -9 (95% CI -13 to -4) mL/min/1.73 m^2 and conditioned -14 (-22 to -6) mL/min/1.73 m^2. The changes are far from the magnitude seen in CIN. Nevertheless, a 10% increase in relative risk of death and non-fatal cardiovascular events was reported for each 10-unit reduction in eGFR below 81 mL/min/1.73 m^2 in 14,527 patients after myocardial infarction [223]. For patients with intracerebral haemorrhage, a 24% reduction in odds of death was associated with each 10 mL/min/1.73 m^2 increase in eGFR [231]. A retrospective study of 29,388 patients reported increased risk of CKD, progression of CKD, and also death if patients experienced an increase in serum creatinine in the first seven days after cardiac surgery [232]. These events occurred even if the increase was only 1-24%. Accumulating evidence illustrates association between reduced renal function and adverse outcomes. These range from increased mortality after stroke [233] and increased all-cause mortality [234] to increased

cardiovascular events [235] and anticoagulation instability during warfarin therapy [236]. Therefore, protecting against small decreases in kidney function should provide benefit.

In this study, patients treated with sulphonylurea were excluded, but not those with comorbidities sometimes associated with loss of condition-mediated protection (advanced age and diabetes mellitus) [237, 238]. In a mouse model of myocardial infarction, hearts from mice with type 1 and type 2 diabetes were not protected by postconditioning [239]. Hence, inclusion of subjects with diabetes mellitus could be problematic. Fifteen percent of the patients in this trial had diabetes mellitus versus 64% in a study that found remote conditioning had no effect on kidney injury after coronary bypass graft surgery [240]. That particular study also included patients treated with sulphonylurea (21%). Moreover, their patients were approximately eight years older than the patients in the current trial. It is proposed that comorbidities be considered when designing future trials. Nevertheless, two trials of remote conditioning and CIN currently enrolling patients [241, 242] do not list diabetes mellitus as an exclusion criterion.

Efforts to translate conditioning-mediated renoprotection from successful animal studies to clinical practice have been disappointing and obstacles remain [243, 244]. The limitations of the current study illustrate some reasons for this disappointment and also some of the obstacles. First, SCr is only a proxy for kidney function and one affected by factors other than changes in function. Additionally, it is not possible to know if the SCr changes observed in the current trial persist; no long-term follow-up was planned. Second, there were few included patients at risk of kidney injury after stratification and therefore it is likely that the curent study was underpowered. Future studies should restrict enrolment to patients with CKD. This is especially important because of the likely weak nephrotoxicity of low-osmolar and iso-osmolar contrast media used in current practice. Third, the primary outcome was biochemical rather than a clinical measure. Recent meta-analysis of cardiovascular surgery application of conditioning highlighted the importance of this difference. Remote conditioning consistently demonstrated protection when biochemical end-points were assessed. However, when clinical outcomes were examined, there was little apparent benefit [102]. Fourth, the chosen conditioning protocol might be suboptimal. For logistical reasons, three minute reperfusion periods

were used rather than the typical five. There is also evidence, based on meta-analysis of animal studies, that conditioning is more effective in kidneys when applied >24 hours before injury [245].

The current study provides support to the hypothesis that remote conditioning protects against contrast-mediated kidney injury. Furthermore, it indicates the importance of risk stratification when analysing conditioning studies. Designing studies to investigate contrast-mediated kidney damage presents challenges because the injury is multifaceted. Nonetheless, conditioning represents an attractive therapy because its protection is multifaceted. Remote conditioning-mediated effects range from the first-reported protection against ischaemia-reperfusion injury, to enhanced endothelial cell function [246], attenuated platelet activation [247] and platelet-mediated thrombosis [248], altered thrombus fibrin organization [249] and enhanced thrombolysis [250], and increased microvascular blood flow [251]. These could all mitigate kidney injury.

Chapter 5: A multi-centre pilot randomized controlled trial of remote ischaemic preconditioning in major vascular surgery

5.1: Abstract

A pilot randomised controlled trial that evaluated the effect of remote ischaemic preconditioning (RIPC) on clinical outcomes following major vascular surgery was performed.

Eligible patients were those scheduled to undergo open abdominal aortic aneurysm (AAA) repair, endovascular aortic aneurysm repair (EVAR), carotid endarterectomy and lower limb revascularisation procedures. Patients were randomised to RIPC or to control groups. The primary outcome was a composite clinical endpoint comprising any of cardiovascular death, myocardial infarction, new onset arrhythmia, cardiac arrest, congestive cardiac failure, cerebrovascular accident, renal failure requiring renal replacement therapy, mesenteric ischaemia and urgent cardiac revascularisation. Secondary outcomes were components of the primary outcome and myocardial injury as assessed by serum troponin values.

The primary outcome occurred in 19/99 controls (19.2%) and 14/99 RIPC group (14.1%) patients (p=0.446). There were no significant differences in secondary outcomes.

The trial generated data that will guide future trials. Further trials are urgently needed.

5.2: Rationale for the use of remote ischaemic preconditioning in major vascular surgery

Patients who require surgery for vascular disease constitute a high-risk group. Perioperative complications such as myocardial infarction (MI), cerebrovascular accident (CVA), renal failure and death are common [252-254]. These complications can be caused by multiple mechanisms such as plaque rupture and hypotension [255]. Therefore it is desirable to have an intervention that can protect against injury via multiple mechanisms. Remote ischaemic preconditioning (RIPC) may be suitable in this regard.

Ischaemic preconditioning is a phenomenon whereby brief periods of non-lethal ischaemia in a tissue can render the tissue resistant to subsequent sustained ischaemic episodes [256] and proof of concept trials have confirmed its efficacy [90]. However ischaemic preconditioning is not clinically attractive as it involves interfering directly with the blood supply of a vital organ such as the heart. RIPC refers to the initiation of an organ-protective phenotype by applying a brief ischaemia-reperfusion (I-R) stimulus to a distant tissue. Any tissue can provide the stimulus and any organ can be protected, although protection is relative and not absolute [256]. Clinically, the most attractive and easily achieved stimulus is skeletal muscle ischaemia induced by blood-pressure cuff inflation. Most of the trials to date have involved cardiac surgery patients although some vascular surgery trials have also been performed. Meta-analyses consistently found benefits in biochemical outcomes although firm data regarding clinical outcomes are lacking [89, 102].

We performed a pilot multi-centre randomised controlled trial to assess whether RIPC could improve clinical outcomes at in patients who were undergoing major vascular surgery. We also examined the effect of RIPC on myocardial injury in these patients.

5.3: Methodology for the randomised controlled trial

This was a prospective, multi-centre, parallel group (1:1 allocation ratio) randomised controlled trial.

It took place from 1st January 2012 to 31st March 2014 in three Irish tertiary vascular centres – University Hospital Limerick, Cork University Hospital and Waterford Regional Hospital. Ethical approval was granted by the institutional review boards of the three participating hospitals and the trial was registered (NCT01691911). The study was compliant with the Declaration of Helsinki and Good Clinical Practice and participants gave written informed consent.

Eligible patients were those who were undergoing elective carotid endarterectomy, open abdominal aortic aneurysm (AAA) repair, endovascular aortic aneurysm repair (EVAR) or surgical lower limb revascularisation (suprainguinal or infra infrainguinal). Patients were excluded for the following reasons: pregnancy, significant upper limb peripheral arterial disease, previous history of upper limb deep venous thrombosis (DVT), therapy with sulphonylurea or nicorandil medication, pre-operative estimated glomerular filtration rate (eGFR) <30ml/min/1.73m^2 using the Modification of Diet in Renal Disease (MDRD) equation, previous history of myocarditis, pericarditis, amyloidosis and the presence of severe hepatic disease defined as an international normalised ratio (INR) >2 in the absence of anticoagulation. Additionally we excluded patients who were undergoing fenestrated or branched EVAR procedures. Patients were recruited consecutively in the participating hospitals before their procedure, either in outpatients' clinics or in hospital wards.

RIPC comprised four cycles of five minutes of forearm ischaemia with five minutes of reperfusion, requiring 35 minutes for an application. This was achieved by inflation and deflation of a blood pressure cuff placed around an upper limb. The cuff was inflated to 200mmHg or to at least 15mmHg higher than systolic pressure for those with systolic blood pressures of >185mmHg. The time of RIPC initiation in relation to onset of anaesthesia and surgery was variable. We aimed to initiate RIPC prior to anaesthesia induction and finish it before or after surgery began. In this way, preconditioned patients were within the initial two hour window of organ protection during their procedures [61]. In most previous trials RIPC was applied uniformly at set times in relation to anaesthesia and surgery – for logistical reasons we had to adopt a flexible approach to timing. Overall, the window between

completion of RIPC and start of surgery was never more than 30 minutes for any patient and for most patients it was 15 minutes or less – thus perioperative organ-protection was potentially achieved for all RIPC patients. Controls received no intervention.

The choice of anaesthetic was at the discretion of the consultant anaesthetist who was responsible for the case. Both regional and general anaesthesia and combinations of the two were utilised in the study. There were no restrictions regarding the use of volatile agents or opiates. The steps involved in surgical procedures were not specified and were left to the discretion of responsible consultant surgeons. Additional procedures such as peripheral angioplasty could be carried out at the surgeon's discretion during EVAR or lower limb revascularisation procedures.

The primary outcome was a composite clinical endpoint comprising any of cardiovascular death, MI, new onset arrhythmia, cardiac arrest, congestive cardiac failure, CVA, renal failure requiring renal replacement therapy, mesenteric ischaemia and urgent cardiac revascularisation within 30 days of operation. These are defined in table 5.1. Pre-specified secondary outcomes were: duration of post-operative hospital and intensive care unit (ICU) stay, unplanned critical care admissions and post-procedure renal injury. The individual components of the composite primary outcomes were further secondary endpoints. Post-operative complications (wound infections, respiratory tract infections, deep venous thromboses, pulmonary emboli, limb ischaemia, limb amputations) were included as post-hoc secondary outcomes. Perioperative myocardial injury, assessed by high sensitivity cardiac troponin t (cTnT-hs) values, was a final secondary outcome. cTnT-hs was measured via serum sampling pre-operatively and on the first, second and third post-operative days. It was measured using an electrochemiluminescence immunoassay on Elecsys and cobas e immunoassay analysers (Roche Diagnostics GmBH, Mannheim, Germany). The reference range was $0 - 5$ng/L and a value of ≥ 5ng/L was considered abnormal.

Table 5.1: Definitions of components of the composite primary outcome in the randomised controlled trial

Outcome	Definition
Cardiovascular death	Death from cardiovascular cause within 30 days of surgery
Myocardial infarction	Following completion of trial recruitment, a blinded cardiologist evaluated all ECGs with corresponding baseline and post-operative cTnT-hs values. Myocardial infarctions were diagnosed based upon the presence of both of the following criteria: i) Electrocardiographic changes including acute ST elevation, the development of new left bundle branch block, new persistent T wave inversion or new ST segment depression ii) Positive cTnT-hs levels with a characteristic rise and fall in levels
New arrhythmia requiring treatment	i) Ventricular fibrillation requiring counter-shock ii) Ventricular tachycardia requiring counter-shock or medication iii) Atrial fibrillation of greater than 15 minutes duration requiring counter-shock or medication
Cardiac arrest	The cessation of cardiac mechanical activity as confirmed by the absence of signs of circulation.
Congestive cardiac failure	Diagnosed clinically with corroborating radiographic evidence
Cerebrovascular accident	New onset neurological deficit, accompanied by evidence of cerebral infarction or intra-cerebral haemorrhage on CT scan, or confirmed at autopsy
Renal failure requiring renal replacement therapy	Haemodialysis, haemofiltration or peritoneal dialysis commenced post-operatively, within 30 days of surgery.
Mesenteric ischaemia	Small or large bowel ischaemia requiring laparotomy or found at autopsy or proven on colonic biopsy
Urgent cardiac revascularisation	Percutaneous or surgical cardiac revascularisation within 30 days of surgery

cTnT-hs – high sensitivity cardiac troponin T; CT – computed tomography; ECG – electrocardiograph.

As no data on treatment effect sizes exist in this population, no sample size calculation was possible for this pilot trial.

A computer generated random sequence was used. Randomisation was stratified by procedure type and by centre and random block sizes of between 4 and 8 were used. Allocation concealment was achieved by the use of sequential sealed opaque envelopes. A third party who was not involved with other aspects of the trial generated the random sequence and the sealed envelopes. Envelopes were opened sequentially prior to operations when patients were within operating theatre complexes. Members of the surgical teams enrolled patients, assigned interventions and applied RIPC where necessary. There was no blinding.

Categorical variables were compared using Fisher's exact test. Continuous variables were compared using the two sample t-test or the Mann Whitney test as appropriate. Results were presented at means with standard deviations or as medians with interquartile ranges as indicated. Minitab version 16 (Pennsylvania, USA) was used for these analyses. Perioperative myocardial injury was compared between groups by comparing area under the curve (AUC) for the first three post-operative days. The troponin measurements were log-transformed before analysis. To account for possible bias due to different missing-data patterns in the preconditioned and non-preconditioned individuals, the R-package norm [257] was used to create 100 different imputations of the missing log-troponin values, assuming a multivariate normal model. For each imputed dataset, the AUC of log-troponin against time post operation (either 0, 1, 2 or 3 days) was computed for each patient. The difference in the average values of the log-troponin AUC for the RIPC group and control groups, and the standard error of these differences was then computed for all 100 imputed datasets. These were then combined (over all 100 imputed datasets) using the technique described in [258] to calculate a single t-test statistic. As sensitivity analyses, we repeated this procedure, but instead based the t-test statistic on comparing (a) the difference in log-troponin on day 3 and pre-operation for each individual and (b) the least-squares regression slope of each individual's log-troponin measurements over time, between the two arms. For all analyses, significance was set at 5%.

5.4: Results of the randomised controlled trial

Figure 5.1 summarises patient flow through the trial. The trial ran between January 2012 and March

2014. It terminated when the 200th trial number was allocated.

Figure 5.1: Trial flow diagram
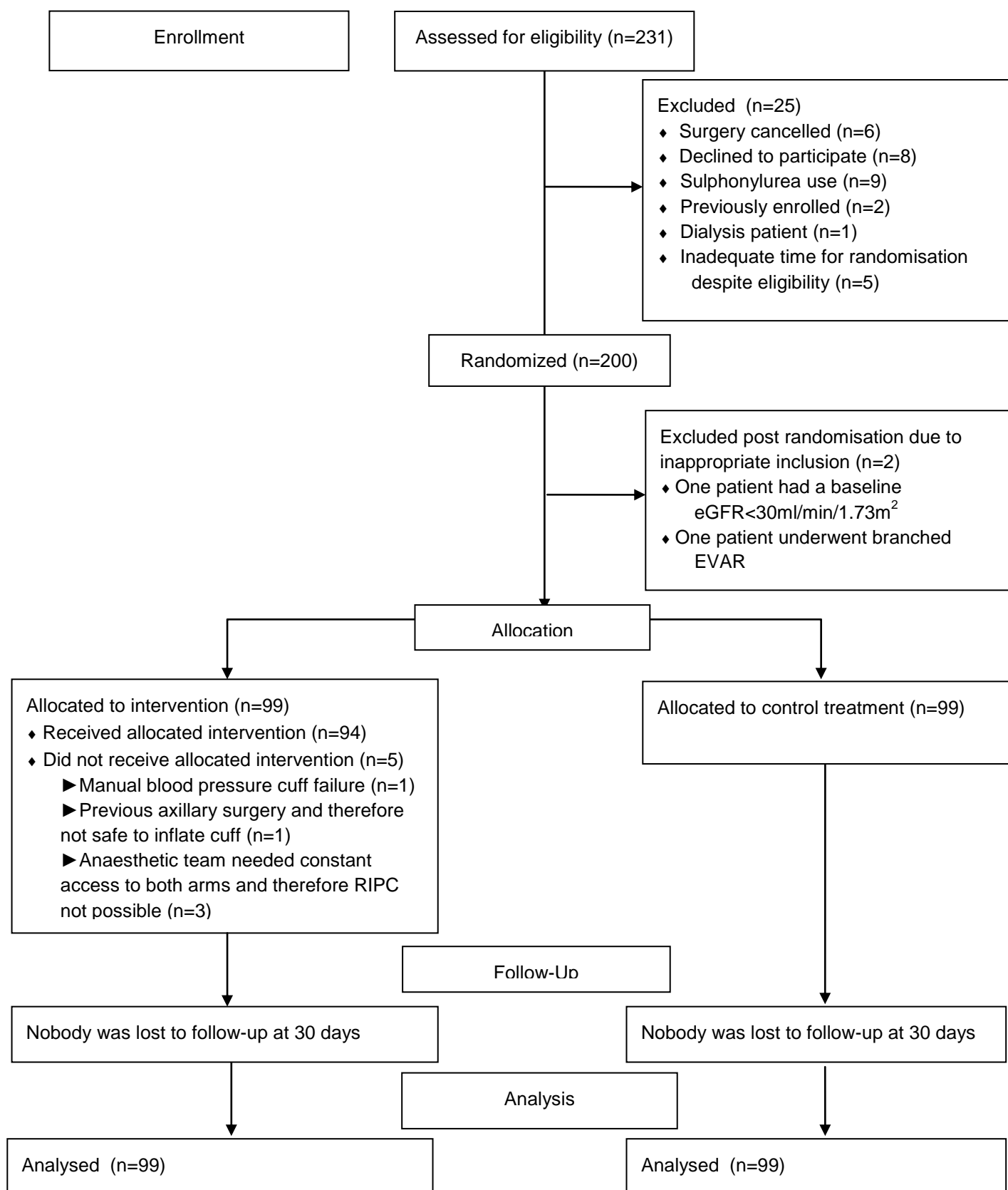
Of 231 patients assessed for eligibility 200 underwent randomisation. Two patients were excluded following randomisation: one underwent a branched EVAR procedure and thus was ineligible for inclusion and another patient had a baseline eGFR<30ml/min/1.73m^2 and thus was ineligible for inclusion. Ninety nine participants were allocated to each treatment arm. Of the 99 patients randomised to the RIPC group 94 received the intervention as described above. Five did not receive the allocated intervention: in one instance the manual blood pressure cuff failed, one patient had previous axillary surgery and on three occasions the anaesthetic team required constant access to both upper limbs. There were no losses to follow up at 30 days. Data from 99 patients in each treatment arm were finally analysed.

Table 5.2 provides details on demographics, comorbidities, medications, baseline laboratory results and operative details for each group. The groups were well matched at baseline. Mean age was 69 years in both groups and 73/99 were male in the control group versus 78/99 in the RIPC group. Similar proportions of patients in each treatment arm underwent open AAA repair, EVAR, carotid endarterectomy and lower limb revascularisation procedures. Medication use and comorbidities were similar.

Table 5.2: Baseline demographics, clinical characteristics and operative data

	Control n=99	RIPC n=99
Baseline demographics		
Mean age in years at last birthday (SD)	69.23 (9.29)	69.21 (8.98)
Proportion with male gender	73/99	78/99
Comorbidities		
Diabetes	18	13
Previous myocardial infarction	16	19
Previous coronary artery bypass graft	15	22
Previous coronary stent/plasty	6	12
Heart failure	8	7
Angina	4	6
Atrial fibrillation	18	13
Other arrhythmia	2	0
Valvular heart disease	3	5
Asthma	2	1
Chronic obstructive airways disease	16	11
Cerebrovascular accident	23	16
Limb amputation	2	0
Claudication	43	48
Critical ischaemia	28	31
Medications		
Antiplatelet	82	84
Warfarin	11	10
Heparin	2	0
Statin	83	83
Beta-blocker	36	39
ACE inhibitor	26	31
Calcium antagonist	18	27
Angiotensin blocker	21	13
Insulin	7	4
Laboratory results at baseline		
Mean pre-operative haemoglobin level in g/dl (SD)	12.93 (1.87)	13.50 (1.53)
Mean white cell count in cells/µL (SD)	8.26 (2.51)	8.00 (2.00)
Mean serum urea in mmol/L (SD)	6.76 (3.46)	5.98 (2.18)
Mean serum creatinine in µmol/L (SD)	94.6 (32.3)	90.5 (29.9)
Median preoperative serum cTnT-hs in ng/L (IQR)	8 (3-15.5)	9 (3-14.00)

Operative characteristics		
Operation type		
Open abdominal aortic aneurysm repair	7	11
Endovascular aortic aneurysm repair	25	22
Carotid endarterectomy	24	21
Lower limb revascularisation	43	45
Median blood loss in ml (IQR)	200 (50-585)	250 (50-900)
Median contrast dose in ml (IQR)	0 (0-88.08)	0 (0-66.10)

cTnT-hs – high sensitivity cardiac troponin T; IQR – interquartile range; SD – standard deviation.

Table 5.3 provides details on the primary outcome and some secondary outcomes. Table 5.4 provides details on the additional secondary outcome of troponin leakage within the first 72 hours. The primary composite clinical endpoint occurred in 19/99 controls and in 14/99 RIPC patients, representing a non-significant difference (p=0.446). There were no significant differences in occurrences of individual components of the composite endpoint or in any of the other secondary outcomes. There was no significant difference between groups regarding mean AUC for troponin over the first 72 hours post-operatively (p=0.4). Regarding our sensitivity analyses, when the t test statistic was based upon the difference in log troponin between the 72 hour point and pre-operative levels, a p value of 0.44 was attained, and when it was based on the least squares regression slope of each individuals log-troponin measurements the p value was 0.54.

Table 5.3: Primary and secondary outcomes

	Control n=99	RIPC n=99	P
Primary composite outcome	19	14	0.446
Secondary outcomes			
Cardiovascular death	2	3	1
Myocardial infarction	13	8	.356
New arrhythmia	5	4	1
Cardiac arrest	3	4	1
Congestive cardiac failure	4	3	1
Cerebrovascular accident	1	0	1
Dialysis	2	1	1
Mesenteric ischaemia	3	0	.246
Urgent cardiac revascularisation	0	1	1
Median hospital length of stay (IQR)	7 (3-12)	6 (3-10)	.174
Median ICU length of stay in days (IQR)	1 (0-2)	0 (0-2)	.525
Unplanned ICU admission	10	4	.164
Vascular graft occlusion	2	1	1
Wound infection	13	13	1.000
Respiratory tract infection	13	6	.146
Urinary infection	1	3	.621
Pulmonary embolus	0	0	N/A
Deep venous thrombosis	1	1	1.000
Limb ischaemia	4	2	.683
Major limb amputation	2	0	.497
Proportion with a creatinine rise >20% from baseline	29/99	22/99	.330

ICU – intensive care unit; IQR – interquartile range; SD – standard deviation.

Table 5.4: Serum troponin values within the first 72 hours post-operatively

	Control	RIPC	P
Median preoperative serum cTnT-hs (IQR)	8 (3-15.5)	9 (3-14.00)	.804
Median serum cTnT-hs 24 hours post surgery	12 (6-26.5)	12 (7-18)	.799
Median serum cTnT-hs 48 hours post surgery	13 (5-29)	12 (4-20.5)	.280
Median serum cTnT-hs 72 hours post surgery	16 (9-43)	14 (7-21)	.400

cTnT-hs – high sensitivity cardiac troponin T; IQR – interquartile range.

5.5: Discussion

In this pilot study of 200 patients undergoing major vascular surgery, the effect of RIPC on clinical outcomes was evaluated. There was no significant effect of RIPC on the predefined primary composite clinical outcome: it occurred in 19/99 control patients (19.2%) and 14/99 RIPC patients (14.1%) (p=0.446). Secondary outcomes included the individual components of the primary outcome – unsurprisingly there was no significant effect of RIPC on any of these. Additionally there was no difference in hospital or ICU length of stay. An additional secondary outcome was perioperative myocardial injury and again there was no significant effect with RIPC when AUC for troponin leakage within 72 hours was compared between groups (p=0.4). Although this was a pilot study that has yielded a negative result, it makes a valuable contribution to knowledge of the clinical effects of RIPC and it can serve to guide future research in this area.

Six other clinical trials have evaluated RIPC in major vascular surgery: four have examined RIPC in the setting of open AAA repair [140-142, 259] and trials have also examined RIPC in EVAR [143] and carotid endarterectomy [144]. Ali *et al.* randomised 82 open AAA patients to a lower limb RIPC stimulus achieved via iliac artery cross clamping or to control groups; they found that RIPC reduced myocardial and renal injury at a biochemical level and also that myocardial infarction rates were reduced with RIPC [140]. Li *et al.* randomised 62 open AAA patients and used an upper limb cuff induced stimulus [142]. They found biochemical evidence for a protective effect of RIPC on pulmonary and intestinal injury but notably they did not measure cardiac enzyme release and they found no difference in rates of myocardial infarction. The studies by Ali and Li both found significant primary outcome results favouring RIPC – this highlights the organ protective potential of RIPC in AAA repair. However the remaining vascular trials yielded negative results as did the current trial. A third open AAA trial [141] involved 40 patients and a lower limb RIPC stimulus achieved by cross clamping iliac arteries. The primary outcome was biochemical renal injury and no difference was found. The final open AAA trial by Murphy *et al.* [259] used an upper limb RIPC stimulus and had a sample size of 62 patients. Post-operative creatinine was the primary outcome and no difference was found between the groups. The trials on EVAR [143] and carotid endarterectomy [144] involved 40 and 70 patients respectively and utilised a lower limb cuff-induced RIPC stimulus. They used

surrogate outcome measures of neurological, cardiac and renal injury and found no difference between groups.

Regarding cardiovascular interventions in general, most of the trials on RIPC have focused on surrogate outcomes and only a few studies [121, 207] have had clinical primary endpoints. Systematic reviews involving cardiac surgery [201], CABG surgery [86], percutaneous coronary intervention (PCI) [203] and cardiovascular surgery combined with PCI [89] have found results favouring RIPC. A recurrent conclusion is that RIPC can reduce myocardial injury as determined by cardiac enzyme release [61, 89, 201, 202] and notably reviews involving PCI only [203] and cardiac and vascular surgery combined with PCI [89] found reduced myocardial infarction rates. A recent large-scale meta-analysis that examined major clinical outcomes following cardiovascular surgery found no evidence for a significant benefit with RIPC [102]but notably the analysis was underpowered to detect clinical outcomes benefits and was limited by study heterogeneity. Nonetheless, the conclusions provided grounds for optimism regarding the potential for RIPC to reduce perioperative MI as the analysis had pooled data from 17 trials (1777 patients) on RIPC in cardiovascular surgery [107-109, 111-113, 115, 121, 122, 140-144, 204, 205, 208] and found a pooled risk ratio of 0.69 (95% CI 0.34 to 1.40) favouring RIPC. When the MIs in the current trial and the 2014 AAA trial by Murphy *et al.* [259] are additionally included, the pooled risk ratio becomes 0.68 (95% CI 0.41 to 1.14) (figure 5.2) (this analysis used RevMan version 5.3, Copenhagen, Denmark).

Figure 5.2: Forest plot for perioperative myocardial infarction including all trials on remote ischaemic preconditioning in cardiovascular surgery

Study or Subgroup	RIPC Events	RIPC Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Ali N	0	50	0	50		Not estimable
Ali ZA	2	41	11	41	10.1%	0.18 [0.04, 0.77]
Candilio	0	89	1	89	2.5%	0.33 [0.01, 8.07]
Choi	0	38	0	38		Not estimable
Hausenloy, Venugopal	0	50	0	50		Not estimable
Healy	8	99	13	99	21.6%	0.62 [0.27, 1.42]
Hong	0	65	0	65		Not estimable
Li AAA	2	31	1	31	4.3%	2.00 [0.19, 20.93]
Li Cardiac	0	26	0	27		Not estimable
Lucchinetti	3	27	1	28	4.9%	3.11 [0.34, 28.09]
Meybohm	4	90	1	90	5.0%	4.00 [0.46, 35.09]
Murphy	4	31	2	31	8.3%	2.00 [0.39, 10.13]
Rahman	4	80	7	82	13.6%	0.59 [0.18, 1.92]
Thielmann	8	162	21	167	23.1%	0.39 [0.18, 0.86]
Walsh Carotid	0	34	0	36		Not estimable
Walsh EVAR	1	18	1	22	3.4%	1.22 [0.08, 18.20]
Walsh Open	1	22	1	18	3.4%	0.82 [0.05, 12.19]
Zimmerman	0	59	0	59		Not estimable
Total (95% CI)		1012		1023	100.0%	0.68 [0.41, 1.14]
Total events	37		60			

Heterogeneity: Tau² = 0.13; Chi² = 12.36, df = 10 (P = 0.26); I² = 19%

Test for overall effect: Z = 1.45 (P = 0.15)

Despite the lack of adequately powered trials with hard clinical outcomes as primary endpoints, convincing "proof of concept" evidence underpins the biological plausibility of achieving cardioprotection via RIPC. In contrast to the situation regarding cardiac surgery, there is a relative paucity of data specifically relating to vascular surgery and conclusions from the available trials are less consistent. Five of the seven vascular trials to date including the current trial have had negative primary outcome results. The reasons underlying this are uncertain although aspects that are probably implicated are small sample sizes and the heterogeneity in terms of populations, outcome measures and procedures. Most of the cardiac surgery trials used cardiac enzyme levels as an outcome measure. In contrast, only four vascular trials evaluated such outcomes ([140],[144, 259] and the current trial). The current report provides important data that may guide the design of future trials involving RIPC and any of the included vascular procedures. Although the major challenge regarding RIPC in the

wider cardiovascular sphere is to generate convincing evidence of clinical benefits, we think that the most feasible next step within the vascular surgery field is to harden the evidence with regard to proof of concept. As such, we think that an adequately powered vascular surgery trial is needed to provide convincing proof of cardioprotection via RIPC.

The principle strength of the current trial is its pragmatic multicentre design which involves an emphasis on clinical as well as surrogate outcomes. Regarding limitations, the chief concern is the omission of a sham intervention. Although blinding surgeons, patients and outcome assessors would have increased the validity of the trial, its omission has allowed for a far greater sample size than would otherwise have been possible for this pilot trial. It is worth highlighting that it was possible to achieve blinding in the current trial in relation to perioperative MI – a blinded cardiologist assessed this outcome, making this analysis quite robust. A further drawback relates to the length of follow up – this was limited to 30 days and therefore conclusions beyond this point cannot be made. Finally, it is important to emphasise that included patients underwent a diverse range of procedures rather than one type of procedure. This allowed enhanced recruitment, although the resultant heterogeneity in terms of the population would reduce the chance of a achieving a significance.

As this was a pilot trial with the purpose of providing data upon which to base the design of a future study, a large-scale feasibility trial of RIPC in vascular surgery is proposed [260]. In this proposed trial there are three main objectives: to fully evaluate the ability of arm-induced RIPC to confer protection in major vascular surgery, to assess the incidence of a proposed composite primary efficacy endpoint and to evaluate the acceptability of the intervention to patients and clinical staff. Recruitment for this trial (NCT02097186) has commenced and we hope that it will yield convincing evidence to confirm the role of RIPC in vascular surgery or to condemn it.

In this pilot trial there was no significant evidence to support the hypothesis that RIPC offers perioperative protective to patients undergoing major vascular surgery. Most of the trials to date on RIPC in vascular surgery have yielded negative results. Despite this, RIPC represents a theoretically attractive risk reduction strategy as convincing mechanistic data confirm its potential in

cardiovascular surgery. Although the long term goal is to evaluate patient important outcomes, for now future trials on RIPC in major vascular surgery should aim to clarify "proof-of-concept".

Chapter 6: Discussion and conclusion

6.1 Discussion

Remote ischaemic preconditioning (RIPC) is a phenomenon that is brought about by brief periods of ischaemia-reperfusion in an organ or tissue, resulting in the induction of a protective phenotype. In this way, a degree of resistance to sustained ischaemic insults can be granted to tissues that have undergone transient ischaemic episodes. RIPC was discovered in 1993 when Przyklenk *et al.* [76] found that transient ischaemia in one coronary artery distribution resulted in resistance to sustained ischaemia in adjacent coronary artery territory. However, the invasive nature of preconditioning limited its utility until a non-invasive stimulus was discovered – in 2002, Kharbanda *et al.* [78] found that transient limb ischaemia induced by inflation of a blood pressure cuff offered organ protection. Following this there was a remarkable increase in the volume of research on RIPC and a notable shift towards clinical studies involving human subjects.

The exact mechanisms underlying RIPC remain unclear. However, evidence suggests the existence of several overlapping aspects – neural, humoral and systemic components have been proposed [92]. These factors converge in the myocardium and induce an anti-apoptotic response by preventing closure of mitochondrial permeability transition pores. It is likely that organ protection outside of the heart is induced in a similar fashion, although most evidence to date has focused on cardioprotection. The potential of RIPC is enormous – any organ can be protected by a simple, cheap, non-invasive stimulus.

The concept of organ-protection is not new – a variety of periprocedural cardioprotective strategies have been investigated and used in the past. These include the use of risk assessment, pharmacological cardioprotection, prophylactic revacularisation and the use of ischaemic preconditioning and post-conditioning techniques. The importance of augmenting organ-protection has risen dramatically over recent years in line with the mounting global burden of cardiovascular disease and in particular the expanding burden of periprocedural cardiovascular disease. Furthermore, with the increasing use of contrast-based imaging techniques, there is an urgent need to augment renoprotection in the setting of contrast administration.

The aim of this thesis was to explore the applications of RIPC with a particular focus on clinical care. The work had four chief components – a systematic review and meta-analysis of RIPC in percutaneous coronary intervention (PCI), a systematic review and meta-analysis of RIPC in major cardiovascular surgery, a single-centre randomised controlled trial on RIPC in the setting of contrast-enhanced computed tomography (CE-CT) scanning and a multi-centre randomised controlled trial on RIPC in major vascular surgery. All four aspects of this thesis have led to advances in knowledge but many uncertainties remain and further research is needed in order to maximise the potential of RIPC.

The second chapter of this thesis was a systematic review and meta-analysis of RIPC in PCI. Each year about 1.5 million people in the United States undergo PCI and about 5-30% develop a periprocedural myocardial infarction (MI). There is some controversy regarding the clinical significance of MIs that occur in the setting of PCI – a body of evidence suggests that there are associated adverse outcomes but there is also evidence that suggests that most periprocedural MIs are too small and localised to be of clinical significance [191]. Regardless of this uncertainty, it is important to minimise the incidence of periprocedural complications.

The systematic review identified eight randomised controlled trials involving 1119 patients who were undergoing both elective and emergency PCI. Six of eight trials (983) had primary outcomes that significantly favoured RIPC. The meta-analysis examined three outcomes – troponin positive events, periprocedural MI and acute kidney injury. There was a significant reduction in periprocedural MI incidence with RIPC (pooled OR = 0.577, 95%CI 0.400 – 0.833, p=0.003) based upon data from four studies and 636 patients. There was no difference in troponin positive events between RIPC and control groups (pooled OR 0.529, 95%CI 0.206 – 1.358, p=0.185) (three studies, 377 patients) and there was no difference in AKI incidence (pooled OR = 0.672, 95%CI 0.252 – 1.787, p=0.425) (two studies, 407 patients).

These results are very encouraging and provide "proof of concept" for the use of RIPC in PCI. Given the number of PCI procedures performed globally, RIPC may become a routine aspect of periprocedural care. The chief limitation of the review was that included studies were small in size

and that there was clinical heterogeneity among the studies. In the future, large scale multi-centre studies are needed before RIPC can be used in PCI outside of research settings. The published article makes an important contribution as it pooled all of the available data on RIPC and PCI and it will thus enhance future research allowing increased accuracy in terms of power calculations and other aspects of design.

The third chapter of this thesis was a systematic review and meta-analysis of RIPC in major cardiovascular surgery. There have been several previous systematic reviews and meta-analyses of RIPC in this setting – all found significant benefits favouring RIPC in relation to reductions in biochemical markers of cardiac injury. However, the effect of RIPC on clinical outcomes following major cardiovascular surgery is less certain because few published trials have reported clinical outcomes as primary endpoints. The aim of this part of the thesis was to complete an extensive review of published and unpublished clinical outcomes data from all cardiothoracic and vascular surgery RIPC trials. The clinical outcomes for the review were predefined, comprising death, myocardial infarction, new arrhythmia requiring treatment, cerebrovascular accident, renal failure requiring renal replacement therapy, mesenteric ischaemia and length of stay in hospital and in intensive care.

In total, data from 23 trials and 2200 patients were included in the review. There were no significant findings in favour of RIPC. However, an encouraging result was found in relation to the outcome of MI – the incidence of perioperative MI in the RIPC group was almost half that of the control group (2.8% versus 4.9%). Based upon these event rates, about 1200 patients would be required in each arm of a trial to confirm that RIPC reduces perioperative MI rates from 4% to 2% with 80% power at the 5% significance level. Several multicentre trials are currently recruiting and such participant numbers are likely to be achieved in the future. It was important to perform such a meta-analysis in order to ethically justify large scale research undertakings. The published article makes an important contribution to the literature as it emphasises the need for data on hard clinical outcomes. The meta-analysis will enhance power calculations in future trials and the systematic review element will improve methodology of future trials as it provides a detailed summary and critical appraisal of the methodology of the included trials.

The fourth chapter described a pilot clinical trial on RIPC for the prevention of contrast induced kidney injury in patients who were undergoing contrast enhanced computed tomography (CE-CT) scanning. Contrast induced kidney injury is a frequent cause of hospital-acquired renal impairment and it is likely that it results in long term consequences for patients because evidence confirms that even small degrees of renal impairment are associated with increased atherosclerotic disease. As contrast-related kidney injury is partly mediated by renal medullary ischaemia, RIPC may confer renoprotection. Two trials involving patients who were undergoing coronary angiography found significant renoprotective effects with RIPC [137, 138] but no study had examined RIPC in the setting of CE-CT.

Eighty seven patients were included (44 RIPC, 43 control). Across the whole cohort of patients in the trial, there were no significant differences in the primary outcome of serum creatinine change or the secondary outcomes of serum urea and incidence of reduced urinary output within 48 hours and length of hospital stay. However, a protective effect of RIPC was found in an exploratory subgroup analysis that examined those who had renal impairment at baseline. In this subgroup, RIPC reduced the relative risk of increased serum creatinine; RR 0.40 (95% confidence intervals 0.17 to 0.95). Logistic regression revealed that RIPC was the only model variable that predicted decreased SCr; odds ratio 0.24 (95% confidence intervals 0.07 to 0.84) in patients with impaired renal function. This pilot trial result provides rationale for a larger trial on RIPC in this setting and it confirms feasibility of the study design that was used. Based on the findings, it seems as though future trials investigating RIPC in this setting should perhaps focus solely on those with renal impairment. It also will improve the methodology of future trials because it highlights the need for analysis stratification based upon renal function. The concept of analysis stratification is not widely practiced in studies on RIPC. This study and a variety of other studies suggest that little additional protection can be offered by RIPC after low- magnitude injuries or in low risk settings.

The final chapter described a pilot clinical trial on RIPC in major vascular surgery. Patients who are undergoing major vascular surgery are a high risk group and frequently suffer complications. Theoretically RIPC offers the potential to confer protection regardless of the mechanism of injury.

This is in contrast to other methods of risk reduction which can target individual mechanisms only. Although RIPC seems attractive in this regard, no definitive evidence for benefits in clinical outcomes exists.

A multi-centre pilot randomised trial was performed in three Irish hospitals. Eligible patients were those who were undergoing elective carotid endarterectomy, open abdominal aortic aneurysm (AAA) repair, endovascular aortic aneurysm repair (EVAR) or surgical lower limb revascularisation (suprainguinal or infrainguinal). The primary outcome was a composite clinical endpoint comprising any of cardiovascular death, myocardial infarction, new onset arrhythmia, cardiac arrest, congestive cardiac failure, cerebrovascular accident, renal failure requiring renal replacement therapy, mesenteric ischaemia and urgent cardiac revascularisation within 30 days of operation. Pre-specified secondary outcomes were: duration of post-operative hospital and intensive care unit (ICU) stay, unplanned critical care admissions and post-procedure renal injury. The individual components of the composite primary outcomes were further secondary endpoints. Post-operative complications (wound infections, respiratory tract infections, deep venous thromboses, pulmonary emboli, limb ischaemia, limb amputations) were included as post-hoc secondary outcomes. Perioperative myocardial injury, assessed by area under the curve of periprocedural high sensitivity cardiac troponin t (cTnT-hs), was a final secondary outcome.

Two hundred patients were recruited and 198 were included in the analysis. The primary outcome occurred in 19/99 controls (19.2%) and 14/99 RIPC group (14.1%) patients (p=0.446). There were no significant differences in secondary outcomes.

Although the trial generated an inconclusive result, it makes an important contribution. Most importantly, it confirmed that it was feasible to recruit, randomise, perform the RIPC intervention and retain subjects for outcome assessment. Experiences from the pilot trial provided the basis for the design of a larger multi-centre funded trial that is currently recruiting [260]. Furthermore, the trial remains the largest trial on RIPC and major vascular surgery to date and it is the largest academic surgical clinical trial to have been completed in Ireland. Given that clinical trial infrastructure is

underdeveloped in Ireland compared with other western countries, confirmation of feasibility is a major achievement. The report emphasises the importance of focusing on clinical outcomes rather than surrogate biochemical outcomes and it provides data that will guide other trials. Data on event rates in the trial were used in sample size estimation for the larger trial mentioned earlier [260]. Although it is clear from proof of concept studies that RIPC can reduce myocardial injury in cardiac surgery, in contrast, many of the vascular surgery trials have yielded negative results. Therefore, within the context of vascular surgery, proof of concept needs to be verified conclusively before large scale studies with clinical primary endpoints can be justified.

6.2 Conclusion

RIPC is undoubtedly an attractive organ-protection strategy and it has potential for risk reduction in a variety of settings. Conclusive evidence of benefits is available from animal studies and also from studies in humans that used surrogate biochemical outcomes. However, before it can be used in routine clinical practice we need definitive evidence of clinical outcomes benefits in humans. The work described in this thesis makes an important contribution towards this goal. The systematic review and meta-analysis on RIPC and PCI highlighted the potential for a reduction in periprocedural myocardial infarction rates with RIPC. Further large scale trials are needed as the review was based upon studies with small sample sizes. The review on RIPC and major cardiovascular surgery included a large amount of previously unpublished data. Although, it did not confirm clinical outcomes benefits, there are grounds for optimism as the incidence of perioperative myocardial infarction in the RIPC arm was almost half that of the control arm (2.8% versus 4.9%). It is hoped that with the completion of other large scale multi-centre trials this may be confirmed. Although the two trials described in this thesis ultimately yielded negative results, they demonstrated feasibility and provided experiences and data that have enormous potential for positively influencing the design of other studies. Both trials suggest that larger sample sizes are needed to generate convincing data.

References

1.   *World Health Report 1999: Making a difference. Geneva.*, 1999.
2.   Omran, A.R., *The epidemiologic transition. A theory of the epidemiology of population change.* Milbank Mem Fund Q, 1971. **49**(4): p. 509-38.
3.   Yusuf, S.*, et al.*, *Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization.* Circulation, 2001. **104**(22): p. 2746-53.
4.   Christopher J. L. Murray, A.D.L., *The global burden of disease : a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020 . Cambridge, Mass. : Harvard University Press.*1996.
5.   Kaplan, G.A. and J.E. Keil, *Socioeconomic factors and cardiovascular disease: a review of the literature.* Circulation, 1993. **88**(4 Pt 1): p. 1973-98.
6.   Marmot, M.G., M. Kogevinas, and M.A. Elston, *Socioeconomic status and disease.* WHO Reg Publ Eur Ser, 1991. **37**: p. 113-46.
7.   Popkin, B.M., *The nutrition transition and its health implications in lower-income countries.* Public Health Nutr, 1998. **1**(1): p. 5-21.
8.   Go, A.S.*, et al.*, *Executive summary: heart disease and stroke statistics--2013 update: a report from the American Heart Association.* Circulation, 2013. **127**(1): p. 143-52.
9.   Roger, V.L.*, et al.*, *Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. 188-97.
10. Lloyd-Jones, D.*, et al.*, *Executive summary: heart disease and stroke statistics--2010 update: a report from the American Heart Association.* Circulation, 2010. **121**(7): p. 948-54.
11. Haynes, A.B.*, et al.*, *A surgical safety checklist to reduce morbidity and mortality in a global population.* N Engl J Med, 2009. **360**(5): p. 491-9.
12. Weiser, T.G.*, et al.*, *An estimation of the global volume of surgery: a modelling strategy based on available data.* Lancet, 2008. **372**(9633): p. 139-44.
13. Devereaux, P.J.*, et al.*, *Perioperative cardiac events in patients undergoing noncardiac surgery: a review of the magnitude of the problem, the pathophysiology of the events and methods to estimate and communicate risk.* CMAJ, 2005. **173**(6): p. 627-34.
14. Group, P.S.*, et al.*, *Effects of extended-release metoprolol succinate in patients undergoing non-cardiac surgery (POISE trial): a randomised controlled trial.* Lancet, 2008. **371**(9627): p. 1839-47.
15. Lee, T.H.*, et al.*, *Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery.* Circulation, 1999. **100**(10): p. 1043-9.
16. Mangano, D.T.*, et al.*, *Long-term cardiac prognosis following noncardiac surgery. The Study of Perioperative Ischemia Research Group.* JAMA, 1992. **268**(2): p. 233-9.
17. Sprung, J.*, et al.*, *Predictors of survival following cardiac arrest in patients undergoing noncardiac surgery: a study of 518,294 patients at a tertiary referral center.* Anesthesiology, 2003. **99**(2): p. 259-69.
18. Charlson, M.*, et al.*, *Long-term prognosis after peri-operative cardiac complications.* J Clin Epidemiol, 1994. **47**(12): p. 1389-400.
19. Fleischmann, K.E.*, et al.*, *Association between cardiac and noncardiac complications in patients undergoing noncardiac surgery: outcomes and effects on length of stay.* Am J Med, 2003. **115**(7): p. 515-20.
20. Falk, E., P.K. Shah, and V. Fuster, *Coronary plaque disruption.* Circulation, 1995. **92**(3): p. 657-71.
21. Thygesen, K.*, et al.*, *Third universal definition of myocardial infarction.* J Am Coll Cardiol, 2012. **60**(16): p. 1581-98.
22. Landesberg, G.*, et al.*, *Perioperative myocardial infarction.* Circulation, 2009. **119**(22): p. 2936-44.

References

23.	Cohen, M.C. and T.H. Aretz, *Histological analysis of coronary artery lesions in fatal postoperative myocardial infarction.* Cardiovasc Pathol, 1999. **8**(3): p. 133-9.
24.	Dawood, M.M.*, et al.*, *Pathology of fatal perioperative myocardial infarction: implications regarding pathophysiology and prevention.* Int J Cardiol, 1996. **57**(1): p. 37-44.
25.	Ellis, S.G.*, et al.*, *Angiographic correlates of cardiac death and myocardial infarction complicating major nonthoracic vascular surgery.* Am J Cardiol, 1996. **77**(12): p. 1126-8.
26.	Fleisher, L.A.*, et al.*, *ACC/AHA 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery): developed in collaboration with the American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Rhythm Society, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, and Society for Vascular Surgery.* Circulation, 2007. **116**(17): p. e418-99.
27.	Fleisher, L.A.*, et al.*, *2009 ACCF/AHA focused update on perioperative beta blockade incorporated into the ACC/AHA 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery: a report of the American college of cardiology foundation/American heart association task force on practice guidelines.* Circulation, 2009. **120**(21): p. e169-276.
28.	Gupta, P.K.*, et al.*, *Development and validation of a risk calculator for prediction of cardiac risk after surgery.* Circulation, 2011. **124**(4): p. 381-7.
29.	Goldman, L., *The revised cardiac risk index delivers what it promised.* Ann Intern Med, 2010. **152**(1): p. 57-8.
30.	2013 15 July 2013]; Available from: http://www.surgicalriskcalculator.com/miorcardiacarrest.
31.	Mangano, D.T., *Perioperative cardiac morbidity.* Anesthesiology, 1990. **72**(1): p. 153-84.
32.	Kumar, R.*, et al.*, *Adverse cardiac events after surgery: assessing risk in a veteran population.* J Gen Intern Med, 2001. **16**(8): p. 507-18.
33.	Garcia, S.*, et al.*, *Perioperative complications after vascular surgery are predicted by the revised cardiac risk index but are not reduced in high-risk subsets with preoperative revascularization.* Circ Cardiovasc Qual Outcomes, 2009. **2**(2): p. 73-7.
34.	Khuri, S.F.*, et al.*, *The National Veterans Administration Surgical Risk Study: risk adjustment for the comparative assessment of the quality of surgical care.* J Am Coll Surg, 1995. **180**(5): p. 519-31.
35.	American College of Cardiology/American Heart Association Task Force on Practice, G.*, et al.*, *ACC/AHA 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery).* Anesth Analg, 2008. **106**(3): p. 685-712.
36.	Leung, M.K. and M.G. Irwin, *Perioperative cardioprotection.* F1000Prime Rep, 2013. **5**: p. 7.
37.	McFalls, E.O.*, et al.*, *Coronary-artery revascularization before elective major vascular surgery.* N Engl J Med, 2004. **351**(27): p. 2795-804.
38.	Schouten, O.*, et al.*, *Long-term outcome of prophylactic coronary revascularization in cardiac high-risk patients undergoing major vascular surgery (from the randomized DECREASE-V Pilot Study).* Am J Cardiol, 2009. **103**(7): p. 897-901.
39.	Poldermans, D.*, et al.*, *A clinical randomized trial to evaluate the safety of a noninvasive approach in high-risk patients undergoing major vascular surgery: the DECREASE-V Pilot Study.* J Am Coll Cardiol, 2007. **49**(17): p. 1763-9.

References

40.   Eagle, K.A. and H.S. Gurm, *We were fishing for TROUT and we caught a CARP: musings on perioperative management in an age of enlightenment.* Circ Cardiovasc Qual Outcomes, 2009. **2**(2): p. 61-2.

41.   Gerstein, N.S.*, et al.*, *Should more patients continue aspirin therapy perioperatively?: clinical impact of aspirin withdrawal syndrome.* Ann Surg, 2012. **255**(5): p. 811-9.

42.   Antithrombotic Trialists, C., *Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients.* BMJ, 2002. **324**(7329): p. 71-86.

43.   Burger, W.*, et al.*, *Low-dose aspirin for secondary cardiovascular prevention - cardiovascular risks after its perioperative withdrawal versus bleeding risks with its continuation - review and meta-analysis.* J Intern Med, 2005. **257**(5): p. 399-414.

44.   Sahebally, S.M.*, et al.*, *Should patients taking aspirin for secondary prevention continue or discontinue the medication prior to elective, abdominal surgery? Best evidence topic (BET).* Int J Surg, 2014. **12**(5): p. 16-21.

45.   O'Riordan, J.M.*, et al.*, *Antiplatelet agents in the perioperative period.* Arch Surg, 2009. **144**(1): p. 69-76; discussion 76.

46.   Patrono, C., *Low-dose aspirin in primary prevention: cardioprotection, chemoprevention, both, or neither?* Eur Heart J, 2013.

47.   *Prevention of pulmonary embolism and deep vein thrombosis with low dose aspirin: Pulmonary Embolism Prevention (PEP) trial.* Lancet, 2000. **355**(9212): p. 1295-302.

48.   Falck-Ytter, Y.*, et al.*, *Prevention of VTE in orthopedic surgery patients: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines.* Chest, 2012. **141**(2 Suppl): p. e278S-325S.

49.   Raj, M.D.*, et al.*, *Stopping anticoagulation before TURP does not appear to increase perioperative cardiovascular complications.* Urology, 2011. **78**(6): p. 1380-4.

50.   Davis, N.F., B.B. McGuire, and H.D. Flood, *Perioperative Management of Antithrombotic Agents in Urological Surgery.* British Journal of Medical and Surgical Urology, 2011. **4**(5): p. 187-196.

51.   Bangalore, S.*, et al.*, *Perioperative beta blockers in patients having non-cardiac surgery: a meta-analysis.* Lancet, 2008. **372**(9654): p. 1962-76.

52.   Dunkelgrun, M.*, et al.*, *Bisoprolol and fluvastatin for the reduction of perioperative cardiac mortality and myocardial infarction in intermediate-risk patients undergoing noncardiovascular surgery: a randomized controlled trial (DECREASE-IV).* Ann Surg, 2009. **249**(6): p. 921-6.

53.   Raby, K.E.*, et al.*, *The effect of heart rate control on myocardial ischemia among high-risk patients after vascular surgery.* Anesth Analg, 1999. **88**(3): p. 477-82.

54.   Brady, A.R.*, et al.*, *Perioperative beta-blockade (POBBLE) for patients undergoing infrarenal vascular surgery: results of a randomized double-blind controlled trial.* J Vasc Surg, 2005. **41**(4): p. 602-9.

55.   Zaugg, M.*, et al.*, *Beneficial effects from beta-adrenergic blockade in elderly patients undergoing noncardiac surgery.* Anesthesiology, 1999. **91**(6): p. 1674-86.

56.   Poldermans, D.*, et al.*, *The effect of bisoprolol on perioperative mortality and myocardial infarction in high-risk patients undergoing vascular surgery. Dutch Echocardiographic Cardiac Risk Evaluation Applying Stress Echocardiography Study Group.* N Engl J Med, 1999. **341**(24): p. 1789-94.

57.   Wallace, A.*, et al.*, *Prophylactic atenolol reduces postoperative myocardial ischemia. McSPI Research Group.* Anesthesiology, 1998. **88**(1): p. 7-17.

58.   Mangano, D.T.*, et al.*, *Effect of atenolol on mortality and cardiovascular morbidity after noncardiac surgery. Multicenter Study of Perioperative Ischemia Research Group.* N Engl J Med, 1996. **335**(23): p. 1713-20.

References

59.   Smith, S.C.*, et al., AHA/ACCF Secondary Prevention and Risk Reduction Therapy for Patients With Coronary and Other Atherosclerotic Vascular Disease: 2011 Update: A Guideline From the American Heart Association and American College of Cardiology Foundation.* Circulation, 2011.

60.   Maroko, P.R.*, et al., Coronary artery reperfusion. I. Early effects on local myocardial function and the extent of myocardial necrosis.* J Clin Invest, 1972. **51**(10): p. 2710-6.

61.   Heusch, G., *Cardioprotection: chances and challenges of its translation to the clinic.* Lancet, 2013. **381**(9861): p. 166-75.

62.   Rezkalla, S.H. and R.A. Kloner, *No-Reflow Phenomenon.* Circulation, 2002. **105**(5): p. 656-662.

63.   Piper, H.M., D. Garcña-Dorado, and M. Ovize, *A fresh look at reperfusion injury.* Cardiovascular Research, 1998. **38**(2): p. 291-300.

64.   Xu, Y.*, et al., Activated platelets contribute importantly to myocardial reperfusion injury.* Am J Physiol Heart Circ Physiol, 2006. **290**(2): p. H692-9.

65.   Saraste, A.*, et al., Apoptosis in human acute myocardial infarction.* Circulation, 1997. **95**(2): p. 320-3.

66.   Reffelmann, T. and R.A. Kloner, *The no-reflow phenomenon: A basic mechanism of myocardial ischemia and reperfusion.* Basic Res Cardiol, 2006. **101**(5): p. 359-72.

67.   Eltzschig, H.K. and C.D. Collard, *Vascular ischaemia and reperfusion injury.* Br Med Bull, 2004. **70**: p. 71-86.

68.   Duane S Pinto, C.M.G., Joanna J Wykrzykowska, *Ischemic reperfusion injury of the heart*. Uptodate, ed. D.E. Basow2013, Waltham, MA.

69.   Murry, C.E., R.B. Jennings, and K.A. Reimer, *Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium.* Circulation, 1986. **74**(5): p. 1124-36.

70.   Geft, I.L.*, et al., Intermittent brief periods of ischemia have a cumulative effect and may cause myocardial necrosis.* Circulation, 1982. **66**(6): p. 1150-3.

71.   Murry, C.E.*, et al., Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode.* Circulation Research, 1990. **66**(4): p. 913-31.

72.   Li, G.C.*, et al., Myocardial protection with preconditioning.* Circulation, 1990. **82**(2): p. 609-19.

73.   Gross, G.J. and J.A. Auchampach, *Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs.* Circ Res, 1992. **70**(2): p. 223-33.

74.   Murry, C.E.*, et al., Myocardial protection is lost before contractile function recovers from ischemic preconditioning.* Am J Physiol, 1991. **260**(3 Pt 2): p. H796-804.

75.   Ovize, M.*, et al., Coronary cyclic flow variations "precondition" ischemic myocardium.* Circulation, 1992. **85**(2): p. 779-89.

76.   Przyklenk, K.*, et al., Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion.* Circulation, 1993. **87**(3): p. 893-9.

77.   Birnbaum, Y., S.L. Hale, and R.A. Kloner, *Ischemic preconditioning at a distance: reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit.* Circulation, 1997. **96**(5): p. 1641-6.

78.   Kharbanda, R.K.*, et al., Transient limb ischemia induces remote ischemic preconditioning in vivo.* Circulation, 2002. **106**(23): p. 2881-3.

79.   Pell, T.J.*, et al., Renal ischemia preconditions myocardium: role of adenosine receptors and ATP-sensitive potassium channels.* Am J Physiol, 1998. **275**(5 Pt 2): p. H1542-7.

80.   Gho, B.C.*, et al., Myocardial protection by brief ischemia in noncardiac tissue.* Circulation, 1996. **94**(9): p. 2193-200.

81.   Oxman, T.*, et al., Limb ischemia preconditions the heart against reperfusion tachyarrhythmia.* Am J Physiol, 1997. **273**(4 Pt 2): p. H1707-12.

82.   Takagi, H.*, et al., Review and meta-analysis of randomized controlled clinical trials of remote ischemic preconditioning in cardiovascular surgery.* Am J Cardiol, 2008. **102**(11): p. 1487-8.

References

83. Takagi, H. and T. Umemoto, *Remote ischemic preconditioning for cardiovascular surgery: an updated meta-analysis of randomized trials.* Vasc Endovascular Surg, 2011. **45**(6): p. 511-3.

84. Yetgin, T.*, et al.*, *Remote ischemic conditioning in percutaneous coronary intervention and coronary artery bypass grafting.* Circ J, 2012. **76**(10): p. 2392-404.

85. Alreja, G., D. Bugano, and A. Lotfi, *Effect of remote ischemic preconditioning on myocardial and renal injury: meta-analysis of randomized controlled trials.* J Invasive Cardiol, 2012. **24**(2): p. 42-8.

86. D'Ascenzo, F.*, et al.*, *Remote ischaemic preconditioning in coronary artery bypass surgery: a meta-analysis.* Heart, 2012. **98**(17): p. 1267-71.

87. Zhou, C.*, et al.*, *beta-Blockers and Volatile Anesthetics May Attenuate Cardioprotection by Remote Preconditioning in Adult Cardiac Surgery: A Meta-analysis of 15 Randomized Trials.* J Cardiothorac Vasc Anesth, 2012.

88. Pilcher, J.M.*, et al.*, *A systematic review and meta-analysis of the cardioprotective effects of remote ischaemic preconditioning in open cardiac surgery.* J R Soc Med, 2012. **105**(10): p. 436-45.

89. Brevoord, D.*, et al.*, *Remote ischemic conditioning to protect against ischemia-reperfusion injury: a systematic review and meta-analysis.* PLoS One, 2012. **7**(7): p. e42179.

90. Walsh, S.R.*, et al.*, *Ischaemic preconditioning during cardiac surgery: systematic review and meta-analysis of perioperative outcomes in randomised clinical trials.* Eur J Cardiothorac Surg, 2008. **34**(5): p. 985-94.

91. Hausenloy, D.J. and D.M. Yellon, *Remote ischaemic preconditioning: underlying mechanisms and clinical application.* Cardiovasc Res, 2008. **79**(3): p. 377-86.

92. Przyklenk, K. and P. Whittaker, *Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities.* J Cardiovasc Pharmacol Ther, 2011. **16**(3-4): p. 255-9.

93. Hausenloy, D.J., S.B. Ong, and D.M. Yellon, *The mitochondrial permeability transition pore as a target for preconditioning and postconditioning.* Basic Res Cardiol, 2009. **104**(2): p. 189-202.

94. Piot, C.*, et al.*, *Effect of cyclosporine on reperfusion injury in acute myocardial infarction.* N Engl J Med, 2008. **359**(5): p. 473-81.

95. Liem, D.A.*, et al.*, *Sites of action of adenosine in interorgan preconditioning of the heart.* Am J Physiol Heart Circ Physiol, 2002. **283**(1): p. H29-37.

96. Loukogeorgakis, S.P.*, et al.*, *Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: role of the autonomic nervous system.* J Am Coll Cardiol, 2005. **46**(3): p. 450-6.

97. Dickson, E.W.*, et al.*, *Ischemic preconditioning may be transferable via whole blood transfusion: preliminary evidence.* J Thromb Thrombolysis, 1999. **8**(2): p. 123-9.

98. Serejo, F.C.*, et al.*, *Cardioprotective properties of humoral factors released from rat hearts subject to ischemic preconditioning.* J Cardiovasc Pharmacol, 2007. **49**(4): p. 214-20.

99. Breivik, L.*, et al.*, *Remote postconditioning by humoral factors in effluent from ischemic preconditioned rat hearts is mediated via PI3K/Akt-dependent cell-survival signaling at reperfusion.* Basic Res Cardiol, 2011. **106**(1): p. 135-45.

100. Bolli, R., *The Late Phase of Preconditioning.* Circulation Research, 2000. **87**(11): p. 972-983.

101. Skyschally, A.*, et al.*, *Bidirectional role of tumor necrosis factor-alpha in coronary microembolization: progressive contractile dysfunction versus delayed protection against infarction.* Circ Res, 2007. **100**(1): p. 140-6.

102. The Remote Preconditioning Trialists' Group*, et al.*, *Remote preconditioning and major clinical complications following adult cardiovascular surgery: systematic review and meta-analysis.* Int J Cardiol, 2014. **176**(1): p. 20-31.

References

103. Botker, H.E.*, et al.*, *Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial.* Lancet, 2010. **375**(9716): p. 727-34.

104. Reiter, R., T.D. Henry, and J.H. Traverse, *Preinfarction angina reduces infarct size in ST-elevation myocardial infarction treated with percutaneous coronary intervention.* Circ Cardiovasc Interv, 2013. **6**(1): p. 52-8.

105. Noda, T.*, et al.*, *Evidence for the delayed effect in human ischemic preconditioning: prospective multicenter study for preconditioning in acute myocardial infarction.* J Am Coll Cardiol, 1999. **34**(7): p. 1966-74.

106. Whittaker, P. and K. Przyklenk, *Remote-conditioning ischemia provides a potential approach to mitigate contrast medium-induced reduction in kidney function: a retrospective observational cohort study.* Cardiology, 2011. **119**(3): p. 145-50.

107. Hausenloy, D.J.*, et al.*, *Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial.* Lancet, 2007. **370**(9587): p. 575-9.

108. Venugopal, V.*, et al.*, *Remote ischaemic preconditioning reduces myocardial injury in patients undergoing cardiac surgery with cold-blood cardioplegia: a randomised controlled trial.* Heart, 2009. **95**(19): p. 1567-71.

109. Ali, N.*, et al.*, *Induced remote ischemic pre-conditioning on ischemia-reperfusion injury in patients undergoing coronary artery bypass.* J Coll Physicians Surg Pak, 2010. **20**(7): p. 427-31.

110. Thielmann, M.*, et al.*, *Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest.* Basic Res Cardiol, 2010. **105**(5): p. 657-64.

111. Rahman, I.A.*, et al.*, *Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment?* Circulation, 2010. **122**(11 Suppl): p. S53-9.

112. Hong, D.M.*, et al.*, *The effect of remote ischaemic preconditioning on myocardial injury in patients undergoing off-pump coronary artery bypass graft surgery.* Anaesth Intensive Care, 2010. **38**(5): p. 924-9.

113. Zimmerman, R.F.*, et al.*, *Ischemic preconditioning at a remote site prevents acute kidney injury in patients following cardiac surgery.* Kidney Int, 2011. **80**(8): p. 861-7.

114. Karuppasamy, P.*, et al.*, *Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and inflammation?* Basic Res Cardiol, 2011. **106**(4): p. 511-9.

115. Lucchinetti, E.*, et al.*, *Remote ischemic preconditioning applied during isoflurane inhalation provides no benefit to the myocardium of patients undergoing on-pump coronary artery bypass graft surgery: lack of synergy or evidence of antagonism in cardioprotection?* Anesthesiology, 2012. **116**(2): p. 296-310.

116. Xie, J.J.*, et al.*, *Remote ischaemic preconditioning reduces myocardial injury in patients undergoing heart valve surgery: randomised controlled trial.* Heart, 2012. **98**(5): p. 384-8.

117. Kottenberg, E.*, et al.*, *Protection by remote ischemic preconditioning during coronary artery bypass graft surgery with isoflurane but not propofol - a clinical trial.* Acta Anaesthesiol Scand, 2012. **56**(1): p. 30-8.

118. Lomivorotov, V.V.*, et al.*, *Remote ischaemic preconditioning does not protect the heart in patients undergoing coronary artery bypass grafting.* Interact Cardiovasc Thorac Surg, 2012. **15**(1): p. 18-22.

119. Gunaydin, B.*, et al.*, *Does remote organ ischaemia trigger cardiac preconditioning during coronary artery surgery?* Pharmacol Res, 2000. **41**(4): p. 493-6.

120. Wagner, R.*, et al.*, *Myocardial injury is decreased by late remote ischaemic preconditioning and aggravated by tramadol in patients undergoing cardiac surgery: a randomised controlled trial.* Interact Cardiovasc Thorac Surg, 2010. **11**(6): p. 758-62.

References

121. Meybohm, P*., et al.*, *Postoperative neurocognitive dysfunction in patients undergoing cardiac surgery after remote ischemic preconditioning: a double-blind randomized controlled pilot study.* PLoS One, 2013. **8**(5): p. e64743.

122. Choi, Y.S*., et al.*, *Effect of remote ischemic preconditioning on renal dysfunction after complex valvular heart surgery: a randomized controlled trial.* J Thorac Cardiovasc Surg, 2011. **142**(1): p. 148-54.

123. Wu, Q*., et al.*, *Effect of limb ischemic preconditioning on myocardial injury in patients undergoing mitral valve replacement surgery. -A randomized controlled trial.* Circ J, 2011. **75**(8): p. 1885-9.

124. Cheung, M.M*., et al.*, *Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans.* J Am Coll Cardiol, 2006. **47**(11): p. 2277-82.

125. Luo, W*., et al.*, *A comparison of cardiac post-conditioning and remote pre-conditioning in paediatric cardiac surgery.* Cardiol Young, 2011. **21**(3): p. 266-70.

126. Zhou, W*., et al.*, *Limb ischemic preconditioning reduces heart and lung injury after an open heart operation in infants.* Pediatr Cardiol, 2010. **31**(1): p. 22-9.

127. Pavione, M.A*., et al.*, *Late remote ischemic preconditioning in children undergoing cardiopulmonary bypass: a randomized controlled trial.* J Thorac Cardiovasc Surg, 2012. **144**(1): p. 178-83.

128. Pepe, S*., et al.*, *Effect of remote ischemic preconditioning on phosphorylated protein signaling in children undergoing tetralogy of fallot repair: a randomized controlled trial.* J Am Heart Assoc, 2013. **2**(3): p. e000095.

129. Jones, B.O*., et al.*, *Remote ischemic preconditioning in cyanosed neonates undergoing cardiopulmonary bypass: A randomized controlled trial.* J Thorac Cardiovasc Surg, 2013.

130. Domanski, M.J*., et al.*, *Association of myocardial enzyme elevation and survival following coronary artery bypass graft surgery.* JAMA, 2011. **305**(6): p. 585-91.

131. Rentoukas, I*., et al.*, *Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention: enhancement by opioid action.* JACC Cardiovasc Interv, 2010. **3**(1): p. 49-55.

132. Prasad, A*., et al.*, *Remote ischemic preconditioning immediately before percutaneous coronary intervention does not impact myocardial necrosis, inflammatory response, and circulating endothelial progenitor cell counts: a single center randomized sham controlled trial.* Catheter Cardiovasc Interv, 2013. **81**(6): p. 930-6.

133. Hoole, S.P*., et al.*, *Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study: a prospective, randomized control trial.* Circulation, 2009. **119**(6): p. 820-7.

134. Iliodromitis, E.K*., et al.*, *Increased C reactive protein and cardiac enzyme levels after coronary stent implantation. Is there protection by remote ischaemic preconditioning?* Heart, 2006. **92**(12): p. 1821-6.

135. Ahmed, R.M*., et al.*, *Effect of remote ischemic preconditioning on serum troponin T level following elective percutaneous coronary intervention.* Catheter Cardiovasc Interv, 2013.

136. Ghaemian, A*., et al.*, *Remote ischemic preconditioning in percutaneous coronary revascularization: a double-blind randomized controlled clinical trial.* Asian Cardiovasc Thorac Ann, 2012. **20**(5): p. 548-54.

137. Er, F*., et al.*, *Ischemic preconditioning for prevention of contrast medium-induced nephropathy: randomized pilot RenPro Trial (Renal Protection Trial).* Circulation, 2012. **126**(3): p. 296-303.

138. Igarashi, G*., et al.*, *Remote Ischemic Pre-Conditioning Alleviates ContrastInduced Acute Kidney Injury in Patients With Moderate Chronic Kidney Disease.* Circ J, 2013.

139. Davies, W.R*., et al.*, *Remote Ischemic Preconditioning Improves Outcome at 6 Years After Elective Percutaneous Coronary Intervention: The CRISP Stent Trial Long-Term Follow-Up.* Circ Cardiovasc Interv, 2013.

References

140. Ali, Z.A.*, et al.*, *Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: a randomized controlled trial.* Circulation, 2007. **116**(11 Suppl): p. I98-105.

141. Walsh, S.R.*, et al.*, *Remote ischemic preconditioning for renal protection during elective open infrarenal abdominal aortic aneurysm repair: randomized controlled trial.* Vasc Endovascular Surg, 2010. **44**(5): p. 334-40.

142. Li, C.*, et al.*, *Limb remote ischemic preconditioning for intestinal and pulmonary protection during elective open infrarenal abdominal aortic aneurysm repair: a randomized controlled trial.* Anesthesiology, 2013. **118**(4): p. 842-52.

143. Walsh, S.R.*, et al.*, *Remote ischemic preconditioning for renal and cardiac protection during endovascular aneurysm repair: a randomized controlled trial.* J Endovasc Ther, 2009. **16**(6): p. 680-9.

144. Walsh, S.R.*, et al.*, *Remote ischemic preconditioning for cerebral and cardiac protection during carotid endarterectomy: results from a pilot randomized clinical trial.* Vasc Endovascular Surg, 2010. **44**(6): p. 434-9.

145. Ren, C.*, et al.*, *Limb remote-preconditioning protects against focal ischemia in rats and contradicts the dogma of therapeutic time windows for preconditioning.* Neuroscience, 2008. **151**(4): p. 1099-103.

146. Malhotra, S., M. Singh, and D. Rosenbaum, *Remote preconditioning protects the rodent brain from focal cerebral ischemia.* Journal of Cerebral Blood FLow & Metabolism, 2005. **25**: p. S308.

147. Stagliano, N.E.*, et al.*, *Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice.* J Cereb Blood Flow Metab, 1999. **19**(7): p. 757-61.

148. Glazier, S.S.*, et al.*, *Induction of ischemic tolerance following brief focal ischemia in rat brain.* J Cereb Blood Flow Metab, 1994. **14**(4): p. 545-53.

149. Hu, S.*, et al.*, *Effects of remote ischemic preconditioning on biochemical markers and neurologic outcomes in patients undergoing elective cervical decompression surgery: a prospective randomized controlled trial.* J Neurosurg Anesthesiol, 2010. **22**(1): p. 46-52.

150. Chan, M.T.*, et al.*, *Effect of ischemic preconditioning on brain tissue gases and pH during temporary cerebral artery occlusion.* Acta Neurochir Suppl, 2005. **95**: p. 93-6.

151. Faries, P.L.*, et al.*, *Ischemic preconditioning during the use of the PercuSurge occlusion balloon for carotid angioplasty and stenting.* Vascular, 2008. **16**(1): p. 1-9.

152. Harder, Y.*, et al.*, *An old dream revitalised: preconditioning strategies to protect surgical flaps from critical ischaemia and ischaemia-reperfusion injury.* J Plast Reconstr Aesthet Surg, 2008. **61**(5): p. 503-11.

153. Wang, W.Z., R.C. Baynosa, and W.A. Zamboni, *Update on ischemia-reperfusion injury for the plastic surgeon: 2011.* Plast Reconstr Surg, 2011. **128**(6): p. 685e-92e.

154. Mounsey, R.A.*, et al.*, *Augmentation of skeletal muscle survival in the latissimus dorsi porcine model using acute ischemic preconditioning.* J Otolaryngol, 1992. **21**(5): p. 315-20.

155. Kuntscher, M.V.*, et al.*, *Acute remote ischemic preconditioning on a rat cremasteric muscle flap model.* Microsurgery, 2002. **22**(6): p. 221-6.

156. Kuntscher, M.V.*, et al.*, *Ischemic preconditioning by brief extremity ischemia before flap ischemia in a rat model.* Plast Reconstr Surg, 2002. **109**(7): p. 2398-404.

157. Rudiger, H.A.*, et al.*, *Comparison of ischemic preconditioning and intermittent and continuous inflow occlusion in the murine liver.* Ann Surg, 2002. **235**(3): p. 400-7.

158. Rudiger, H.A., R. Graf, and P.A. Clavien, *Sub-lethal oxidative stress triggers the protective effects of ischemic preconditioning in the mouse liver.* J Hepatol, 2003. **39**(6): p. 972-7.

159. Gurusamy, K.S.*, et al.*, *Ischaemic pre-conditioning for elective liver resections performed under vascular occlusion.* Cochrane Database Syst Rev, 2009(1): p. CD007629.

160. Rahbari, N.N.*, et al.*, *Systematic review and meta-analysis of the effect of portal triad clamping on outcome after hepatic resection.* Br J Surg, 2008. **95**(4): p. 424-32.

References

161. O'Neill, S.*, et al.*, *Meta-analysis of ischaemic preconditioning for liver resections.* Br J Surg, 2013. **100**(13): p. 1689-700.

162. Meybohm, P.*, et al.*, *Remote ischaemic preconditioning for heart surgery. The study design for a multi-center randomized double-blinded controlled clinical trial--the RIPHeart-Study.* Eur Heart J, 2012. **33**(12): p. 1423-6.

163. Landoni, G.*, et al.*, *Desflurane and sevoflurane in cardiac surgery: a meta-analysis of randomized clinical trials.* J Cardiothorac Vasc Anesth, 2007. **21**(4): p. 502-11.

164. Hausenloy, D.J.*, et al.*, *Effect of remote ischemic preconditioning on clinical outcomes in patients undergoing coronary artery bypass graft surgery (ERICCA): rationale and study design of a multi-centre randomized double-blinded controlled clinical trial.* Clin Res Cardiol, 2012. **101**(5): p. 339-48.

165. *The Renal Protection Against Ischaemia Reperfusion in Transplantation (REPAIR) trial*. 2013 20th May 2013]; Available from: http://repair.lshtm.ac.uk/.

166. Szijarto, A.*, et al.*, *Remote ischemic perconditioning--a simple, low-risk method to decrease ischemic reperfusion injury: models, protocols and mechanistic background. A review.* J Surg Res, 2012. **178**(2): p. 797-806.

167. Schmidt, M.R.*, et al.*, *Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic perconditioning.* Am J Physiol Heart Circ Physiol, 2007. **292**(4): p. H1883-90.

168. Na, H.S.*, et al.*, *Ventricular premature beat-driven intermittent restoration of coronary blood flow reduces the incidence of reperfusion-induced ventricular fibrillation in a cat model of regional ischemia.* Am Heart J, 1996. **132**(1 Pt 1): p. 78-83.

169. Zhao, Z.Q.*, et al.*, *Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning.* Am J Physiol Heart Circ Physiol, 2003. **285**(2): p. H579-88.

170. Andreka, G.*, et al.*, *Remote ischaemic postconditioning protects the heart during acute myocardial infarction in pigs.* Heart, 2007. **93**(6): p. 749-52.

171. Carrasco-Chinchilla, F.*, et al.*, *Remote ischaemic postconditioning: does it protect against ischaemic damage in percutaneous coronary revascularisation? Randomised placebo-controlled clinical trial.* Heart, 2013. **99**(19): p. 1431-7.

172. Heusch, G., *PostconditioningOld wine in a new bottle?*. Journal of the American College of Cardiology, 2004. **44**(5): p. 1111-1112.

173. Hausenloy, D.J. and D.M. Yellon, *New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway.* Cardiovasc Res, 2004. **61**(3): p. 448-60.

174. *ESUR guidelines on contrast media. Contrast media safety guidelines of the Contrast Media Safety Committee of the European Society of Urogenital Radiology*. 2007; Available from: http://www.esur.org/fileadmin/Guidelines/ESUR_2007_Guideline_6_Kern_Ubersicht.pdf.

175. Nash, K., A. Hafeez, and S. Hou, *Hospital-acquired renal insufficiency.* Am J Kidney Dis, 2002. **39**(5): p. 930-6.

176. Rudnick, M.R., *Pathogenesis, clinical features, and diagnosis of contrast-induced nephropathy*. UpToDate in Medicine2013, Waltham, MA.

177. Newhouse, J.H.*, et al.*, *Frequency of serum creatinine changes in the absence of iodinated contrast material: implications for studies of contrast nephrotoxicity.* AJR Am J Roentgenol, 2008. **191**(2): p. 376-82.

178. Oduncu, V.*, et al.*, *Relation of the severity of contrast induced nephropathy to SYNTAX score and long term prognosis in patients treated with primary percutaneous coronary intervention.* Int J Cardiol, 2013.

179. Wickenbrock, I.*, et al.*, *Contrast medium induced nephropathy in patients undergoing percutaneous coronary intervention for acute coronary syndrome: differences in STEMI and NSTEMI.* Clin Res Cardiol, 2009. **98**(12): p. 765-72.

180. Marenzi, G*., et al.*, *Contrast volume during primary percutaneous coronary intervention and subsequent contrast-induced nephropathy and mortality.* Ann Intern Med, 2009. **150**(3): p. 170-7.

181. Finn, W.F., *The clinical and renal consequences of contrast-induced nephropathy.* Nephrol Dial Transplant, 2006. **21**(6): p. i2-10.

182. O'Sullivan, S*., et al.*, *The Role of N-Acetylcysteine in the Prevention of Contrast-Induced Nephropathy in Patients Undergoing Peripheral Angiography: A Structured Review and Meta-Analysis.* Angiology, 2012.

183. Sudarsky, D. and E. Nikolsky, *Contrast-induced nephropathy in interventional cardiology.* Int J Nephrol Renovasc Dis, 2011. **4**: p. 85-99.

184. Asif, A. and M. Epstein, *Prevention of radiocontrast-induced nephropathy.* Am J Kidney Dis, 2004. **44**(1): p. 12-24.

185. Katzberg, R.W. and C. Haller, *Contrast-induced nephrotoxicity: clinical landscape.* Kidney Int Suppl, 2006(100): p. S3-7.

186. Russo, D*., et al.*, *Early effects of contrast media on renal hemodynamics and tubular function in chronic renal failure.* J Am Soc Nephrol, 1995. **6**(5): p. 1451-8.

187. Tumlin, J.A*., et al.*, *Fenoldopam mesylate blocks reductions in renal plasma flow after radiocontrast dye infusion: a pilot trial in the prevention of contrast nephropathy.* Am Heart J, 2002. **143**(5): p. 894-903.

188. Dauerman, H.L., *In search of an algorithm to prevent acute kidney injury.* JACC Cardiovasc Interv, 2009. **2**(11): p. 1125-7.

189. Brezis, M. and S. Rosen, *Hypoxia of the renal medulla--its implications for disease.* N Engl J Med, 1995. **332**(10): p. 647-55.

190. Levine, G.N*., et al.*, *2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions.* J Am Coll Cardiol, 2011. **58**(24): p. e44-122.

191. Prasad, A. and J. Herrmann, *Myocardial infarction due to percutaneous coronary intervention.* N Engl J Med, 2011. **364**(5): p. 453-64.

192. Moher, D*., et al.*, *Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.* Ann Intern Med, 2009. **151**(4): p. 264-9, W64.

193. DerSimonian, R. and N. Laird, *Meta-analysis in clinical trials.* Control Clin Trials, 1986. **7**(3): p. 177-88.

194. Luo, S.J*., et al.*, *Remote Ischemic Preconditioning Reduces Myocardial Injury in Patients Undergoing Coronary Stent Implantation.* Can J Cardiol, 2013.

195. Thygesen, K*., et al.*, *Universal definition of myocardial infarction.* Circulation, 2007. **116**(22): p. 2634-53.

196. Davies, W.R*., et al.*, *Remote ischemic preconditioning improves outcome at 6 years after elective percutaneous coronary intervention: the CRISP stent trial long-term follow-up.* Circ Cardiovasc Interv, 2013. **6**(3): p. 246-51.

197. Whittaker, P. and K. Przyklenk, *Reduction of infarct size in vivo with ischemic preconditioning: mathematical evidence for protection via non-ischemic tissue.* Basic Res Cardiol, 1994. **89**(1): p. 6-15.

198. Thielmann, M., *Remote ischemic preconditioning in cardiac surgery: caught between clinical relevance and statistical significance?* Basic Res Cardiol, 2012. **107**(3): p. 259.

199. Iliodromitis, E.K., D. Farmakis, and D.T. Kremastinos, *Biomarkers of myocardial injury as surrogate end-points for remote ischemic conditioning trials.* Int J Cardiol, 2011. **149**(3): p. 379.

200. Hausenloy, D.J. and D.M. Yellon, *The therapeutic potential of ischemic conditioning: an update.* Nat Rev Cardiol, 2011. **8**(11): p. 619-29.

References

201.   Yang, L.*, et al.*, *Remote Ischemic Preconditioning Reduces Cardiac Troponin I Release in Cardiac Surgery: A Meta-Analysis.* J Cardiothorac Vasc Anesth, 2013.

202.   Haji Mohd Yasin, N.A.*, et al.*, *The role of remote ischemic preconditioning in organ protection after cardiac surgery: a meta-analysis.* J Surg Res, 2014. **186**(1): p. 207-16.

203.   Healy, D.A.*, et al.*, *Systematic review and meta-analysis of remote ischaemic preconditioning in percutaneous coronary intervention.* IJC Metabolic & Endocrine, 2013. **1**: p. 13-19.

204.   Thielmann, M.*, et al.*, *Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial.* Lancet, 2013. **382**(9892): p. 597-604.

205.   Li, L.*, et al.*, *Remote perconditioning reduces myocardial injury in adult valve replacement: a randomized controlled trial.* J Surg Res, 2010. **164**(1): p. e21-6.

206.   Young, P.J.*, et al.*, *A pilot study investigating the effects of remote ischemic preconditioning in high-risk cardiac surgery using a randomised controlled double-blind protocol.* Basic Res Cardiol, 2012. **107**(3): p. 256.

207.   Joung, K.W.*, et al.*, *Effect of remote ischemic preconditioning on cognitive function after off-pump coronary artery bypass graft: a pilot study.* Korean J Anesthesiol, 2013. **65**(5): p. 418-24.

208.   Candilio, L.*, et al.*, *177: The protective effects of remote ischaemic preconditioning on patients undergoing elective cardiac surgery.* 2013 Annual Meeting of the Society of Cardiothoracic Surgery in Great Britain and Ireland, 2013.

209.   Yokoyama, Y.*, et al.*, *Association between new electrocardiographic abnormalities after coronary revascularization and five-year cardiac mortality in BARI randomized and registry patients.* Am J Cardiol, 2000. **86**(8): p. 819-24.

210.   Chaitman, B.R.*, et al.*, *Use of survival analysis to determine the clinical significance of new Q waves after coronary bypass surgery.* Circulation, 1983. **67**(2): p. 302-9.

211.   Hu, Z.Y. and J. Liu, *Mechanism of cardiac preconditioning with volatile anaesthetics.* Anaesth Intensive Care, 2009. **37**(4): p. 532-8.

212.   Schultz, J.E., A.K. Hsu, and G.J. Gross, *Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart.* Circ Res, 1996. **78**(6): p. 1100-4.

213.   Kottenberg, E.*, et al.*, *Interference of propofol with signal transducer and activator of transcription 5 activation and cardioprotection by remote ischemic preconditioning during coronary artery bypass grafting.* J Thorac Cardiovasc Surg, 2014. **147**(1): p. 376-82.

214.   Ateş, E.*, et al.*, *Renal protection by brief liver ischemia in rats.* Transplantation, 2002. **74**(9): p. 1247-51.

215.   Wever, K.E.*, et al.*, *Remote ischaemic preconditioning by brief hind limb ischaemia protects against renal ischaemia-reperfusion injury: the role of adenosine.* Nephrol Dial Transplant, 2011. **26**(10): p. 3108-17.

216.   Venugopal, V.*, et al.*, *Effect of remote ischemic preconditioning on acute kidney injury in nondiabetic patients undergoing coronary artery bypass graft surgery: a secondary analysis of 2 small randomized trials.* Am J Kidney Dis, 2010. **56**(6): p. 1043-9.

217.   Wu, J.*, et al.*, *Remote ischemic conditioning enhanced the early recovery of renal function in recipients after kidney transplantation: a randomized controlled trial.* J Surg Res, 2014. **188**(1): p. 303-8.

218.   Veighey, K. and R. MacAllister, *Clinical applications of remote ischaemic preconditioning in native and transplant acute kidney injury.* Pediatr Nephrol, 2014.

219.   Sendeski, M.M., *Pathophysiology of renal tissue damage by iodinated contrast media.* Clin Exp Pharmacol Physiol, 2011. **38**(5): p. 292-9.

220.   Heyman, S.N.*, et al.*, *Reactive oxygen species and the pathogenesis of radiocontrast-induced nephropathy.* Invest Radiol, 2010. **45**(4): p. 188-95.

References

221. Yamanaka, T.*, et al.*, *Remote ischemic preconditioning reduces contrast-induced acute kidney injury in patients with ST-elevation myocardial infarction: A randomized controlled trial.* Int J Cardiol, 2014. **178**: p. 136-141.

222. Deftereos, S.*, et al.*, *Renoprotective effect of remote ischemic post-conditioning by intermittent balloon inflations in patients undergoing percutaneous coronary intervention.* J Am Coll Cardiol, 2013. **61**(19): p. 1949-55.

223. Anavekar, N.S.*, et al.*, *Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction.* N Engl J Med, 2004. **351**(13): p. 1285-95.

224. National Kidney Foundation, *K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification.* Am J Kidney Dis, 2002. **39**(2 Suppl 1): p. S1-266.

225. Bøtker, H.E.*, et al.*, *Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial.* Lancet, 2010. **375**(9716): p. 727-34.

226. Ovize, M., H. Thibault, and K. Przyklenk, *Myocardial conditioning: opportunities for clinical translation.* Circ Res, 2013. **113**(4): p. 439-50.

227. Crimi, G.*, et al.*, *Remote ischemic postconditioning as a strategy to reduce acute kidney injury during primary PCI: A post-hoc analysis of a randomized trial.* Int J Cardiol, 2014. **177**(2): p. 500-2.

228. Yang, Y.*, et al.*, *Remote ischemic preconditioning for prevention of acute kidney injury: a meta-analysis of randomized controlled trials.* Am J Kidney Dis, 2014. **64**(4): p. 574-83.

229. Pedersen, K.R.*, et al.*, *Failure of remote ischemic preconditioning to reduce the risk of postoperative acute kidney injury in children undergoing operation for complex congenital heart disease: a randomized single-center study.* J Thorac Cardiovasc Surg, 2012. **143**(3): p. 576-83.

230. Luo, S.J.*, et al.*, *Remote ischemic preconditioning reduces myocardial injury in patients undergoing coronary stent implantation.* Can J Cardiol, 2013. **29**(9): p. 1084-9.

231. Rhoney, D.H.*, et al.*, *Kidney dysfunction at the time of intracerebral hemorrhage is associated with increased in-hospital mortality: a retrospective observational cohort study.* Neurol Res, 2012. **34**(5): p. 518-21.

232. Ishani, A.*, et al.*, *The magnitude of acute serum creatinine increase after cardiac surgery and the risk of chronic kidney disease, progression of kidney disease, and death.* Arch Intern Med, 2011. **171**(3): p. 226-33.

233. MacWalter, R.S.*, et al.*, *Does renal dysfunction predict mortality after acute stroke? A 7-year follow-up study.* Stroke, 2002. **33**(6): p. 1630-5.

234. Go, A.S.*, et al.*, *Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization.* N Engl J Med, 2004. **351**(13): p. 1296-305.

235. Manjunath, G.*, et al.*, *Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community.* J Am Coll Cardiol, 2003. **41**(1): p. 47-55.

236. Kleinow, M.E.*, et al.*, *Effect of chronic kidney disease on warfarin management in a pharmacist-managed anticoagulation clinic.* J Manag Care Pharm, 2011. **17**(7): p. 523-30.

237. Przyklenk, K., *Efficacy of cardioprotective 'conditioning' strategies in aging and diabetic cohorts: the co-morbidity conundrum.* Drugs Aging, 2011. **28**(5): p. 331-43.

238. McCafferty, K.*, et al.*, *The challenge of translating ischemic conditioning from animal models to humans: the role of comorbidities.* Dis Model Mech, 2014. **7**(12): p. 1321-1333.

239. Przyklenk, K.*, et al.*, *Cardioprotection with postconditioning: loss of efficacy in murine models of type-2 and type-1 diabetes.* Antioxid Redox Signal, 2011. **14**(5): p. 781-90.

240. Gallagher, S.M.*, et al.*, *Remote ischemic preconditioning has a neutral effect on the incidence of kidney injury after coronary artery bypass graft surgery.* Kidney Int, 2015. **(in press)**: p. doi: 10.1038/ki.2014.259.

241. Sterenborg, T.B.*, et al.*, *Remote ischemic preconditioning to reduce contrast-induced nephropathy: study protocol for a randomized controlled trial.* Trials, 2014. **15**: p. 119.

References

242. Bell, R.M*., et al., Effect of remote ischaemic conditioning on contrast-induced nephropathy in patients undergoing elective coronary angiography (ERICCIN): rationale and study design of a randomised single-centre, double-blind placebo-controlled trial.* Clin Res Cardiol, 2014. **103**(3): p. 203-9.

243. Healy, D.A*., et al., Remote ischaemic preconditioning as a method for perioperative cardioprotection: concepts, applications and future directions.* Int J Surg, 2014. **12**(10): p. 1093-9.

244. McCafferty, K., C. Byrne, and M.M. Yaqoob, *Ischaemic conditioning strategies for the nephrologist: a promise lost in translation?* Nephrol Dial Transplant, 2014. **29**(10): p. 1827-40.

245. Wever, K.E*., et al., Ischemic preconditioning in the animal kidney, a systematic review and meta-analysis.* PLoS One, 2012. **7**(2): p. e32296.

246. Jones, H*., et al., Seven-day remote ischemic preconditioning improves local and systemic endothelial function and microcirculation in healthy humans.* Am J Hypertens, 2014. **27**(7): p. 918-25.

247. Linden, M.D*., et al., Preconditioning ischemia attenuates molecular indices of platelet activation-aggregation.* J Thromb Haemost, 2006. **4**(12): p. 2670-7.

248. Hata, K*., et al., Brief myocardial ischemia attenuates platelet thrombosis in remote, damaged, and stenotic carotid arteries.* Circulation, 1999. **100**(8): p. 843-8.

249. Whittaker, P. and K. Przyklenk, *Fibrin architecture in clots: a quantitative polarized light microscopy analysis.* Blood Cells Mol Dis, 2009. **42**(1): p. 51-6.

250. Przyklenk, K. and P. Whittaker, *Brief antecedent ischemia enhances recombinant tissue plasminogen activator-induced coronary thrombolysis by adenosine-mediated mechanism.* Circulation, 2000. **102**(1): p. 88-95.

251. Kolbenschlag, J*., et al., Upper extremity ischemia is superior to lower extremity ischemia for remote ischemic conditioning of antero-lateral thigh cutaneous blood flow.* Microsurgery, 2015. **35**(3): p. 211-7.

252. *Endovascular aneurysm repair versus open repair in patients with abdominal aortic aneurysm (EVAR trial 1): randomised controlled trial.* Lancet, 2005. **365**(9478): p. 2179-86.

253. Chambers, B.R. and G.A. Donnan, *Carotid endarterectomy for asymptomatic carotid stenosis.* Cochrane Database Syst Rev, 2005(4): p. CD001923.

254. Adam, D.J*., et al., Bypass versus angioplasty in severe ischaemia of the leg (BASIL): multicentre, randomised controlled trial.* Lancet, 2005. **366**(9501): p. 1925-34.

255. Landesberg, G*., et al., Perioperative Myocardial Infarction.* Circulation, 2009. **119**(22): p. 2936-2944.

256. Healy, D.A*., et al., Remote ischaemic preconditioning as a method for perioperative cardioprotection: Concepts, applications and future directions.* Int J Surg, 2014.

257. *Ported to R by Alvaro A. Novo. Original by Joseph L. Schafer <jls@stat.psu.edu> : Analysis of multivariate normal datasets with missing values. R package version 1.0-9.5: "http://CRAN.R-project.org/package=norm".* 2013.

258. Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Vehtari, A., & Rubin, D. B., *Bayesian data analysis.* 2013: CRC press.

259. Murphy, N*., et al., Remote ischemic preconditioning does not affect the incidence of acute kidney injury after elective abdominal aortic aneurysm repair.* J Cardiothorac Vasc Anesth, 2014. **28**(5): p. 1285-92.

260. Healy, D*., et al., Preconditioning Shields Against Vascular Events in Surgery (SAVES), a multicentre feasibility trial of preconditioning against adverse events in major vascular surgery: study protocol for a randomised control trial.* Trials, 2015. **16**(1): p. 185.