

# Ischaemic conditioning – are we there yet?

Heerajnarain Bulluck<sup>1</sup> & Derek J Hausenloy<sup>1,2</sup>

<sup>1</sup>The Hatter Cardiovascular Institute, University College London, London, UK

<sup>2</sup> Cardiovascular and Metabolic Disorders Program, Duke-NUS Graduate Medical School, Singapore, Singapore.

## Corresponding author:

Professor Derek Hausenloy  
The Hatter Cardiovascular Institute, Institute of Cardiovascular Science,  
NIHR University College London Hospitals Biomedical Research Centre,  
University College London Hospital & Medical School,  
67 Chenies Mews, London,  
WC1E 6HX, UK.  
Tel: +44 (203) 447 9888  
Fax: +44 (203) 447 9505  
E-mail: [d.hausenloy@ucl.ac.uk](mailto:d.hausenloy@ucl.ac.uk)

**Word Count:** 3938 not including references and tables

**Keywords:** reperfusion injury, ischemic preconditioning, ischemic postconditioning, remote ischemic conditioning, myocardial ischemia, cardioprotection

## **Learning objectives:**

1. To recognise that acute myocardial ischaemia/reperfusion injury is a neglected therapeutic target for cardioprotection that is responsible for the ongoing morbidity and mortality of patients with ischaemic heart disease.
2. To be aware that cardiac bypass surgery and STEMI are the major clinical settings in which the heart is subjected to acute ischaemia/reperfusion injury.
3. To be familiar with the concept of 'ischaemic conditioning', in which the heart is protected against acute ischaemia/reperfusion injury by subjecting it to cycles of brief ischaemia and reperfusion, a therapeutic strategy which has been demonstrated in proof-of-concept studies to be beneficial in patients with ischaemic heart disease.

## **1. INTRODUCTION - THE NEED FOR CARDIOPROTECTION**

Ischaemic heart disease (IHD) is the leading cause of death and disability worldwide. Despite current therapies, patients still experience significant morbidity and mortality when undergoing cardiac bypass surgery or when presenting with an ST-segment elevation myocardial infarction (STEMI). This is partly attributed to the detrimental effects of acute ischaemia/reperfusion injury (IRI) on the heart, which in combination mediate cardiomyocyte death, resulting in impaired left ventricular (LV) systolic function and increased risk of heart failure. Although a number of strategies exist for reducing the ischaemic component of acute IRI injury in cardiac bypass surgery (such as cardioplegia and hypothermia) and STEMI (such as prompt reperfusion with primary percutaneous intervention - PPCI), paradoxically, reperfusing previously ischaemic myocardium leads to further cardiomyocyte death – termed ‘myocardial reperfusion injury’ and for which there is currently no effective therapy. Therefore, novel therapeutic interventions are required to protect the heart from acute IRI in these clinical settings in order to improve clinical outcomes. In this regard, ‘ischaemic conditioning’, in which the heart is rendered tolerant to acute IRI by subjecting it to cycles of brief ischaemia and reperfusion, provides an endogenous form of cardioprotection. In this article, we review the role for ischaemic conditioning as a therapeutic strategy for attenuating cardiomyocyte death, preserving myocardial function, and improving clinical outcomes in patients subjected to acute myocardial IRI.

## **2. MYOCARDIAL REPERFUSION INJURY – A NEGLECTED THERAPEUTIC TARGET**

Although myocardial reperfusion is essential to salvage viable myocardium following the onset of acute myocardial ischaemia, the restoration of coronary blood flow comes at a price, paradoxically inducing myocardial injury and cardiomyocyte death – termed

'myocardial reperfusion injury'. Four types of myocardial reperfusion injury have been described:

1. *Reperfusion-induced arrhythmias*: These occur on reperfusing previously ischaemic myocardium and comprise idioventricular rhythm and ventricular arrhythmias, the majority of which are self-terminating or are easily treated.
2. *Myocardial stunning*: This refers to the reversible contractile dysfunction that occurs on reperfusing acute ischaemic myocardium and is believed due to be due to myocardial oxidative stress and intracellular calcium overload.
3. *Coronary no-reflow and microvascular obstruction*: Coronary no-reflow is indicative of underlying microvascular obstruction (MVO) – defined as the “inability to reperfuse a previously ischaemic region”<sup>1</sup>. MVO is present on cardiovascular magnetic resonance imaging (CMR) in 40 to 60% of PPCI patients, despite the presence of normal coronary flow (TIMI 3) within the infarct-related artery post-PPCI<sup>2 3</sup>. The presence of MVO is associated with adverse LV remodelling and worse clinical outcomes post-PPCI.
4. *Lethal myocardial reperfusion injury*: Ischaemia/Reperfusion induces cytosolic and mitochondrial calcium overload, oxidative stress, rapid restoration in intracellular pH, which on a background of relative adenosine triphosphate (ATP) depletion, culminates in the opening of the mitochondrial permeability transition pore (MPTP) and irreversible cardiomyocyte hypercontracture – the hallmark of lethal myocardial reperfusion injury. Cardiomyocytes that were still viable at the end of the index ischaemic insult undergoes necrosis and this accounts for up to 50% of the final MI size, thereby mitigating the benefits of reperfusion, and making lethal myocardial reperfusion injury a therapeutic target for cardioprotection (Figure 1).

Crucially, there is currently no effective therapy for reducing either MVO or lethal myocardial reperfusion injury. In the following sections we review the therapeutic potential

of ischaemic conditioning, a collective term given to the different forms of endogenous cardioprotection, which have been described, and these include ischaemic preconditioning, ischaemic postconditioning and remote ischaemic conditioning.

### **3. ISCHAEMIC PRECONDITIONING – LIMITED CLINICAL APPLICATION**

In 1986, Murry et al<sup>4</sup> made the intriguing observation that following an acute coronary artery occlusion the resultant myocardial infarct (MI) size could be significantly reduced by 'preconditioning' the heart with brief episodes of ischaemia and reperfusion. In that landmark experimental study, four cycles of 5-minutes alternating left anterior descending (LAD) coronary artery occlusion and reflow applied immediately prior to a 90-minutes LAD occlusion and a 3-days reperfusion period, led to a 25% reduction in MI size in the canine heart<sup>4</sup>. This endogenous form of cardioprotection, which was termed ischaemic preconditioning (IPC), has been shown to be ubiquitous in several other organs and all species tested (reviewed in <sup>5 6</sup>). The IPC stimulus is known to elicit two windows of cardioprotection, the first one occurring immediately and lasting 2-3 hours, and the second one appearing 12-24 hours later, and lasting up to 72 hours. In several clinical scenarios the heart is able to protect itself by IPC – e.g. (1) 'Warm-up angina' refers to the phenomenon in which a patient with stable IHD is able to exercise more following an episode of angina followed by a period of rest<sup>7</sup>; (2) 'Pre-infarct angina' refers to the cardioprotective effects of an episodes of angina immediately prior to an MI resulting in smaller MI size and better clinical outcomes<sup>8</sup>. As a cardioprotective strategy for protecting the heart against acute IRI, its clinical application has been limited by the need to apply the IPC stimulus directly to the heart, and prior to the index ischaemia, which cannot be predicted in acute MI patients. In this regard, the discovery of ischaemic postconditioning has provided a cardioprotective strategy which can be applied at the time of reperfusion to protect the heart against acute IRI.

#### **4. ISCHAEMIC POSTCONDITIONING – PROTECTION AT TIME OF REPERFUSION**

In 2003, Zhao et al<sup>9</sup> discovered that by interrupting myocardial reperfusion with several short-lived episodes of myocardial ischaemia could reduce MI size to a similar extent as IPC – a phenomenon which has been termed ‘ischaemic postconditioning’ (IPost)<sup>6 10</sup>. They<sup>9</sup> found that by applying three cycles of 30-seconds LAD coronary artery occlusion and reflow at the onset of myocardial reperfusion could reduce MI size by 44% in the canine heart<sup>9</sup>. The idea of modifying reperfusion as a strategy to limit MI size was first proposed in the 1980s and 1990s using gentle<sup>11</sup> and gradual<sup>12</sup> reperfusion instead of sudden reperfusion. The discovery of IPost as a therapeutic strategy which could be applied at the onset of myocardial reperfusion has rekindled interest in lethal myocardial reperfusion injury as a target for cardioprotection, and was rapidly translated into the clinical setting within two years of discovery<sup>13</sup>. It has also provided confirmatory evidence for the existence of lethal myocardial reperfusion injury in man. However, both IPC and IPost require an intervention applied to the heart directly, which may not always be feasible depending on the clinical situation, and therefore, the strategy of remote ischaemic conditioning, in which the cardioprotective stimulus is applied to an organ or tissue away from the heart is vastly more attractive as a clinical application.

#### **5. REMOTE ISCHAEMIC CONDITIONING – CARDIOPROTECTION MADE EASY**

In 1993, Przyklenk et al<sup>14</sup> made the interesting observation that the cardioprotective effect of IPC was not restricted to one particular coronary artery territory as it could be transferred to another coronary artery territory. This gave rise to the concept of ‘remote ischaemic conditioning’ (RIC), the term given to the cardioprotection induced by applying cycles of brief ischaemia and reperfusion to an organ or tissue away from the heart. Using the canine heart, these authors found that brief occlusions and reflow in the circumflex

coronary artery were able to reduce MI size by 63% following LAD coronary artery occlusion<sup>14</sup>. This idea was soon extended beyond the heart with the demonstration that the RIC stimulus (cycles of brief ischaemia and reperfusion) could be applied to the kidney to limit MI size, giving rise to the concept of inter-organ protection against acute IRI<sup>15</sup>. Two key properties of RIC have facilitated its translation into the clinical setting:

(1) **Feasibility:** The discovery in an experimental animal MI model that the RIC stimulus could be applied to the hind limb to protect the heart against acute IRI<sup>16 17</sup>, and the finding that the RIC stimulus could be delivered non-invasively in human volunteers by simply inflating a blood pressure cuff on the upper arm to induce cycles of brief ischaemia and reperfusion<sup>18</sup>, has greatly increased its feasibility in the clinical setting.

(2) **Flexibility:** The application of IPC and IPost are restricted in terms of their 'timings', as the protective stimulus has to be applied either prior to ischaemia or at the onset of reperfusion, respectively) and 'target' (the protective stimulus has to be applied to the heart directly), whereas RIC can be applied at any time (either prior to, after the onset of, or at the end of ischaemia) and to an organ or tissue away from the heart, thereby making it much more flexible as a cardioprotective strategy (Figure 2).

Therefore, most clinical studies applying RIC as a cardioprotective strategy have used cycles of brief ischaemia and reperfusion in the upper or lower limb (henceforth referred to as limb RIC).

## **6. OVERVIEW OF MECHANISMS UNDERLYING ISCHAEMIC CONDITIONING**

The mechanisms underlying ischaemic conditioning are rather complex and have been the subject of intensive investigation over the last 20 to 30 years (for comprehensive reviews please see <sup>5 6 19 20</sup>). With respect to IPC and IPost, the current paradigm proposes that the cycles of brief ischaemia and reperfusion, which make up the protective stimulus generate autacoids in the interstitium which then activate a number of signal transduction pathways

by binding to their respective receptors on the cardiomyocyte plasma membrane. This in turn results in the recruitment of a number of pro-survival protein kinase pathways (such as the Reperfusion Injury Salvage Kinase [RISK]<sup>21 22</sup>, Survivor Activator Factor Enhancement [SAFE]<sup>23</sup>, and cGMP-PKG pathways<sup>24</sup>), which converge on and protect mitochondria from dysfunction induced by acute IRI. However, it is important to appreciate that there are some differences between IPC and IPost in terms of its signalling components. With IPC there is an additional signalling pathway responsible for the 'memory' effect of classical IPC (the activation of protein kinases such as protein kinase C) and of the second window of protection (the transcription of new proteins such as cyclooxygenase-2, inducible nitric oxide synthase and so on)<sup>6 20</sup>. The mechanisms underlying RIC are even more complex given the added dimension of having to convey the cardioprotective signal from the remote organ or tissue to the heart. Once at the heart, the signalling pathways alluded to above for IPC and IPost are also recruited by RIC. The details of the pathway linking the remote organ or tissue to the heart remain unresolved, but are believed to involve the release of local autacoids stimulating the sensory afferent neural pathway in the remote organ or tissue leading to the production of a circulating transferrable blood-borne factor(s), which is able to confer cross-species cardioprotection. The identity of the cardioprotective factor(s) remains unknown, but is probably a peptide <30 kilodaltons in size (reviewed in <sup>25 26</sup>).

## **7. CLINICAL APPLICATION OF ISCHAEMIC CONDITIONING**

Ischaemic conditioning has been investigated in several clinical settings in which the heart is subjected to acute myocardial IRI including cardiac bypass surgery, elective percutaneous coronary intervention (PCI) and PPCI (Figure 2).

### ***7.1. Cardiac bypass surgery as a clinical setting for cardioprotection***



In patients undergoing cardiac bypass surgery the heart is subjected to global ischaemic injury (when the aorta is clamped and the heart is put onto cardiopulmonary bypass) followed by global reperfusion injury (when the aorta is unclamped and the heart is taken off cardiopulmonary bypass)<sup>27</sup>. However, acute IRI is not the only cause of myocardial injury during cardiac bypass surgery as direct handling of the heart, coronary embolisation, and the inflammatory response to cardiopulmonary bypass can all contribute. The perioperative myocardial injury (PMI) which occurs during cardiac bypass surgery can be quantified by measuring serum cardiac enzymes (Creatine Kinase MB isoenzyme, Troponin T and I)<sup>28 29</sup>, and can be detected as late gadolinium enhancement on cardiovascular magnetic resonance imaging (CMR)<sup>30</sup>. Given that the presence of PMI has been associated with worse clinical outcomes post-cardiac surgery<sup>28 29</sup>, the measurement of serum cardiac enzymes has been used as a surrogate endpoint to assess the cardioprotective efficacy of novel therapies in patients undergoing cardiac bypass surgery.

### *IPC and IPost in cardiac bypass surgery*

IPC was the first cardioprotective strategy to be investigated in the setting of cardiac bypass surgery. In 1993, Yellon's group<sup>31</sup> first demonstrated that brief episodes of global ischaemia induced by clamping and declamping the aorta prior to cardiopulmonary bypass preserved myocardial ATP levels in patients with IHD. Since then a number of studies have investigated the role of IPC as a cardioprotective strategy in cardiopulmonary bypass surgery. In a recent meta-analysis it was shown that IPC significantly reduced ventricular arrhythmias, decreased inotrope requirements and shortened the intensive care unit stay<sup>32</sup>. However, despite these potential beneficial effects, the need to intervene on the heart directly and the risk of embolisation arising from clamping an atherosclerotic aorta have prevented IPC from being adopted in this clinical setting.

Using a similar approach to cardioprotection at the time of aorta unclamping it has been reported that IPost induced by re-clamping and declamping the aorta to stutter global myocardial reperfusion attenuated perioperative myocardial injury in terms of serum cardiac enzyme release<sup>33</sup>. There is the potential to apply this cardioprotective strategy to children (in whom the aorta is not yet atherosclerotic) undergoing cardiopulmonary bypass surgery to correct congenital cardiac defects<sup>34</sup>.

### *Limb RIC in cardiac bypass surgery*

Limb RIC was first demonstrated to show benefit in the clinical setting of cardiac bypass surgery in 2006 by Cheung et al<sup>35</sup> in children undergoing corrective cardiac surgery for congenital heart defects. Subsequent studies have reported beneficial effects of RIC in adult patients undergoing coronary artery bypass graft surgery (CABG) and/or valve surgery in terms of attenuated perioperative myocardial injury as evidenced by decreased serum cardiac enzyme release (Table 1). However, there have been several neutral studies<sup>36-38</sup> including at least one very large study<sup>39</sup>. The reasons for this discrepancy are not clear but may relate to the following factors: patient selection (CABG vs valve surgery, stable vs unstable IHD patients); timing of the limb RIC protocol (prior to vs after surgical incision); blinding of the RIC protocol (proper vs limited blinding); the intensity of the RIC protocol (3 vs 4 cycles of limb RIC and inflation of cuff to 200mmHg vs 15mmHg above systolic blood pressure); and the presence of confounding factors (Table 5). The results of several recent meta-analyses have confirmed the cardioprotective effects of RIC in this clinical setting in terms of attenuating perioperative myocardial injury<sup>40 41</sup>. The ongoing RIPHeart<sup>42</sup> and ERICCA<sup>43</sup> multicentre clinical trials, which are currently investigating the effect of RIC on clinical outcomes post cardiac bypass surgery (Table 1), should hopefully provide definitive evidence of the cardioprotective effects of RIC in this clinical setting.

### *7.2. Elective PCI as a clinical setting for cardioprotection*

Ischaemic conditioning has been investigated as a cardioprotective strategy for protecting the heart against periprocedural myocardial injury in patients undergoing elective PCI. It is important to appreciate that PCI-related injury, which occurs in about 20 to 30% of patients undergoing elective PCI and measured by the release of serum cardiac enzymes, is not due to acute IRI, but mainly by acute ischaemic injury (distal branch occlusions, and coronary embolisation) - complications which are more frequent following multi-vessel and complex PCI<sup>44</sup>. Limb RIC administered prior to elective PCI has been reported to be beneficial in IHD patients, in terms of reducing serum levels of cardiac enzymes (Table 2), although there have been a number of neutral studies<sup>45 46</sup> and one negative study<sup>47</sup>. The reasons for this discrepancy are not clear but may relate to the following factors: the setting itself (acute IRI not a major component of myocardial injury during elective PCI); patient selection (stable vs unstable IHD patients); the timing of RIC in relation to PCI procedure (prior to vs after) and the PCI procedure itself (simple vs multi-vessel or complex PCI). A recent meta-analysis has suggested that limb RIC may be beneficial if lower limb RIC is used and in the setting of multi-vessel or complex PCI<sup>48</sup>.

### *7.3. PPCI as a clinical setting for cardioprotection*

For patients presenting with STEMI, the most effective therapy for limiting MI size and preserve LV systolic function is myocardial reperfusion by PPCI. However, the restoration of coronary blood flow in the infarct-related artery induces myocardial reperfusion injury, thereby providing a target for cardioprotection for ischaemic conditioning strategies such as IPost and RIC.

### *IPost in STEMI patients*

IPost was rapidly translated into the clinical setting by Staat et al in 2005 only 2 years after its discovery in the original animal experimental study<sup>13</sup>. They demonstrated in a small proof-of-concept study of 30 patients that IPost could reduce enzymatic MI size by 36% (Table 3). The IPost protocol was applied following direct stenting of the infarct-related artery by inflation of the angioplasty balloon to low pressure upstream of the stent to interrupt coronary flow for one minute followed by deflation of the angioplasty balloon for one minute to allow coronary reflow and repeated 4 times in total. A number of clinical studies have gone on to confirm the cardioprotective effect of IPost using echocardiographic, myocardial SPECT and CMR endpoints (Table 3). However, not all the studies have been positive<sup>49 50</sup>, and the reasons for this are not clear but may be due to a number of factors including: patient selection (only patients with complete occlusion of the infarct-related artery and no coronary collateralisation should be included), the stenting technique (most benefit seen with direct stenting), the IPost protocol itself (which should not be delivered within the stent); the endpoints used to assess cardioprotection (ST-segment resolution vs MI size). The results of recent meta-analyses of IPost in PPCI patients have also produced mixed results<sup>51-54</sup>. Whether IPost can actually improve clinical outcomes following PPCI is currently being investigated in the DANAMI-3 trial (ClinicalTrials.gov Identifier: NCT01435408)(Table 3).

### *Limb RIC in STEMI patients*

Several proof-of-concept studies have reported cardioprotective effects with limb RIC in STEMI patients treated by PPCI (Table 3). It appears to be effective when given in the ambulance by paramedics<sup>55</sup>, on arrival at the hospital prior to PPCI<sup>56 57</sup>, and even at the onset of reperfusion at the time of PPCI<sup>58</sup>. Whether limb RIC can improve clinical outcomes in PPCI patients is currently being investigated in the ERIC-PPCI and CONDI2 trials (ClinicalTrials.gov Identifier: NCT01857414), which are investigation, in collaboration,

whether RIC can reduce the rates of cardiac death and hospitalisation for heart failure at 12 months.

### *Pharmacological conditioning – mimicking ischaemic conditioning*

The elucidation of the signal transduction pathways underlying ischaemic conditioning have resulted in the identification of new targets for cardioprotection, some of which can be modulated by pharmacological agents (reviewed in <sup>59 60</sup>). In this regard, the most promising of these pharmacological conditioning strategies include atrial natriuretic peptide<sup>61</sup>, cyclosporin-A (CsA)<sup>62</sup>, exenatide<sup>63</sup>, and metoprolol<sup>64</sup>, all of which have been reported in proof-of-concept clinical studies to reduce MI size and preserve LV systolic function (Table 4). Whether CsA therapy can improve clinical outcomes post-PPCI is currently being tested in two clinical outcome studies (the CYCLOsporinE A in Reperfused Acute Myocardial Infarction (CYCLE) (ClinicalTrials.gov NCT01650662) and Cyclosporine and Prognosis in Acute Myocardial Infarction Patients (CIRCUS) (ClinicalTrials.gov NCT01502774) multi-centre randomised clinical trials.

## **8. NEW AVENUES FOR ISCHAEMIC CONDITIONING**

### *8.1. Other clinical settings of acute myocardial IRI*

There are other clinical settings in which acute myocardial IRI is a critical determinant of outcome. In patients having a cardiac arrest the whole body including the heart is subjected to acute global ischaemia, and in those patients which are successfully resuscitated, the whole body is then subjected to acute global reperfusion injury. Whether limb RIC is a therapeutic option in patients who are successfully resuscitated following a cardiac arrest remains to be tested. The added benefit of limb RIC in this setting is multi-organ protection against acute IRI.

For patients undergoing cardiac transplantation acute IRI is a major determinant of graft dysfunction post-transplantation. In this setting, the heart is subjected to global ischaemic injury as it is removed from the donor, followed by global reperfusion injury as it is transplanted into the recipient. In this setting there is the opportunity to perform limb RIC to the donor prior to organ harvesting and to the recipient prior to transplantation, but whether this approach is beneficial remains to be tested.

### *8.2. Limb RIC and cardiac function*

A number of clinical studies are investigating the cardioprotective effects of limb RIC on cardiac function. Limb RIC has been reported to attenuate ST-segment depression and prevent myocardial stunning in chronic renal failure patients undergoing haemodialysis during which the heart is subjected to repeated bouts of acute myocardial ischemia resulting in myocardial stunning and chronic LV systolic impairment <sup>65</sup>.

Whether repeated episodes of limb RIC, applied as a daily therapy, is beneficial in the clinical setting is not known. One experimental study demonstrated that repeating limb RIC daily for 28 days prevented adverse post-MI LV remodelling in the rat heart<sup>66</sup>. There are currently two clinical studies investigating the effect of daily RIC continued for 4 weeks on post-MI LV remodelling (Daily REmote Ischaemic Conditioning following Acute Myocardial Infarction (DREAM, ClinicalTrials.gov Identifier: NCT01664611) and the Chronic Remote Ischemic Conditioning to Modify Post-MI Remodeling (CRIC-RCT; ClinicalTrials.gov Identifier:NCT01817114) trials. The CONDI-HF study (ClinicalTrials.gov Identifier:NCT02248441) is currently investigating the effect of daily RIC on LV ejection fraction in chronic heart failure patients.

### *8.3. Increasing exercise performance by limb RIC*

Interestingly, limb RIC has been reported to improve exercise performance in elite swimmers<sup>67</sup>, presumably by rendering skeletal muscle more tolerant to acute ischaemia, although the actual mechanism is not clear. In the setting of heart failure however, limb RIC failed to improve exercise capacity and oxygen consumption, with the suggestion that heart failure patients were already chronically preconditioned, as plasma dialysate obtained from both sham and RIC patients reduced murine MI size compared to plasma dialysate from historical healthy controls<sup>68</sup>.

#### ***8.4. Limb RIC protection of other organs***

The key advantage of limb RIC as a therapeutic strategy is that it offers multi-organ protection against acute IRI. As such limb RIC has been shown to be beneficial in a number of non-cardiac organs including the brain (against acute ischaemic stroke<sup>69</sup>), the kidney (protection against acute kidney injury induced by cardiac bypass surgery<sup>38 70 71</sup>, and induced by contrast following coronary angiography<sup>72</sup>) and the liver (during acute liver resection [ClinicalTrials.gov Identifier: NCT007965880] and liver transplantation [Remote Ischaemic PreCOnditioning in Liver Transplant or RIPCOLT. The recently completed REnal Protection Against Ischaemia-Reperfusion in transplantation (REPAIR) trial (ISRCTN30083294) has found that limb RIC of both the donor and recipient preserved estimated glomerular filtration rate of the transplanted kidney at 6 months in recipient patients undergoing live-donor related renal transplantation, suggesting limb RIC to be a potential therapeutic strategy for preserving renal graft function post-transplantation.

### **9. OPTIMISING THE TRANSLATION OF CARDIOPROTECTION**

The field of cardioprotection has a chequered history with a disappointing large number of neutral clinical studies in which a novel cardioprotective therapy has failed to improve clinical outcomes in IHD patients subjected to acute IRI. The failure to translate the large

number of cardioprotective therapies discovered in laboratory studies into patient benefit has been the subject of several recent articles <sup>73-76</sup>- the major issues are highlighted in Table 5.

## **10. SUMMARY AND CONCLUSIONS**

Ischaemic conditioning is an endogenous form of cardioprotection which can be elicited by cycles of brief ischaemia and reperfusion to the heart directly or to an organ or tissue away from the heart. A number of proof-of-concept clinical studies have shown beneficial effects with ischaemic conditioning in the settings of cardiac bypass surgery, elective PCI and PPCI, with reduced myocardial injury and preservation of cardiac function. Whether ischaemic conditioning can actually improve clinical outcomes in CABG and PPCI patients is currently being investigated in several large multi-centre randomised clinical trials. As a result, in the next couple of years we should know whether ischaemic conditioning could benefit IHD patients in terms of reducing morbidity and mortality and potentially change clinical practice.

## **ACKNOWLEDGMENTS**

DJH is funded by the British Heart Foundation (grant number FS/10/039/28270), the Rosetrees Trust, and is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.



## REFERENCES

1. Krug A, Du Mesnil dR, Korb G. Blood supply of the myocardium after temporary coronary occlusion. *Circ.Res.* 1966;19:57-62.
2. White SK, Hausenloy DJ, Moon JC. Imaging the myocardial microcirculation post-myocardial infarction. *Curr.Heart Fail.Rep.* 2012;9:282-92.
3. Bogaert J, Kalantzi M, Rademakers FE *et al.* Determinants and impact of microvascular obstruction in successfully reperfused ST-segment elevation myocardial infarction. Assessment by magnetic resonance imaging. *Eur.Radiol.* 2007;17:2572-80.
4. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-36.
5. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev.* 2003;83:1113-51.
6. Hausenloy DJ. Cardioprotection techniques: preconditioning, postconditioning and remote conditioning (basic science). *Curr.Pharm.Des* 2013;19:4544-63.
7. Williams RP, Manou-Stathopoulou V, Redwood SR *et al.* 'Warm-up Angina': harnessing the benefits of exercise and myocardial ischaemia. *Heart* 2014;100:106-14.
8. Eitel I, Thiele H. Cardioprotection by pre-infarct angina: training the heart to enhance myocardial salvage. *Eur.Heart J.Cardiovasc.Imaging* 2013;14:1115-6.
9. Zhao ZQ, Corvera JS, Halkos ME *et al.* Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am.J.Physiol Heart Circ.Physiol* 2003;285:H579-H588.
10. Vinten-Johansen J, Shi W. The science and clinical translation of remote postconditioning. *J.Cardiovasc.Med.(Hagerstown.)* 2013;14:206-13.
11. Okamoto F, Allen BS, Buckberg GD *et al.* Reperfusion conditions: importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. *J.Thorac.Cardiovasc Surg.* 1986;92:613-20.
12. Sato H, Jordan JE, Zhao ZQ *et al.* Gradual reperfusion reduces infarct size and endothelial injury but augments neutrophil accumulation. *Ann.Thorac.Surg.* 1997;64:1099-107.
13. Staat P, Rioufol G, Piot C *et al.* Postconditioning the human heart. *Circulation* 2005;112:2143-8.
14. Przyklenk K, Bauer B, Ovize M *et al.* Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993;87:893-9.
15. McClanahan T, Nao B, Wolke L, Martin BJ, Mezt TE. Brief renal occlusion and reperfusion reduces myocardial infarct size in rabbits (abstract). *FASEB J* 1993;7:A18.
16. Birnbaum Y, Hale SL, Kloner RA. 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;96:1641-6.
17. Oxman T, Arad M, Klein R *et al.* Limb ischemia preconditions the heart against reperfusion tachyarrhythmia. *Am J Physiol* 1997;273:H1707-H1712.
18. Kharbanda RK, Mortensen UM, White PA *et al.* Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation* 2002;106:2881-3.
19. Hausenloy DJ, Yellon DM. Survival kinases in ischemic preconditioning and postconditioning. *Cardiovasc.Res.* 2006;70:240-53.
20. Hausenloy DJ, Yellon DM. The second window of preconditioning (SWOP) where are we now? *Cardiovasc.Drugs Ther.* 2010;24:235-54.
21. Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. *Cardiovasc Res.* 2004;61:448-60.
22. Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail.Rev.* 2007;12:217-34.
23. Hausenloy DJ, Lecour S, Yellon DM. Reperfusion injury salvage kinase and survivor activating factor enhancement pro-survival signaling pathways in ischemic postconditioning: two sides of the same coin. *Antioxid.Redox.Signal.* 2011;14:893-907.
24. Inserte J, Hernando V, Vilardosa U *et al.* Activation of cGMP/protein kinase G pathway in postconditioned myocardium depends on reduced oxidative stress and preserved endothelial nitric oxide synthase coupling. *J.Am.Heart Assoc.* 2013;2:e005975.
25. Hausenloy DJ, Yellon DM. Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc Res.* 2008;79:377-86.
26. V.Sivaraman, JMJ Pickard, D.J Hausenloy. Remote ischaemic conditioning: From academic curiosity to clinical reality. *Anaesthesia* 2014.
27. Venugopal V, Ludman A, Yellon DM *et al.* 'Conditioning' the heart during surgery. *Eur.J.Cardiothorac.Surg.* 2009;35:977-87.

28. Croal BL, Hillis GS, Gibson PH *et al.* Relationship between postoperative cardiac troponin I levels and outcome of cardiac surgery. *Circulation* 2006;114:1468-75.
29. Wang TK, Stewart RA, Ramanathan T *et al.* Diagnosis of MI after CABG with high-sensitivity troponin T and new ECG or echocardiogram changes: relationship with mortality and validation of the universal definition of MI. *Eur.Heart J.Acute.Cardiovasc.Care* 2013;2:323-33.
30. Selvanayagam JB, Porto I, Channon K *et al.* Troponin elevation after percutaneous coronary intervention directly represents the extent of irreversible myocardial injury: insights from cardiovascular magnetic resonance imaging. *Circulation* 2005;111:1027-32.
31. Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. *Lancet* 1993;342:276-7.
32. Walsh SR, Tang TY, Kullar P *et al.* Ischaemic preconditioning during cardiac surgery: systematic review and meta-analysis of perioperative outcomes in randomised clinical trials. *Eur.J.Cardiothorac.Surg.* 2008;34:985-94.
33. Luo W, Li B, Chen R *et al.* Effect of ischemic postconditioning in adult valve replacement. *Eur.J Cardiothorac.Surg.* 2008;33:203-8.
34. Luo W, Li B, Lin G *et al.* Postconditioning in cardiac surgery for tetralogy of Fallot. *J Thorac.Cardiovasc Surg.* 2007;133:1373-4.
35. Cheung MM, Kharbada RK, Konstantinov IE *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;47:2277-82.
36. Karupphasamy P, Chaubey S, Dew T *et al.* Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and inflammation? *Basic Res.Cardiol.* 2011;106:511-9.
37. Young PJ, Dalley P, Garden A *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;107:1-10.
38. Rahman IA, Mascaro JG, Steeds RP *et al.* Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment? *Circulation* 2010;122:S53-S59.
39. McCrindle BW, Clarizia NA, Khaikin S *et al.* Remote ischemic preconditioning in children undergoing cardiac surgery with cardiopulmonary bypass: a single-center double-blinded randomized trial. *J.Am.Heart Assoc.* 2014;3.
40. Haji Mohd Yasin NA, Herbison P, Saxena P *et al.* The role of remote ischemic preconditioning in organ protection after cardiac surgery: a meta-analysis. *J.Surg.Res.* 2014;186:207-16.
41. Healy DA, Khan WA, Wong CS *et al.* Remote preconditioning and major clinical complications following adult cardiovascular surgery: systematic review and meta-analysis. *Int.J.Cardiol.* 2014;176:20-31.
42. Meybohm P, Zacharowski K, Cremer J *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;33:1423-6.
43. Hausenloy DJ, Candilio L, Laing C *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;101:339-48.
44. Babu GG, Walker JM, Yellon DM *et al.* Peri-procedural myocardial injury during percutaneous coronary intervention: an important target for cardioprotection. *Eur Heart J* 2011;32:23-31.
45. Prasad A, Gossel M, Hoyt J *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.* 2012.
46. Lavi S, D'Alfonso S, Diamantouros P *et al.* Remote ischemic postconditioning during percutaneous coronary interventions: remote ischemic postconditioning-percutaneous coronary intervention randomized trial. *Circ.Cardiovasc.Interv.* 2014;7:225-32.
47. Iliodromitis EK, Kyrzopoulos S, Paraskevaidis IA *et al.* Increased C reactive protein and cardiac enzyme levels after coronary stent implantation. Is there protection by remote ischaemic preconditioning? *Heart* 2006;92:1821-6.
48. D'Ascenzo F, Moretti C, Omede P *et al.* Cardiac remote ischaemic preconditioning reduces periprocedural myocardial infarction for patients undergoing percutaneous coronary interventions: a meta-analysis of randomised clinical trials. *EuroIntervention.* 2014;9:1463-71.
49. Tarantini G, Favaretto E, Marra MP *et al.* Postconditioning during coronary angioplasty in acute myocardial infarction: the POST-AMI trial. *Int.J.Cardiol.* 2012;162:33-8.

50. Hahn JY, Song YB, Kim EK *et al.* Ischemic postconditioning during primary percutaneous coronary intervention: the effects of postconditioning on myocardial reperfusion in patients with ST-segment elevation myocardial infarction (POST) randomized trial. *Circulation* 2013;128:1889-96.
51. Zhou C, Yao Y, Zheng Z *et al.* Stenting technique, gender, and age are associated with cardioprotection by ischaemic postconditioning in primary coronary intervention: a systematic review of 10 randomized trials. *Eur.Heart J.* 2012;33:3070-7.
52. Favaretto E, Roffi M, Frigo AC *et al.* Meta-Analysis of Randomized Trials of Postconditioning in ST-Elevation Myocardial Infarction. *Am.J.Cardiol.* 2014;114:946-52.
53. Abdelnoor M, Sandven I, Limalanathan S *et al.* Postconditioning in ST-elevation myocardial infarction: a systematic review, critical appraisal, and meta-analysis of randomized clinical trials. *Vasc.Health Risk Manag.* 2014;10:477-91.
54. Khan AR, Binabdulhak AA, Alastal Y *et al.* Cardioprotective role of ischemic postconditioning in acute myocardial infarction: A systematic review and meta-analysis. *Am.Heart J.* 2014;168:512-21.
55. Botker HE, Kharbanda R, Schmidt MR *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;375:727-34.
56. Rentoukas I, Giannopoulos G, Kaoukis A *et al.* Cardioprotective role of remote ischemic perconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *JACC.Cardiovasc.Interv.* 2010;3:49-55.
57. White SK, Frohlich GM, Sado DM *et al.* Remote Ischemic Conditioning Reduces Myocardial Infarct Size and Edema in Patients With ST-Segment Elevation Myocardial Infarction. *JACC.Cardiovasc.Interv.* 2014.
58. Crimi G, Pica S, Raineri C *et al.* Remote ischemic post-conditioning of the lower limb during primary percutaneous coronary intervention safely reduces enzymatic infarct size in anterior myocardial infarction: a randomized controlled trial. *JACC.Cardiovasc.Interv.* 2013;6:1055-63.
59. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N.Engl.J Med.* 2007;357:1121-35.
60. Sharma V, Bell RM, Yellon DM. Targeting reperfusion injury in acute myocardial infarction: a review of reperfusion injury pharmacotherapy. *Expert.Opin.Pharmacother.* 2012;13:1153-75.
61. Kitakaze M, Asakura M, Kim J *et al.* Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 2007;370:1483-93.
62. Piot C, Croisille P, Staat P *et al.* Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N.Engl.J Med.* 2008;359:473-81.
63. Lonborg J, Vejlsstrup N, Kelbaek H *et al.* Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 2012;33:1491-9.
64. Ibanez B, Macaya C, Sanchez-Brunete V *et al.* Effect of Early Metoprolol on Infarct Size in ST-Segment-Elevation Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention: The Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) Trial. *Circulation* 2013;128:1495-503.
65. Crowley LE, McIntyre CW. Remote ischaemic conditioning-therapeutic opportunities in renal medicine. *Nat.Rev.Nephrol.* 2013;9:739-46.
66. Wei M, Xin P, Li S *et al.* Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. *Circ.Res.* 2011;108:1220-5.
67. Jean-St-Michel E, Manlihot C, Li J *et al.* Remote preconditioning improves maximal performance in highly trained athletes. *Med.Sci.Sports Exerc.* 2011;43:1280-6.
68. McDonald MA, Braga JR, Li J *et al.* A randomized pilot trial of remote ischemic preconditioning in heart failure with reduced ejection fraction. *PLoS.One.* 2014;9:e105361.
69. Hougaard KD, Hjort N, Zeidler D *et al.* Remote ischemic perconditioning in thrombolysed stroke patients: randomized study of activating endogenous neuroprotection - design and MRI measurements. *Int.J.Stroke* 2013;8:141-6.
70. Venugopal V, Laing CM, Ludman A *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;56:1043-9.
71. Candilio L, Malik A, Ariti C *et al.* Effect of remote ischaemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery: a randomised controlled clinical trial. *Heart* 2014.
72. Er F, Nia AM, Dopp H *et al.* Ischemic preconditioning for prevention of contrast medium-induced nephropathy: randomized pilot RenPro Trial (Renal Protection Trial). *Circulation* 2012;126:296-303.
73. Hausenloy DJ, Baxter G, Bell R *et al.* Translating novel strategies for cardioprotection: the Hatter Workshop Recommendations. *Basic Res.Cardiol* 2010;105:677-86.
74. Schwartz LL, Kloner RA, Arai AE *et al.* New horizons in cardioprotection: recommendations from the 2010 national heart, lung, and blood institute workshop. *Circulation* 2011;124:1172-9.

75. Hausenloy DJ, Erik BH, Condorelli G *et al.* Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc.Res.* 2013;98:7-27.
76. Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet* 2013;381:166-75.
77. Hausenloy DJ, Mwamure PK, Venugopal V *et al.* Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *Lancet* 2007;370:575-9.
78. Thielmann M, Kottenberg E, Kleinbongard P *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;382:597-604.
79. Hoole S, Heck PM, Sharples L *et al.* Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study: a prospective, randomized control trial. *Circulation* 2009;119:820-7.
80. Davies WR, Brown AJ, Watson W *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;6:246-51.
81. Zografos TA, Katritsis GD, Tsiafoutis I *et al.* Effect of one-cycle remote ischemic preconditioning to reduce myocardial injury during percutaneous coronary intervention. *Am.J.Cardiol.* 2014;113:2013-7.
82. Liu Z, Wang YL, Hua Q *et al.* Late remote ischemic preconditioning provides benefit to patients undergoing elective percutaneous coronary intervention. *Cell Biochem.Biophys.* 2014;70:437-42.
83. Moretti C, Cavallero E, D'Ascenzo F *et al.* The EUROpean and Chinese cardiac and renal Remote Ischemic Preconditioning Study (EURO-CRIPS): study design and methods. *J.Cardiovasc.Med.(Hagerstown.)* 2014.
84. Thibault H, Piot C, Staat P *et al.* Long-term benefit of postconditioning. *Circulation* 2008;117:1037-44.
85. Lonborg J, Kelbaek H, Vejlstrup N *et al.* Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ.Cardiovasc.Interv.* 2010;3:34-41.
86. Thuny F, Lairez O, Roubille F *et al.* Post-conditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. *J.Am.Coll.Cardiol.* 2012;59:2175-81.
87. Sloth AD, Schmidt MR, Munk K *et al.* Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote ischaemic conditioning as an adjunct to primary percutaneous coronary intervention. *Eur.Heart J.* 2014;35:168-75.
88. Newton N, Croisille P, Gahide G *et al.* Effect of cyclosporine on left ventricular remodeling after reperfused myocardial infarction. *J Am.Coll.Cardiol* 2010;55:1200-5.
89. Lonborg J, Kelbaek H, Vejlstrup N *et al.* Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ.Cardiovasc Interv.* 2012;5:288-95.
90. Pizarro G, Fernandez-Friera L, Fuster V *et al.* Long-term benefit of early pre-reperfusion metoprolol administration in patients with acute myocardial infarction: results from the METOCARD-CNIC trial (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction). *J.Am.Coll.Cardiol.* 2014;63:2356-62.

## **Figure legends**

### **Figure 1**

#### **The clinical impact of myocardial reperfusion injury in reperfused STEMI patients**

This hypothetical scheme depicts the magnitude and clinical impact of myocardial reperfusion injury on patients with ischaemic heart disease who are subjected to acute ischaemia/reperfusion injury. The thick blue curve shows the extent of myocardial salvage (which equates to the area-at-risk subtract the myocardial infarct size and is expressed as the % of the left ventricular volume) in a theoretical patient presenting with an acute ST-segment elevation myocardial infarction (STEMI) reperfused by primary percutaneous coronary intervention (PPCI) or thrombolysis – as expected in the absence of reperfusion the extent of myocardial salvage declines with time. Although myocardial reperfusion is essential for myocardial salvage following a STEMI, the process of restoring coronary blood flow within the infarct-related artery, can paradoxically induce cardiomyocyte death – a phenomenon which has been termed ‘myocardial reperfusion injury’. As a result, following reperfusion, the extent of myocardial salvage is actually smaller than expected given the duration of acute myocardial ischaemia – this attenuation in myocardial salvage is due to the presence of myocardial reperfusion injury, which can contribute up to 50% of the final myocardial infarct size.

## Figure 2

### **Ischaemic conditioning in the clinical setting**

This figure illustrates the variety of clinical settings in which ischaemic conditioning has been tested (in orange) including: cardiac bypass surgery, elective percutaneous coronary intervention (PCI) and non-ST-segment elevation myocardial infarction (NSTEMI) patients undergoing PCI, and STEMI patients. There is the potential to investigate the role of ischaemic conditioning in other clinical settings (in yellow) such as heart transplantation, heart failure and cardiac arrest. The term ischaemic conditioning encompasses a number of endogenous forms of cardioprotection including ischaemic preconditioning (IPC, which has to be delivered within 2-3 hours of the index ischaemia), ischaemic postconditioning (IPost, which has to be delivered in the first minute of reperfusion) and remote ischaemic conditioning (RIC, using transient limb ischaemia and reperfusion). RIC can be divided according to the timing of the intervention into remote ischaemic preconditioning (RIC stimulus applied prior to index ischaemia), RIPerC (RIC stimulus applied after the onset of index ischaemia but prior to reperfusion) and RIPost (RIC stimulus applied at the onset of reperfusion).

**Table 1: Major clinical studies investigating limb RIC in cardiac bypass surgery**

Study	RIC protocol	Number	Main outcomes	Comments
Cheung et al 2006 <sup>35</sup>	Four x 5 minutes thigh cuff inflations/deflations	37	Smaller peak Trop T, less inotrope support and lower airway pressures	First study to report beneficial effect of limb RIC in CABG
Hausenloy et al 2007 <sup>77</sup>	Three x 5 minutes upper arm cuff inflations/deflations	53	43% reduction in 72 hr AUC Trop-T	First study to report beneficial effect of limb RIC patients undergoing CABG
Candilio et al 2014 <sup>71</sup>	Two x 5 minutes simultaneous upper arm and thigh cuff inflations/deflations	180	27% less 72 hr AUC Trop T. 54% Less AF 48% Less AKI and 1 day less ICU stay	First study to report beneficial effect of limb RIC on short-term clinical outcomes
Heusch et al 2013 <sup>78</sup>	Three x 5 minutes upper arm cuff inflations/deflations	329	21% less 72 hr AUC Trop I. 73% reduction in all-cause mortality	First study to report beneficial effect of limb RIC on long-term clinical outcomes.
<b>Ongoing studies</b>				
Meybohm et al RIPHEART <sup>42</sup>	Four x 5 minutes cycles of upper arm cuff inflation/deflation	2070	Primary endpoint of death, non-fatal MI, stroke, AKI at 30 days	First study which will test effect of RIC on 30 days primary endpoint following CABG Results available March 2015
Hausenloy et al ERICCA <sup>43</sup>	Four x 5 minutes cycles of upper arm cuff inflation/deflation	1610	Primary endpoint of death, non-fatal MI, revascularisation, stroke at 12 months	First study which will test effect of RIC on 12 months primary endpoint following CABG Results available March 2015

RIC: remote ischaemic conditioning; Trop T: troponin T; CABG: cardiac bypass surgery; AUC: area under curve; AF: atrial fibrillation;

AKI: acute kidney injury; ICU: intensive care unit; MI: myocardial infarct

**Table 2: Major clinical studies investigating limb RIC in elective PCI**

Clinical study	RIC protocol	Number of patients	Outcome	Comments
Hoole et al 2009 <sup>79</sup>	Three x 5 minutes upper arm cuff inflations/deflations immediately prior to PCI	242	63% reduction in median Trop I	First study to test effect of limb RIC in PCI
Davies et al 2013 <sup>80</sup>	Three x 5 minutes upper arm cuff inflations/deflations immediately prior to PCI	192	42% reduction in all-cause mortality, nonfatal MI, TIA or stroke, HHF at 6 years	First study to test effect of limb RIC on long-term clinical outcomes following PCI
Zografos et al 2014 <sup>81</sup>	One x 5 minutes upper arm cuff inflations/deflations Immediately prior to PCI	94	80% reduction in 24 hr serum levels of Trop I. Also reduced incidence of PCI-related MI by 56%.	First study to test effect of one cycle of limb RIC
Liu et al 2014 <sup>82</sup>	Three x 5 minutes upper arm cuff inflations/deflations 18 hours prior to PCI	200	40-60% reduction in 24 hr serum levels of Trop I and CK-MB. Also less chest pain and ST-segment deviation with PCI.	First study to test effect of second window of protection of limb RIC
EURO-CRIPS <sup>83</sup>	Three x 5 minutes upper arm cuff inflations/deflations immediately prior to PCI	Planned		Also investigating the effect of limb RIC on contrast-induced AKI

RIC: remote ischaemic conditioning; PCI: percutaneous coronary intervention, Trop I: troponin I; MI: myocardial infarction; TIA: transient ischaemic attack; HHF: hospitalisation for heart failure; CK-MB: creatine kinase MB isoenzyme; AKI: acute kidney injury



**Table 3: Major clinical studies investigating IPost and limb RIC in STEMI patients**

Clinical study	Treatment protocol	Number of patients	Outcome	Comments
<b>Ischaemic postconditioning in STEMI patients</b>				
Staat et al 2005 <sup>13</sup>	Four x 60 seconds angioplasty balloon inflations/deflations	30	36% reduction in 72 hours AUC CK	First study to report beneficial effect of IPost in PPCI patients
Thibault et al 2008 <sup>84</sup>	Four x 60 seconds angioplasty balloon inflations/deflations	38	41% reduction in 72 hours AUC CK-MB 39% reduction in MI size at 6 months by SPECT LVEF improved by 7% echocardiogram at one year	First study to report long-term cardioprotective effects of IPost in PPCI patients
Lonborg et al 2010 <sup>85</sup>	Four x 30 seconds angioplasty balloon inflations/deflations	118	9% reduction in MI size at 3 months by CMR 31% increase in myocardial salvage index	First study to report cardioprotective effects of IPost in PPCI patients using CMR
Thuny et al <sup>86</sup>	Four x 60 seconds angioplasty balloon inflations/deflations	50	40% reduction in MI size and 21% reduction in myocardial oedema on CMR	First study to report effect of IPost on myocardial oedema on CMR
Engstrom et al DANAMI-3 ClinicalTrials.gov Identifier: NCT01435408	Four x 30 seconds angioplasty balloon inflations/deflations	2000 Completed recruitment	All-cause mortality, heart failure at 4 years Results awaited	First study which will report effects of IPost on long-term clinical outcomes
<b>Remote ischaemic conditioning in STEMI patients</b>				
Botker et al 2010 <sup>55</sup>	Four x 5 minutes upper arm cuff inflations/deflations in the ambulance prior to PPCI	142	Increase in myocardial salvage index at 30 days No difference in MI size (SPECT or peak Troponin)	First study to test effect of RIC in PPCI-treated STEMI patients. Reduced MI size in LAD STEMI.
Rentoukas et al 2010 <sup>56</sup>	Three x 4 minutes cuff inflations/deflations at the hospital prior to PPCI	93	Better ST resolution and lower peak Troponin I. Synergistic effects with morphine.	
Crimi et al 2013 <sup>58</sup>	Three x 5 minutes thigh cuff inflations/ deflations at onset of reperfusion	100 anterior STEMI only	20% reduction in 72 hours AUC CK-MB. % reduction in myocardial oedema by CMR	First study to show effect of RIC given at onset of reperfusion via PPCI. Also, first study to report effect of RIC on enzymatic MI size and myocardial oedema.
White et al 2014 <sup>57</sup>	Four x 5 minutes upper arm cuff inflations/deflations at the hospital prior to PPCI	197	27% reduction in MI size by CMR 19% reduction in myocardial oedema by CMR	First study to show effect of RIC given prior to PPCI on MI size and myocardial oedema by CMR
Hausenloy et al ERIC-LYSIS	Four x 5 minutes upper arm cuff	519	17% reduction in enzymatic MI size (CK-	Only study to test effect of RIC in

(ClinicalTrials.gov Identifier: NCT02197117)	inflations/deflations at hospital prior to thrombolysis		MB and Trop-T)	thrombolysed STEMI patients
Sloth et al 2014 <sup>87</sup>	Four x 5 minutes upper arm cuff inflations/deflations in the ambulance prior to PPCI	251	51% reduction in all-cause mortality, nonfatal MI, TIA or stroke, HHF at 3.8 years	First study to test effect of RIC on long-term outcomes following PPCI
Botker CONDI-2 Hausenloy ERIC-PPCI  ClinicalTrials.gov Identifier: NCT01857414	Four x 5 minutes upper arm cuff inflations/deflations prior to PPCI	4300 ongoing	Primary endpoint of cardiac death and HHF at 12 months	Collaboration between UK, Denmark. First study to test effect of RIC on long-term clinical outcomes at primary endpoint following PPCI

*I*Post: Ischaemic postconditioning; *RIC*: remote ischaemic conditioning; *STEMI*: ST-segment elevation myocardial infarction; *AUC*: area under curve; *CK*: creatine kinase; *PPCI*: primary percutaneous coronary intervention; *CK-MB*: creatine kinase MB isoenzyme; *SPECT*: single-photon emission computed tomography; *LVEF*: left ventricular ejection fraction; *PPCI*: primary percutaneous coronary intervention; *MI*: myocardial infarct; *LAD*: left anterior descending artery; *CMR*: cardiovascular magnetic resonance imaging; *Trop I*: Troponin I; *TIA*: transient ischaemic attack; *HHF*: hospitalisation for heart failure;

**Table 4:** Major clinical studies investigating promising pharmacological conditioning agents in STEMI patients

Clinical study	Therapeutic intervention	Number	Outcome	Potential mechanisms underlying cardioprotection
Kitakaze et al 2007 <sup>61</sup> J-WIND	72 hours IV carperitide (atrial natriuretic peptide analogue) infusion started prior to PPCI	569	15% reduction in 72 hours AUC total CK 2.0% absolute increase in LVEF	Atrial natriuretic peptide targets pro-survival kinase pathways such as the cGMP and RISK pathways
Piot et al 2008 <sup>62</sup>	IV cyclosporin A (2.5mg/kg) bolus 10 minutes prior to PPCI	58	44% reduction in MI size (72 hours AUC total CK) 20% reduction in MI size (CMR subset)  28% reduction in MI size and smaller LVESV on CMR at 6 months <sup>88</sup>	Cyclosporin-A inhibits the opening of the mitochondrial permeability transition pore, a critical determinant of lethal myocardial reperfusion injury
Lonborg et al 2012 <sup>63</sup>	IV infusion of exenatide started 15 minutes prior to PPCI and continued for 6 hr	107	Increase in myocardial salvage index (0.62 to 0.71) 23% reduction in MI size at 3 months on CMR  Patients presenting with short ischaemic times ( $\leq 132$ minutes) had greater myocardial salvage <sup>89</sup>	Exenatide, a GLP-1 analogue, targets pro-survival kinase pathways such as the RISK pathway
Ibanez et al 2013 <sup>64</sup>	IV metoprolol (3x5mg) in ambulance prior to PPCI	270	Reduction in MI size by CMR at one week. Increased LVEF at 6 months Improvement in clinical outcome at 2 years  Reduced: incidence of severely depressed LVEF (<35%) at 6 months by 60%; less need for ICD by 65% at 6 months and reduced HF at 2 years <sup>90</sup>	The mechanism of cardioprotection is not currently clear

STEMI: ST-segment elevation myocardial infarction; IV: intravenous; AUC: area under curve; CK: creatine kinase; LVEF: left ventricular ejection fraction; cGMP: cyclic guanosine monophosphate; RISK: reperfusion injury salvage kinase; MI: myocardial infarct; CMR: cardiovascular magnetic resonance imaging; LVESV: left ventricular end-systolic volume; GLP-1: glucagon-like peptide-1; AAR: area at risk; HF: heart failure

**Table 5: Improving the translation of cardioprotection for patient benefit**

	<b>STEMI-PPCI trials</b>	<b>Cardiac bypass surgery trials</b>
<b>Patient selection</b>	<p>Select patients which are most likely to benefit from the cardioprotective therapy:</p> <ol style="list-style-type: none"> <li>1. Large AAR (&gt;30% of the left ventricle)</li> <li>2. No coronary collateralisation (Rentrop&lt;1)</li> <li>3. Fully occluded artery prior to PPCI (TIMI&lt;1)</li> <li>4. Onset of symptoms 3-6 hours</li> </ol>	<p>Select patients which are most likely to benefit from the cardioprotective therapy:</p> <ol style="list-style-type: none"> <li>1. Patients undergoing on-pump cardiac surgery who are subjected to global acute IRI.</li> <li>2. Patients with longer cardiopulmonary bypass times are at greater risk of perioperative myocardial injury.</li> <li>3. Higher risk patients who are at greater risk of perioperative myocardial injury.</li> </ol>
<b>Confounding factors</b>	<p>Be aware of confounding factors such as prior chest pain, age, diabetes mellitus, hypertension, hyperlipidaemia and concomitant medication (nitrates, morphine), which can interfere with cardioprotection.</p>	<p>Be aware of confounding factors such as prior chest pain, age, diabetes mellitus, hypertension, hyperlipidaemia and concomitant medication (sulphonylureas, nitrates, morphine, nicorandil, volatile anaesthetics), which can interfere with cardioprotection.</p>
<b>The intervention</b>	<ol style="list-style-type: none"> <li>1. Only test therapies having shown conclusive cardioprotection in pre-clinical studies.</li> <li>2. Administer the therapy prior to myocardial reperfusion via PPCI.</li> </ol>	<ol style="list-style-type: none"> <li>1. Only test therapies having shown conclusive cardioprotection in pre-clinical studies</li> <li>2. Option to administer therapy prior to surgical incision, in cardioplegic solution, or at time of aortic declamping.</li> </ol>
<b>Clinical endpoints</b>	<p>Select relevant clinical endpoints for assessing cardioprotective efficacy:</p> <ol style="list-style-type: none"> <li>1. MI size (enzymatic or CMR)</li> <li>2. Myocardial salvage index (more sensitive than MI size reduction)</li> <li>3. Microvascular obstruction</li> <li>4. LV remodelling (LVH, LVEF and indexed LVEDV or LVESV)</li> <li>5. Cardiac death</li> <li>6. Hospitalisation for heart failure.</li> </ol>	<p>Select relevant clinical endpoints for assessing cardioprotective efficacy:</p> <ol style="list-style-type: none"> <li>1. Perioperative myocardial injury (enzymatic or CMR)</li> <li>2. LVEF</li> <li>3. Amount of inotrope support required</li> <li>4. Cardiac death</li> <li>5. Hospitalisation for heart failure</li> </ol>

STEMI: ST-elevation myocardial infarction; PPCI: percutaneous coronary intervention; AAR: area at risk; CMR: cardiovascular magnetic resonance imaging; AUC: area under the curve; MI: myocardial infarction, LVH: left ventricular hypertrophy; LVEF: left ventricular ejection fraction; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume

**Figure 1**

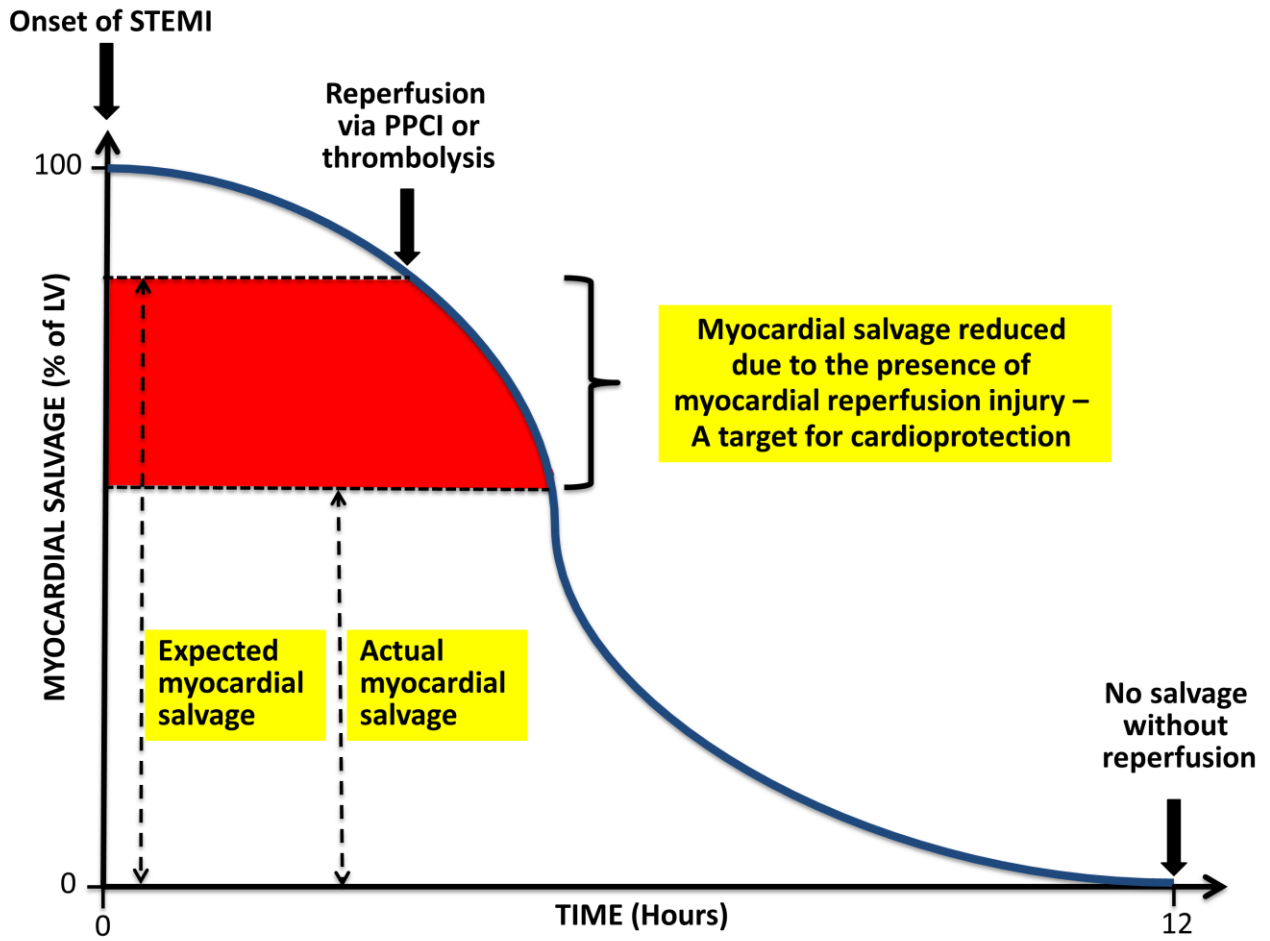
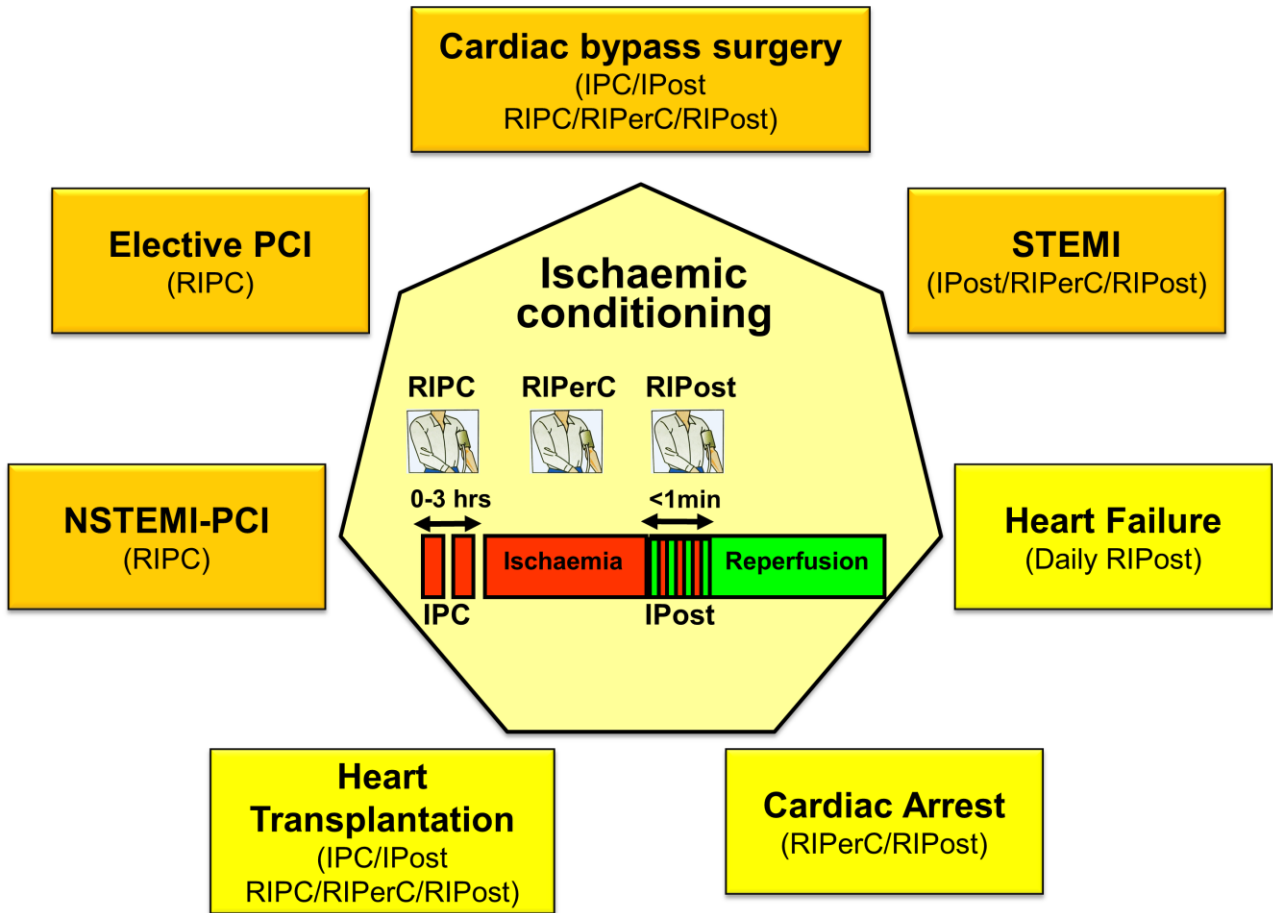


Figure 2



## Ischaemic conditioning in the clinical setting: key points

- 'Ischaemic conditioning' has the therapeutic potential to protect the heart against acute ischaemia/reperfusion (I/R) injury and improve clinical outcomes in patients with ischaemic heart disease (IHD), the leading cause of death and disability worldwide.
- Ischaemic conditioning is mediated by applying cycles of brief ischaemia and reperfusion to either the heart itself or to an organ/tissue remote from the heart - it can be reproduced by certain pharmacological agents (termed 'pharmacological conditioning').
- Ischaemic and pharmacological conditioning have been reported in proof-of-concept studies to be beneficial in three major clinical settings in which the heart is subjected to acute I/R injury: cardiac bypass surgery, elective percutaneous coronary intervention (PCI), and ST-segment elevation myocardial infarction (STEMI) patients treated by primary PCI.
- Whether this therapeutic strategy can improve patient outcomes in these clinical settings should be known in the next few years with the availability of results from several large multi-centre clinical trials.
- The translation of promising cardioprotective therapies discovered in the research laboratory into the clinic has been hampered by the use of inadequate animal models and poorly designed clinical studies – this can be overcome by increased interaction between basic scientists and clinicians, thereby facilitating the translation of novel cardioprotective therapies into the clinical setting for patient benefit.