REVIEW

Cardioprotection by remote ischemic conditioning: Mechanisms and clinical evidences

Alberto Aimo, Chiara Borrelli, Alberto Giannoni, Luigi Emilio Pastormerlo, Andrea Barison, Gianluca Mirizzi, Michele Emdin, Claudio Passino

Alberto Aimo, Chiara Borrelli, Michele Emdin, Claudio Passino, Institute of Life Sciences, Scuola Superiore Sant'Anna, 56126 Pisa, Italy

Alberto Giannoni, Luigi Emilio Pastormerlo, Andrea Barison, Gianluca Mirizzi, Michele Emdin, Claudio Passino, Division of Cardiology and Cardiovascular Medicine, Fondazione Toscana Gabriele Monasterio, 56124 Pisa, Italy

Correspondence to: Claudio Passino, MD, Division of Cardiology and Cardiovascular Medicine, Fondazione Toscana Gabriele Monasterio, via Giuseppe Moruzzi 1, 56124 Pisa, Italy. passino@ftgm.it
Telephone: +39-050-3152191
Fax: +39-050-3152109

Abstract

In remote ischemic conditioning (RIC), several cycles of ischemia and reperfusion render distant organ and tissues more resistant to the ischemia-reperfusion injury. The intermittent ischemia can be applied before the ischemic insult in the target site (remote ischemic preconditioning), during the ischemic insult (remote ischemic perconditioning) or at the onset of reperfusion (remote ischemic postconditioning). The mechanisms of RIC have not been completely defined yet; however, these mechanisms must be represented by the release of humoral mediators and/or the activation of a neural reflex. RIC has been discovered in the heart, and has been arising great enthusiasm in the cardiovascular field. Its efficacy has been evaluated in many clinical trials, which provided controversial results. Our incomplete comprehension of the mechanisms underlying the RIC could be impairing the design of clinical trials and the interpretation of their results. In the present review we summarize current knowledge about RIC pathophysiology and the data about its cardioprotective efficacy.

Key words: Remote ischemic conditioning; Ischemic heart disease; Percutaneous coronary intervention; Cardiac surgery; Atherosclerosis

Core tip: Remote ischemic conditioning (RIC) is a safe, non-invasive, and inexpensive technique that has the potential to protect the heart against the ischemia-reperfusion injury. Its cardioprotective efficacy is currently being evaluated, and diverging results are emerging. It is thus worth resuming current understanding of RIC pathophysiology and clinical efficacy.

INTRODUCTION

The myocardium can tolerate brief periods (up to 15 min) of severe and even total ischemia. Such ischemic episodes occur in the settings of angina, coronary vasospasm, and balloon angioplasty, and are not associated with concomitant myocyte cell death. With increasing duration and severity of ischemia, greater myocardial damage, and the predisposition to further damage during reperfusion develop. The combined deleterious effects of coronary occlusion and revascularization configure the "ischemia-reperfusion (IR) injury"[1].

The counterintuitive idea to apply several brief episodes of IR cycles to protect the myocardium against IR injury was firstly advanced in 1986, when Murry *et al*[2] reported that the infarcted area following a 40-min coronary occlusion was reduced if preceded by four 5-min IR cycles. This phenomenon was called "ischemic preconditioning". Its clinical application is hindered by the unpredictable timing of acute myocardial infarction (AMI), and by the necessity to intervene on coronary vessels[3]. However, several IR cycles were found to confer cardioprotection even when applied at the onset of coronary revascularization (ischemic postconditioning)[4,5], both in animals and in human patients undergoing primary percutaneous coronary intervention (PCI).

In 1993, it was demonstrated in anesthetized dogs that 4 episodes of 5-min ischemia and reperfusion in the left circumflex coronary territory, followed by a 1-h occlusion of the left anterior descending coronary artery, significantly reduced the infarct size[6]. The "remote ischemic conditioning (RIC)" paradigm has been progressively extended[7]. At present, RIC is defined as the phenomenon by which brief episodes of ischemia and reperfusion in one vascular bed, tissue, or organ render distant sites resistant to the ischemia-reperfusion injury[7,8]. The IR cycles are effective when applied before myocardial ischemia (remote ischemic preconditioning), during coronary occlusion (remote ischemic perconditioning), and during cardiac revascularization (remote ischemic post-conditioning)[7-12].

The mechanisms conferring protection at distance have not been completely defined[7], yet their characterization would be relevant to achieve a full comprehension of the phenomenon, and to exploit its full potential in clinical practice. In fact, understanding whether humoral mediators, neural mechanisms, or their combination mediate remote ischemic conditioning would be crucial to determine the optimal number of IR cycles, the better site and timing of their application, to select the patients according to age, comorbidities, and medical treatment, and to optimize the overall therapeutic management of the patient.

In the first part of the present review, we analyze current knowledge of the mechanisms underlying RIC, comparing humoral and reflex-mediated mechanisms.

In the second half of this work, we attempt a critical analysis of the available literature concerning the cardioprotective potential of RIC in different settings.

THE "HUMORAL HYPOTHESIS" OF RIC, AND POTENTIAL CIRCULATING MEDIATORS

The "humoral hypothesis" has been formulated in the setting of remote ischemic preconditioning (RIPC). It postulates that the IR cycles in a distant site cause the local accumulation of mediators which are then released into the bloodstream and finally reach the heart[7] (Figure 1).

Several data from animal models support this hypothesis. In particular, it has been demonstrated that the effluent from preconditioned hearts could transfer the protection to naïve recipients[13-15]; this protection seems to be mediated by small hydrophobic proteins whose molecular weight ranges between 3.5 and 15-30 kDa[16].

Since these humoral mediators must be effective in remote sites after dilution into the bloodstream, their release from the peripheral tissue has to be massive[16]. The identification of humoral mediators should therefore be relatively easy to perform in animals or humans undergoing a RIPC protocol[16]. Indeed, proteomic approaches have been attempted in both animals and humans, still they have yielded controversial results[17-19].

Among the proteins potentially involved, there are kallistatin, apolipoprotein A-I, and stromal-derived factor 1α (SDF-1α)[16].

Kallistatin is a serine protease which reduces inflammation, apoptosis, and oxidative stress in endothelial cells[20]. It has been recently characterized as a protective factor against renal ischemia-reperfusion injury in mice[21], and has been found to be increased in the plasma of healthy humans undergoing a RIPC protocol[16]. However, its role as a humoral mediator of RIPC has not been properly evaluated yet.

Apolipoprotein A-I has anti-inflammatory properties, which could prove useful in the protection against ischemia-reperfusion injury[18]. In humans, its circulating levels have been found to be either increased[18] or decreased[17,22] after a RIPC protocol; therefore, its exact role is still debated.

Finally, SDF-1α has been proposed to be an important, and possibly the main, mediator of RIPC[23]. In a study on rats, a 50% plasma increment was detected in rats subjected to a RIPC protocol compared to control animals (890 ± 70 pg/mL *vs* 590 ± 50 pg/mL; $n = 8$, $P < 0.01$)[23]. Nevertheless, the administration of a selective inhibitor of SDF-1α did not completely abrogate the reduction in infarct size following RIPC[24], suggesting the existence of other mechanisms of cardioprotection[24].

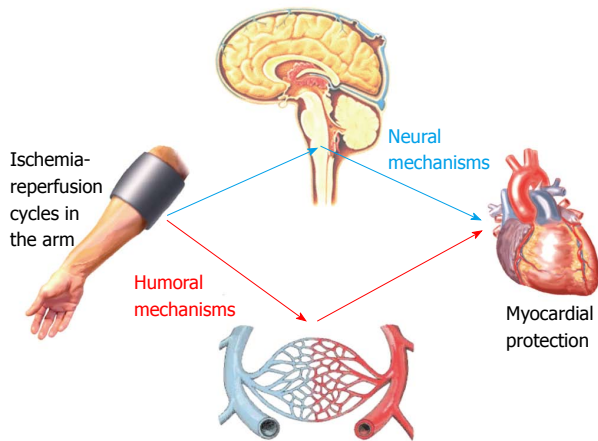Several potential other mediators have been identified: among them, there are microRNAs (miRNAs),

Figure 1 Mechanisms of remote ischemic conditioning. The cardioprotective effects of several ischemia/reperfusion cycles applied in a distant site (most commonly the upper limb) have been ascribed to the activation of humoral and/or neural pathways. The pathogenesis of remote ischemic conditioning is incompletely known, however it is possi-ble that both humoral and neural mechanisms underlie this response.

bradykinin, adenosine, and nitric oxide (NO).

miRNAs have been involved in both muscle ischemia[25] and protection against myocardial IR injury[26]. The circulating levels of miR-144 have been found to increase by 1.6 folds in healthy human subjects undergoing a RIPC protocol, even though the exact mechanism of its action is still unknown[27].

Bradykinin is released by damaged tissues and can activate afferent fibers (see below), possibly contri-buting to a cardioprotective effect known as "remote preconditioning of trauma"[28]. A release of bradykinin from ischemic tissues into the bloodstream has been reported[29]; the involvement of bradykinin in the RIPC response has been postulated, but the data from an animal study were inconclusive[30].

Finally, both adenosine and NO have been extensively studied in the setting of ischemic preconditioning[31], and have been considered potential mediators of RIPC as well[7,32], although their extremely short half-life makes unlikely that they could exert a significant cardioprotective effect.

To summarize, it is quite established the existence of humoral mechanisms underlying RIPC, but the nature of the mediator(s) is currently unclear. Potential humoral mediators should be assessed with respect to their potential mechanism of action, the increase in their circulating levels following a conditioning protocol, and their half-life; in fact, all these parameters should be compatible with a cardioprotective role. Further studies are required to define the existence and the role of humoral mechanisms underlying RIPC, as well as the other forms of remote ischemic conditioning.

NEURALLY-MEDIATED CARDIOPROTECTION

Neural control of the heart
The autonomic nervous system consists of an afferent pathway, integrating centers located into the central nervous system, and two efferent limbs, the sympathetic and the parasympathetic nervous systems[33]. In the heart, sensory innervation is provided by afferent neurons located into the nodose and dorsal root ganglia, and projecting to brainstem areas controlling the activity of both sympathetic and parasympathetic nuclei[33]. Sympathetic efferent fibers innervate the sinoatrial and atrioventricular nodes, the atria, and the ventricles[33]. Parasympathetic efferent fibers are traditionally believed to control exclusively the nodal tissue and the atria[33]. Nevertheless, the presence of cholinergic innervations have been detected in both ventricles, and it has been demonstrated that vagal activation decreases the force of ventricular contraction irrespective of its effect on heart rate, in both animals and humans[33].

The "neural hypothesis" of RIPC, and potential role of the sympathetic system
The "neural hypothesis" of remote ischemic preconditioning (RIPC) postulates that ischemia-reperfusion (IR) cycles in peripheral sites might activate a neural reflex resulting in myocardial protection against a subsequent myocardial insult[34] (Figure 1).

In animals, cycles of occlusion and reopening of the renal artery, mesenteric artery, or femoral artery resulted in significant cardioprotection; in all these cases, the resection of the afferent fibers projecting to the ischemized territories abolished the cardioprotective effect[35-37]. In rats, IR cycles in the mesenteric artery conferred a cardioprotection similar in entity to that provided by ischemic preconditioning (*i.e.*, IR cycles of the coronary vessel before sustained occlusion); the systemic administration of hexametonium, a blocker of both sympathetic and parasympathetic ganglia, abolished this effect[38].

Conflicting results have been provided by Kingma *et al*[39], who reported that, in dogs anesthetized with isoflurane, a RIPC protocol conferred robust myocardial protection against a subsequent ischemic injury even during autonomic blockade or surgical denervation of the heart. It should be noted that in this study the animals were anesthetized with isoflurane[39], which is *per se* a powerful preconditioning agent (see below).

The activation of neural afferents during RIPC has been ascribed to local accumulation of mediators such as calcitonin-gene related peptide (CGRP), adenosine, and bradykinin[40]. Interestingly, the accumulation of adenosine[41,42], bradykinin[43], and other mediators[44,45] in the exercising muscle has been implied as a deter-minant of the metaboreflex[46], which is a neural mechanism coupling sympathetic tone to exercise requirements[47,48]. It could then be speculated that the IR cycles of a conditioning protocol cause metaboreflex activation. The subsequent increase in sympathetic outflow could confer myocardial protection through the activation of β1 and/or β2 adrenergic receptors; this phenomenon has been discovered in animal hearts perfused with β-agonists, and has been named

"β-adrenergic preconditioning"[49-51].

Evidences of vagal activation following RIPC

As discussed above, a theoretical framework could be provided for sympathetic outflow as the final mediator of RIPC. Nevertheless, a growing body of evidences points to RIPC as a vagal reflex.

The possibility to precondition the heart by the infusion of acetylcholine (ACh) was demonstrated in 1993 by Yao *et al*[52] The preconditioning potential of ACh has been confirmed by several other studies[53-55]. It has been demonstrated that cardioprotection by RIPC is suppressed by spinal cord section, bilateral vagotomy or the systemic administration of the muscarinic receptor antagonist atropine, while vagal stimulation closely recapitulates the effects of RIPC[56,57].

Using a viral transfer gene approach in rats, Mastitskaya *et al*[58] confirmed that an intact parasympathetic outflow is crucial for myocardial protection by RIPC. The neurons in the dorsal motor nucleus of the vagus nerve were selectively silenced, thus abolishing the cardioprotective effect of a RIPC protocol[58]. The selective activation of the same neurons closely recapitulated the cardioprotective effect of RIPC; this response was suppressed by atropine[58]. Again in rats, Basalay *et al*[59] reported that IR cycles in the limb conferred cardioprotection when applied 25 min prior to myocardial ischemia. The authors then found that the cardioprotective effect was abolished by the denervation of the peripheral ischemic organ or bilateral vagotomy[59].

Autonomic function in RIPC: What happens in humans?

To our knowledge, only two studies have evaluated the consequences of a RIPC protocol on the autonomic function in humans. In 2005, Loukogeorgakis *et al*[60] evaluated the possibility to protect against endothelial ischemia-reperfusion injury by RIPC. A cuff inflation to 200 mmHg for 20 min in the non-dominant arm was used as the ischemic insult; the subsequent endothelial damage was denoted by reduced flow-mediated dilation (FMD)[60]. When arm ischemia was preceded by a RIPC protocol in the dominant arm, the ischemic insult in the other arm caused no significant reduction in FMD compared to baseline, suggesting endothelial protection by RIPC[60]. Such response was abolished by the autonomic ganglion blocker trimetaphan[60]. These results suggested an autonomic activation underlying the endothelial protection by RIPC; however, being trimetaphan an aspecific autonomic blocker, it was not possible to ascertain if either the sympathetic or the parasympathetic system accounted for the protection by RIPC[60].

Parasympathetic activation was detected as the underlying mechanism by Enko *et al*[61] in 2011. After 3 cycles of 5 min ischemia and 5 min reperfusion in the left arm, a significant dilation of the right brachial artery was observed; in the power spectral analysis of heart rate, the high frequency domain displayed a simultaneous increase, denoting increased parasympathetic outflow[61].

Remote ischemic perconditioning and postconditioning: does vagal activation play a role?

The first demonstration of remote ischemic perconditioning was provided in 2007: in pigs, four 5-min cycles of lower limb ischemia during a 40-min left anterior descending coronary artery occlusion caused a significant reduction in infarct size, improved indexes of systolic and diastolic function, and less arrhythmic events during the reperfusion phase[11]. With respect to remote ischemic postconditioning, a cardioprotective effect of IR cycles at the beginning of reperfusion was demonstrated for the first time in 2005[12], and subsequently corroborated by other animal studies[62,63].

To our knowledge, only Basalay *et al*[59] assessed the pathophysiology of remote ischemic perconditioning and postconditioning. These authors reported that deafferenting the site of IR cycles or cutting both vagus nerves abolished the preconditioning and perconditioning responses in rats, but did not alter the postconditioning effect[59]. These results suggest that remote ischemic perconditioning relies on neural mechanisms, while remote ischemic postconditioning is mediated by humoral mediators[59].

Further studies are required to assess this hypothesis. However, it should be noted that neural mechanisms are more qualified than humoral mechanisms to protect the ischemic myocardium in the setting of remote ischemic perconditioning, at least when the coronary flow is completely blocked. In the same setting, an activation of the parasympathetic system would probably be more effective than a sympathetic response.

Excessive concentrations of catecholamines have been detected in the ischemic area during an acute myocardial infarction[64]. Increased cardiac sympathetic outflow is due to pain, anxiety, and a fall of cardiac output or arterial blood pressure; a further release of catecholamines is promoted by the ischemic damage of nerve endings[64]. As a result, extracellular norepinephrine reaches up to 100-1000 times its normal plasma concentrations within 30 min of coronary occlusion[64]. Far from being protective, local concentrations of this magnitude are capable of producing myocardial necrosis even in nonischemic myocardium, and might promote malignant arrhythmias[64]. This mechanism accounts for the positive effects of early administration of β-blockers during acute myocardial infarction[65,66], and probably excludes increased sympathetic outflow as the final mediator of cardioprotection by remote ischemic perconditioning.

PROTECTING THE HEART IN THE SETTING OF PCI

Stable coronary artery disease (SCAD) is associated with impaired quality of life, reduced physical endurance,

recurrent hospitalizations and outpatient visits[67]. Revascularization by either elective PCI or CABG can relieve symptoms, reduce the use of anti-ischemic drugs, improve exercise capacity and quality of life, compared to medical therapy alone[67]. The efficacy of elective PCI in addition to medical therapy in patients with SCAD has been demonstrated in a large number of randomized controlled trials, meta-analyses, and large-scale registries[67].

Albeit elective PCI is becoming increasingly safe, balloon inflation during PCI often causes transient ischemia[68]. Myocardial injury with necrosis may derive from recognizable peri-procedural events such as coronary dissection, occlusion of a major coronary artery or a side-branch, disruption of collateral flow, slow flow or no-reflow, distal embolization, and microvascular plugging; alternatively, the ischemic insult can have no detectable cause[68]. Myocardial ischemia is attested by a rise and fall of cardiac biomarkers after the procedure, with values rising five or more folds over the 99th percentile being indicative of peri-procedural myocardial infarction (PMI)[68].

Four recent meta-analyses have demonstrated that RIPC is effective in reducing PMIs in patients undergoing elective PCI[69-72]. For example, in the meta-analysis by Zografos *et al*[71], PMI occurred in 40.3% of patients in the RIPC group and in 51.3% of patients in the control group (odds ratio 0.57).

Several trials have assessed the long-term outcomes after elective PCI. An improvement in prognosis was not found by Prasad *et al*[73] over 1 year follow-up. By contrast, in the Cardiac Remote Ischemic Preconditioning in Coronary Stenting study, a significant reduction of major adverse cardiac and cerebral events (MACCE; a composite of all-cause mortality, myocardial infarction, readmission for heart failure, and ischemic stroke or transient ischemic attack) was found at 6 mo[74]. A recent follow-up study evaluating the same cohort demonstrated that the MACCE rate at 6 years remained lower in the RIPC group[75].

The significant heterogeneity of the study protocols could be hindering a careful assessment of RIPC efficacy in the setting of elective PCI. For example, the studies assessed in the meta-analysis by Zografos *et al*[71] differed with regard to the RIPC procedure (number of IR cycles, duration of the IR periods, site of application of the IR cycles), the percentage of patients with multivessel disease, and the positivity or the negativity of cardiac troponin I (cTnI) before PCI[71]. By contrast, in all the studies evaluated in this meta-analysis the IR cycles were performed immediately before elective PCI[74-80], so that a different time span between the IR cycles and the angioplasty procedure cannot be regarded as a potential confounding factor.

On the whole, a protective role for RIPC in the setting of elective PCI is emerging, even though its efficacy seems to be lower than in primary PCI. Nevertheless, only one long-term follow-up study has been published so far[75]; the prognostic role of RIPC should therefore receive extensive evaluation, as well as the optimal RIPC protocol to achieve effective cardioprotection.

Remote ischemic perconditioning refers to the application of ischemia-reperfusion cycles in a distant site shortly before revascularization. The first evidences of a protective role of remote ischemic perconditioning in human patients was provided in 2010 by Bøtker *et al*[81], who assessed 333 patients with suspected first ST-elevation myocardial infarction. The primary endpoint was myocardial salvage index (MSI), quantified as the proportion of the area at risk preserved by the treatment, 30 d after primary PCI. MSI was significant higher in the conditioning group than in controls; the protective effect of remote ischemic perconditioning seemed to be strongest in patients with more severe infarctions, *i.e.*, presenting with occluded vessels or infarcts in the left anterior descending artery[81].

The long-term outcome of remote ischemic perconditioning was assessed in the same study population[82]. A significant reduction of MACCE and all cause mortality was observed in the conditioning group over a median follow-up of 3.8 years. There was also a trend toward reduced myocardial reinfarction, readmission for heart failure, and ischemic stroke or transient ischemic attack[82].

In 2013, remote ischemic postconditioning was assessed on 232 patients undergoing elective PCI[83]. In the conditioning group, the patients underwent three 5-min cycles of cuff inflation in the nondominant arm just after the end of the angioplasty. No significant difference was found between the conditioning group and the control group in terms of peak troponin I levels, PMI rate, recurrence of myocardial ischemia[83]. In another study, the incidence of PMIs was similar between all groups, and no difference was remarked with respect to the creatine kinase (CK) levels or the incidence of acute kidney failure[84]. In other studies, significant reductions in the incidence of acute kidney failure were observed; the prevention of acute kidney failure is currently regarded as the most promising perspective for the application of remote ischemic postconditioning during PCI[85,86].

For the details of the studies cited in the present paragraph, see Table 1.

REMOTE ISCHEMIC PRECONDITIONING BEFORE ELECTIVE CARDIAC SURGERY

Elective coronary artery bypass surgery (CABG) stands as an alternative to elective PCI for the management of SCAD[87]. The safety and efficacy of both techniques are similar, as well as the incidence of PMIs[87].

In 2007, a randomized controlled study enrolled 57 adult patients undergoing elective CABG surgery[88]. In the RIPC group, three IR cycles were performed

Table 1 Clinical studies on remote ischemic conditioning in percutaneous coronary intervention

Ref.	Patients *n* (CTRLS/RIPC)	ST or LT outcome	Conditioning protocol			Primary endpoint	Results		
			I/R cycles	Cuff pressure	Limb		RIPC	CTRLS	*P*
Remote ischemic preconditioning									
Prasad *et al*[73], 2013	48/47	ST	3 × 3′	200 mmHg	Upper	Post-PCI myonecrosis (cTnT ⩾ 0.03 ng/dL)	40%	47%	0.42
Ahmed *et al*[76], 2013	72/77	ST	3 × 5′	200 mmHg	Upper	cTnT 16 h post-PCI	0.02 ng/mL	0.047 ng/mL	0.047
Ghaemian *et al*[77], 2010	40/40	ST	2 × 5′	Above systolic	Lower	TnT 24 h post-PCI	12.50%	40%	0.01
Hoole *et al*[74], 2009	117/125	ST	3 × 5′	200 mmHg	Upper	cTnI 24 h post-PCI	0.06 ng/mL	0.16 ng/mL	0.04
Luo e *et al*[78], 2013	104/101	ST	3 × 5′	200 mmHg	Upper	hs-cTnI 16 h post-PCI	0.11 ng/mL	0.21 ng/mL	< 0.01
Xu *et al*[79], 2014	98/102	ST	3 × 5′	200 mmHg	Upper	hs-cTnI 16 h post-PCI	0.29 ng/mL	0.38 ng/mL	0.256
Davies *et al*[75], 2013	117/125	LT	3 × 5′	200 mmHg	Upper	MACCE (6 yr follow up)	23	36	0.039
Bøtker *et al*[81], 2010	167/166	ST	4 × 5′	200 mmHg	Upper	MSI after 30 d	0.75	0.55	0.033
Sloth *et al*[82], 2014	167/166	LT	4 × 5′	200 mmHg	Upper	MACCE rates (5 yr follow-up)	25.60%	13.50%	0.018
Carrasco-Chinchilla *et al*[83], 2013	114/118	ST	3 × 5′	200 mmHg	Upper	TnI 24 h post-PCI	0.476 ng/mL	0.478 ng/mL	0.378

cTnI: Cardiac troponin I; cTnT: Cardiac Tro-ponin T; CTRLS: Controls; hs-cTnT: High sensitivity cardiac troponin T; I/R: Ische-mia/reperfusion; MACCE: Major adverse cardiac and cerebral events; RIPC: Remote ischemic preconditioning; ST: Short term; LT: Long term.

after the induction of anesthesia, resulting in a 43% reduction in the 72 h area under the curve (AUC) of cTnT compared with the control group[88]. Other randomized trials confirmed a cardioprotective role of RIPC, in terms of reduced cTnT[89], cTnI[90], and CK isoenzyme MB[91] levels. By contrast, several studies failed to detect significant differences among the RIPC group and the control group[92-95]; the use of volatile anesthetics with preconditioning potential (isoflurane, enflurane, sevoflurane) possibly accounts for discrepant results[96-98].

In a meta-analysis, D'Ascenzo *et al*[99] reported a significant reduction in cTnI and cTnT levels in the RIPC group after elective CABG surgery. Such diff-erence persisted after excluding the trials with potentially confounding factors (among them, the use of isoflurane)[99]. It has been suggested that the cardioprotective effect of RIPC could be masked by the administration of volatile anesthetics and blunted by the perioperative administration of β-blockers[99,100]. Indeed, previous studies on animals or isolated human atrial trabeculae had demonstrated that β-blockers could attenuate ischemic preconditioning-induced cardioprotection[100,101], perhaps since even the activation of β-adrenergic receptor is protective against ischemia-reperfusion injury (β-adrenergic preconditioning; see paragraph The "neural hypothesis" of RIPC, and potential role of the sympathetic system).

Two studies evaluating the long-term efficacy of RIPC provided diverging results. Lucchinetti *et al*[94] did not find any difference at 6 mo in terms of deaths or revascularizations, whereas Thielmann *et al*[102] found significantly lower mortality rates in the RIPC group than in controls over a mean 1.54 year follow-up.

With respect to the settings of elective valve replacement surgery and congenital cardiac surgery, a recent metanalysis detected a significant cardio-protective role for RIPC[103]. Nevertheless, the small number of studies, and the high heterogeneity among them[103] might undermine the reliability of these con-clusions. Another recent meta-analysis considered cumulatively CABG, valve replacement surgery, and congenital cardiac surgery, and detected a significant reduction in the post-operative cTnI levels among the patients undergoing RIPC[104]. No subgroup analysis was performed, and the heterogeneity among studies assessing non-CABG surgery was marked[104]. A third meta-analysis took into consideration the studies evaluating RIPC efficacy in adult patients undergoing "major elective or emergency cardiac or vascular surgery"[105]. In such a broad and mixed setting, no significant efficacy of RIPC was detected with regard to several outcomes: perioperative death, myocardial infarction, new-onset cardiac arrhythmias requiring treatment, cerebrovascular accidents, renal failure requiring renal replacement therapy, mesenteric

Table 2 Clinical studies on remote ischemic preconditioning before elective coronary artery bypass graft surgery

Ref.	Patients *n* (CTRLS/RIPC)	RIPC protocol			Primary endpoint	Results			Notes	
		I/R cycles	Cuff pressure	Limb		RIPC	CTRLS	*P*	β-blockers	Isoflurane, desflurane
Short terms results										
Hausenloy *et al*[88], 2007	30/27	3 × 5 min	200 mmHg	Upper	cTnT at				+	+
					6 h	0.31 mcg/L	0.59 mcg/L	0.039		
					12 h	0.37mcg/L	0.69 mcg/L	0.002		
					24 h	0.30 mcg/L	0.52 mcg/L	0.003		
					48 h	0.30 mcg/L	0.52 mcg/L	0.036		
					72 h	0.25 mcg/L	0.48 mcg/L	0.111		
Wagner *et al*[90], 2010	35/33	3 × 5 min	40 mmHg above systolic pressure	Upper	cTnI at 8 h	2.54 mcg/L	2.90 mcg/L	0.043	-	-
Ali *et al*[91], 2010	50/50	3 × 5 min	200 mmHg	Upper	CK-MB at				+	NS
					8 h	27.22 IU/L	30.24 IU/L	0.026		
					16 h	33.3 IU/L	37.2 IU/L	0.021		
					24 h	22.74 IU/L	25.22 IU/L	0.052		
					48 h	17.20 IU/L	19.72 IU/L	0.003		
Lomivorotov *et al*[93], 2012	40/40	3 × 5 min	200 mmHg	Upper	48 h cTnI AUC	54.4 ng/mL	53.3 ng/mL	> 0.05	+	+
Thielmann *et al*[102], 2013	167/162	3 × 5 min	200 mmHg	Upper	72 h cTnI AUC	266 ng/mL	321 ng/mL	0.022	+	+
Rahman *et al*[95], 2010	55	3 × 5 min	200 mmHg	Upper	48 h cTnT AUC	30 ng/mL	28 ng/mL	0.721	+	+
Lucchinetti *et al*[94], 2012	28/27	4 × 5 min	400 mmHg	Lower	72 h hs-cTnT AUC	11708 pg/mL	9574 pg/mL	0.33	+	+
Long term results										
Thielmann *et al*[102], 2013	167/162	3 × 5 min	200 mmHg	Upper	ACE at 1.54 yr	3	11	0.046	+	+
					death MACCE	8	23	0.005		
Lucchinetti *et al*[94], 2012	28/27	4 × 5 min	400 mmHg	Lower	ACE at 6 mo death	0	1	1.00	+	+
					rehospitalization	3	3	1.00		

ACE: Adverse cardiovascular events; AUC: Area under the curve; cTnI: Cardiac troponin I; cTnT: Cardiac Troponin T; CTRLS: Controls; hs-cTnT: High sensitivity cardiac troponin T; I/R: Ischemia/reperfusion; MACCE: Major adverse cardiac and cerebral events; NS: Not specified; RIPC: Remote ischemic preconditioning.

ischemia, length of hospital stay and intensive care unit stay[105].

The few studies evaluating the effects of RIPC in the sole setting of valve replacement yielded conflicting results. For example, Wu *et al*[106] did not find a significant effect of a standard RIPC protocol on cTnI release after mitral valve replacement surgery, whereas Xie *et al*[107] reported a significant reduction of the 72 h cTnI-AUC in patients undergoing mitral valve, aortic valve or tricuspid valve surgery.

On the whole, RIPC seems to exert a protective role against PMIs caused by elective CABG surgery, while its long term effects are still uncertain. Furthermore, no definite statement can be made about the RIPC efficacy in other forms of elective cardiac surgery, namely valve replacement surgery and congenital cardiac surgery (Table 2). Finally, volatile anesthetics and β-blockers are emerging as potential confounding factors, although the mechanisms are still unclear.

CONCLUSION

Remote ischemic conditioning (RIC) has been proposed as a "non-invasive, simple, safe, and cheap"[108] strategy to protect the heart against ischemic insults. A great research effort has been performed in order to verify the existence of a myocardial protection by RIC, and to evaluate the extent of such protection. Nevertheless, clinical studies have provided conflicting results. A deeper comprehension of the mechanisms underlying RIC is advisable in order to correctly assess the cardioprotective potential of RIC, and to guide future clinical research.

REFERENCES

1   **Verma S**, Fedak PW, Weisel RD, Butany J, Rao V, Maitland A, Li RK, Dhillon B, Yau TM. Fundamentals of reperfusion injury for the clinical cardiologist. *Circulation* 2002; **105**: 2332-2336 [PMID: 12021216 DOI: 10.1161/01.CIR.0000016602.96363.36]
2   **Murry CE**, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74**: 1124-1136 [PMID: 3769170 DOI: 10.1161/01.CIR.74.5.1124]
3   **Ovize M**, Thibault H, Przyklenk K. Myocardial conditioning: opportunities for clinical translation. *Circ Res* 2013; **113**: 439-450 [PMID: 23908331 DOI: 10.1161/CIRCRESAHA.113.300764]
4   **Zhao ZQ**, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton

RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003; **285**: H579-H588 [PMID: 12860564 DOI: 10.1152/ajpheart.01064.2002]

5   **Touboul C**, Angoulvant D, Mewton N, Ivanes F, Muntean D, Prunier F, Ovize M, Bejan-Angoulvant T. Ischaemic postconditioning reduces infarct size: systematic review and meta-analysis of randomized controlled trials. *Arch Cardiovasc Dis* 2015; **108**: 39-49 [PMID: 25453717 DOI: 10.1016/j.acvd.2014.08.004]

6   **Przyklenk K**, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993; **87**: 893-899 [PMID: 7680290 DOI: 10.1161/01.CIR.87.3.893]

7   **Heusch G**, Bøtker HE, Przyklenk K, Redington A, Yellon D. Remote ischemic conditioning. *J Am Coll Cardiol* 2015; **65**: 177-195 [PMID: 25593060 DOI: 10.1016/j.jacc.2014.10.031]

8   **Lim SY**, Hausenloy DJ. Remote ischemic conditioning: from bench to bedside. *Front Physiol* 2012; **3**: 27 [PMID: 22363297 DOI: 10.3389/fphys.2012.00027]

9   **Kharbanda RK**, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R. Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation* 2002; **106**: 2881-2883 [PMID: 12460865 DOI: 10.1161/01.CIR.0000043806.51912.9B]

10 **Schmidt MR**, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, White PA, Kristiansen SB, Sorensen K, Dzavik V, Redington AN, Kharbanda RK. 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**: H1883-H1890 [PMID: 17172279 DOI: 10.1152/ajpheart.00617.2006]

11 **Kerendi F**, Kin H, Halkos ME, Jiang R, Zatta AJ, Zhao ZQ, Guyton RA, Vinten-Johansen J. Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. *Basic Res Cardiol* 2005; **100**: 404-412 [PMID: 15965583 DOI: 10.1007/s00395-005-0539-2]

12 **Vinten-Johansen J**, Shi W. Perconditioning and postconditioning: current knowledge, knowledge gaps, barriers to adoption, and future directions. *J Cardiovasc Pharmacol Ther* 2011; **16**: 260-266 [PMID: 21821526 DOI: 10.1177/1074248411415270]

13 **Breivik L**, Helgeland E, Aarnes EK, Mrdalj J, Jonassen AK. 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**: 135-145 [PMID: 21103992 DOI: 10.1007/s00395-010-0133-0]

14 **Dickson EW**, Lorbar M, Porcaro WA, Fenton RA, Reinhardt CP, Gysembergh A, Przyklenk K. Rabbit heart can be "preconditioned" via transfer of coronary effluent. *Am J Physiol* 1999; **277**: H2451-H2457 [PMID: 10600868]

15 **Serejo FC**, Rodrigues LF, da Silva Tavares KC, de Carvalho AC, Nascimento JH. Cardioprotective properties of humoral factors released from rat hearts subject to ischemic preconditioning. *J Cardiovasc Pharmacol* 2007; **49**: 214-220 [PMID: 17438406 DOI: 10.1097/FJC.0b013e3180325ad9]

16 **Helgeland E**, Breivik LE, Vaudel M, Svendsen ØS, Garberg H, Nordrehaug JE, Berven FS, Jonassen AK. Exploring the human plasma proteome for humoral mediators of remote ischemic preconditioning--a word of caution. *PLoS One* 2014; **9**: e109279 [PMID: 25333471 DOI: 10.1371/journal.pone.0109279]

17 **Hepponstall M**, Ignjatovic V, Binos S, Monagle P, Jones B, Cheung MH, d'Udekem Y, Konstantinov IE. Remote ischemic preconditioning (RIPC) modifies plasma proteome in humans. *PLoS One* 2012; **7**: e48284 [PMID: 23139772 DOI: 10.1371/journal.pone.0048284]

18 **Hibert P**, Prunier-Mirebeau D, Beseme O, Chwastyniak M, Tamareille S, Lamon D, Furber A, Pinet F, Prunier F. Apolipoprotein a-I is a potential mediator of remote ischemic preconditioning. *PLoS One* 2013; **8**: e77211 [PMID: 24155931 DOI: 10.1371/journal.pone.0077211]

19 **Hibert P**, Prunier-Mirebeau D, Beseme O, Chwastyniak M, Tamareille S, Pinet F, Prunier F. Modifications in rat plasma proteome after remote ischemic preconditioning (RIPC) stimulus: identification by a SELDI-TOF-MS approach. *PLoS One* 2014; **9**: e85669 [PMID: 24454915 DOI: 10.1371/journal.pone.0085669]

20 **Gao L**, Li P, Zhang J, Hagiwara M, Shen B, Bledsoe G, Chang E, Chao L, Chao J. Novel role of kallistatin in vascular repair by promoting mobility, viability, and function of endothelial progenitor cells. *J Am Heart Assoc* 2014; **3**: e001194 [PMID: 25237049 DOI: 10.1161/JAHA.114.001194]

21 **Zhou S**, Sun Y, Zhuang Y, Zhao W, Chen Y, Jiang B, Guo C, Zhang Z, Peng H, Chen Y. Effects of kallistatin on oxidative stress and inflammation on renal ischemia-reperfusion injury in mice. *Curr Vasc Pharmacol* 2015; **13**: 265-273 [PMID: 25654330]

22 **Pang T**, Zhao Y, Zhang NR, Jin SQ, Pan SQ. Transient limb ischemia alters serum protein expression in healthy volunteers: complement C3 and vitronectin may be involved in organ protection induced by remote ischemic preconditioning. *Oxid Med Cell Longev* 2013; **2013**: 859056 [PMID: 24363825 DOI: 10.1155/2013/859056]

23 **Davidson SM**, Selvaraj P, He D, Boi-Doku C, Yellon RL, Vicencio JM, Yellon DM. Remote ischaemic preconditioning involves signalling through the SDF-1α/CXCR4 signalling axis. *Basic Res Cardiol* 2013; **108**: 377 [PMID: 23917520 DOI: 10.1007/s00395-013-0377-6]

24 **Przyklenk K**. 'Going out on a limb': SDF-1α/CXCR4 signaling as a mechanism of remote ischemic preconditioning? *Basic Res Cardiol* 2013; **108**: 382 [PMID: 24002083 DOI: 10.1007/s00395-013-0382-9]

25 **Yang JC**, Wu SC, Rau CS, Chen YC, Lu TH, Wu YC, Tzeng SL, Wu CJ, Hsieh CH. TLR4/NF-κB-responsive microRNAs and their potential target genes: a mouse model of skeletal muscle ischemia-reperfusion injury. *Biomed Res Int* 2015; **2015**: 410721 [PMID: 25692136 DOI: 10.1155/2015/410721]

26 **Kukreja RC**, Yin C, Salloum FN. MicroRNAs: new players in cardiac injury and protection. *Mol Pharmacol* 2011; **80**: 558-564 [PMID: 21737570 DOI: 10.1124/mol.111.073528]

27 **Li J**, Rohailla S, Gelber N, Rutka J, Sabah N, Gladstone RA, Wei C, Hu P, Kharbanda RK, Redington AN. MicroRNA-144 is a circulating effector of remote ischemic preconditioning. *Basic Res Cardiol* 2014; **109**: 423 [PMID: 25060662 DOI: 10.1007/s00395-014-0423-z]

28 **Gross GJ**, Baker JE, Moore J, Falck JR, Nithipatikom K. Abdominal surgical incision induces remote preconditioning of trauma (RPCT) via activation of bradykinin receptors (BK2R) and the cytochrome P450 epoxygenase pathway in canine hearts. *Cardiovasc Drugs Ther* 2011; **25**: 517-522 [PMID: 21786213 DOI: 10.1007/s10557-011-6321-9]

29 **Baumgarten CR**, Linz W, Kunkel G, Schölkens BA, Wiemer G. Ramiprilat increases bradykinin outflow from isolated hearts of rat. *Br J Pharmacol* 1993; **108**: 293-295 [PMID: 8448580 DOI: 10.1111/j.1476-5381.1993.tb12797.x]

30 **Goto M**, Liu Y, Yang XM, Ardell JL, Cohen MV, Downey JM. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ Res* 1995; **77**: 611-621 [PMID: 7641331 DOI: 10.1161/01.RES.77.3.611]

31 **Iliodromitis EK**, Andreadou I, Iliodromitis K, Dagres N. Ischemic and postischemic conditioning of the myocardium in clinical practice: challenges, expectations and obstacles. *Cardiology* 2014; **129**: 117-125 [PMID: 25227478 DOI: 10.1159/000362499]

32 **Küntscher MV**, Kastell T, Altmann J, Menke H, Gebhard MM, Germann G. Acute remote ischemic preconditioning II: the role of nitric oxide. *Microsurgery* 2002; **22**: 227-231 [PMID: 12375287 DOI: 10.1002/micr.10042]

33 **Gourine A**, Gourine AV. Neural mechanisms of cardioprotection. *Physiology* (Bethesda) 2014; **29**: 133-140 [PMID: 24583769 DOI: 10.1152/physiol.00037.2013]

34 **Gill R**, Kuriakose R, Gertz ZM, Salloum FN, Xi L, Kukreja RC. Remote ischemic preconditioning for myocardial protection: update on mechanisms and clinical relevance. *Mol Cell Biochem* 2015; **402**:

41-49 [PMID: 25552250 DOI: 10.1007/s11010-014-2312-z]

35   **Ding YF**, Zhang MM, He RR. Role of renal nerve in cardioprotection provided by renal ischemic preconditioning in anesthetized rabbits. *ShenglLixue Bao* 2001; **53**: 7-12 [PMID: 11354802]

36   **Liem DA**, Verdouw PD, Ploeg H, Kazim S, Duncker DJ. Sites of action of adenosine in interorgan preconditioning of the heart. *Am J Physiol Heart Circ Physiol* 2002; **283**: H29-H37 [PMID: 12063271 DOI: 10.1152/ajpheart.01031.2001]

37   **Dong JH**, Liu YX, Ji ES, He RR. [Limb ischemic preconditioning reduces infarct size following myocardial ischemia-reperfusion in rats]. *Sheng Li Xue Bao* 2004; **56**: 41-46 [PMID: 14985828]

38   **Gho BC**, Schoemaker RG, van den Doel MA, Duncker DJ, Verdouw PD. Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* 1996; **94**: 2193-2200 [PMID: 8901671]

39   **Kingma JG**, Simard D, Voisine P, Rouleau JR. Role of the autonomic nervous system in cardioprotection by remote preconditioning in isoflurane-anaesthetized dogs. *Cardiovasc Res* 2011; **89**: 384-391 [PMID: 20876586 DOI: 10.1093/cvr/cvq306]

40   **Tang ZL**, Dai W, Li YJ, Deng HW. Involvement of capsaicin-sensitive sensory nerves in early and delayed cardioprotection induced by a brief ischaemia of the small intestine. *Naunyn Schmiedebergs Arch Pharmacol* 1999; **359**: 243-247 [PMID: 10208312 DOI: 10.1007/PL00005348]

41   **Costa F**, Biaggioni I. Role of adenosine in the sympathetic activation produced by isometric exercise in humans. *J Clin Invest* 1994; **93**: 1654-1660 [PMID: 8163667 DOI: 10.1172/JCI117147]

42   **Notarius CF**, Atchison DJ, Rongen GA, Floras JS. Effect of adenosine receptor blockade with caffeine on sympathetic response to handgrip exercise in heart failure. *Am J Physiol Heart Circ Physiol* 2001; **281**: H1312-H1318 [PMID: 11514302]

43   **Scott AC**, Wensel R, Davos CH, Kemp M, Kaczmarek A, Hooper J, Coats AJ, Piepoli MF. Chemical mediators of the muscle ergoreflex in chronic heart failure: a putative role for prostaglandins in reflex ventilatory control. *Circulation* 2002; **106**: 214-220 [PMID: 12105161 DOI: 10.1161/01.CIR.0000021603.36744.5E]

44   **Scott AC**, Wensel R, Davos CH, Georgiadou P, Kemp M, Hooper J, Coats AJ, Piepoli MF. Skeletal muscle reflex in heart failure patients: role of hydrogen. *Circulation* 2003; **107**: 300-306 [PMID: 12538432 DOI: 10.1161/01.CIR.0000042704.37387.29]

45   **Rotto DM**, Kaufman MP. Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *J Appl Physiol* (1985) 1988; **64**: 2306-2313 [PMID: 3136123]

46   **Nobrega AC**, O'Leary D, Silva BM, Marongiu E, Piepoli MF, Crisafulli A. Neural regulation of cardiovascular response to exercise: role of central command and peripheral afferents. *Biomed Res Int* 2014; **2014**: 478965 [PMID: 24818143 DOI: 10.1155/2014/478965]

47   **Coats AJ**, Clark AL, Piepoli M, Volterrani M, Poole-Wilson PA. Symptoms and quality of life in heart failure: the muscle hypothesis. *Br Heart J* 1994; **72**: S36-S39 [PMID: 7946756 DOI: 10.1136/hrt.72.2_Suppl.S36]

48   **Piepoli M**, Clark AL, Volterrani M, Adamopoulos S, Sleight P, Coats AJ. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation* 1996; **93**: 940-952 [PMID: 8598085 DOI: 10.1161/01.CIR.93.5.940]

49   **Salie R**, Moolman JA, Lochner A. The mechanism of beta-adrenergic preconditioning: roles for adenosine and ROS during triggering and mediation. *Basic Res Cardiol* 2012; **107**: 281 [PMID: 22797560 DOI: 10.1007/s00395-012-0281-5]

50   **Robinet A**, Hoizey G, Millart H. PI 3-kinase, protein kinase C, and protein kinase A are involved in the trigger phase of beta1-adrenergic preconditioning. *Cardiovasc Res* 2005; **66**: 530-542 [PMID: 15914118 DOI: 10.1016/j.cardiores.2005.02.010]

51   **Bhushan S**, Kondo K, Predmore BL, Zlatopolsky M, King AL, Pearce C, Huang H, Tao YX, Condit ME, Lefer DJ. Selective β2-adrenoreceptor stimulation attenuates myocardial cell death and preserves cardiac function after ischemia-reperfusion injury. *Arterioscler Thromb Vasc Biol* 2012; **32**: 1865-1874 [PMID: 22652602 DOI: 10.1161/ATVBAHA.112.251769]

52   **Yao Z**, Gross GJ. Acetylcholine mimics ischemic preconditioning via a glibenclamide-sensitive mechanism in dogs. *Am J Physiol* 1993; **264**: H2221-H2225 [PMID: 8322953]

53   **Richard V**, Blanc T, Kaeffer N, Tron C, Thuillez C. Myocardial and coronary endothelial protective effects of acetylcholine after myocardial ischaemia and reperfusion in rats: role of nitric oxide. *Br J Pharmacol* 1995; **115**: 1532-1538 [PMID: 8564215 DOI: 10.1111/j.1476-5381.1995.tb16647.x]

54   **Qian YZ**, Levasseur JE, Yoshida K, Kukreja RC. KATP channels in rat heart: blockade of ischemic and acetylcholine-mediated preconditioning by glibenclamide. *Am J Physiol* 1996; **271**: H23-H28 [PMID: 8760153]

55   **Yamaguchi F**, Nasa Y, Yabe K, Ohba S, Hashizume Y, Ohaku H, Furuhama K, Takeo S. Activation of cardiac muscarinic receptor and ischemic preconditioning effects in in situ rat heart. *Heart Vessels* 1997; **12**: 74-83 [PMID: 9403311 DOI: 10.1007/BF02820870]

56   **Katare RG**, Ando M, Kakinuma Y, Arikawa M, Handa T, Yamasaki F, Sato T. Vagal nerve stimulation prevents reperfusion injury through inhibition of opening of mitochondrial permeability transition pore independent of the bradycardic effect. *J Thorac Cardiovasc Surg* 2009; **137**: 223-231 [PMID: 19154929 DOI: 10.1016/j.jtcvs.2008.08.020]

57   **Gourine A**, Gourine AV, Mastistkaya S, Ackland G. "Remote preconditioning reflex"- a neural pathway of cardioprotection during myocardial ischaemia and reperfusion induced by remote ischaemic preconditioning. *Eur Heart J* 2010; **31**: 319

58   **Mastitskaya S**, Marina N, Gourine A, Gilbey MP, Spyer KM, Teschemacher AG, Kasparov S, Trapp S, Ackland GL, Gourine AV. Cardioprotection evoked by remote ischaemic preconditioning is critically dependent on the activity of vagal pre-ganglionic neurones. *Cardiovasc Res* 2012; **95**: 487-494 [PMID: 22739118 DOI: 10.1093/cvr/cvs212]

59   **Basalay M**, Barsukevich V, Mastitskaya S, Mrochek A, Pernow J, Sjöquist PO, Ackland GL, Gourine AV, Gourine A. Remote ischaemic pre- and delayed postconditioning - similar degree of cardioprotection but distinct mechanisms. *Exp Physiol* 2012; **97**: 908-917 [PMID: 22427438 DOI: 10.1113/expphysiol.2012.064923]

60   **Loukogeorgakis SP**, Panagiotidou AT, Broadhead MW, Donald A, Deanfield JE, MacAllister RJ. 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**: 450-456 [PMID: 16053957 DOI: 10.1016/j.jacc.2005.04.044]

61   **Enko K**, Nakamura K, Yunoki K, Miyoshi T, Akagi S, Yoshida M, Toh N, Sangawa M, Nishii N, Nagase S, Kohno K, Morita H, Kusano KF, Ito H. Intermittent arm ischemia induces vasodilatation of the contralateral upper limb. *J Physiol Sci* 2011; **61**: 507-513 [PMID: 21901641 DOI: 10.1007/s12576-011-0172-9]

62   **Andreka G**, Vertesaljai M, Szantho G, Font G, Piroth Z, Fontos G, Juhasz ED, Szekely L, Szelid Z, Turner MS, Ashrafian H, Frenneaux MP, Andreka P. Remote ischaemic postconditioning protects the heart during acute myocardial infarction in pigs. *Heart* 2007; **93**: 749-752 [PMID: 17449499 DOI: 10.1136/hrt.2006.114504]

63   **Gritsopoulos G**, Iliodromitis EK, Zoga A, Farmakis D, Demerouti E, Papalois A, Paraskevaidis IA, Kremastinos DT. Remote postconditioning is more potent than classic postconditioning in reducing the infarct size in anesthetized rabbits. *Cardiovasc Drugs Ther* 2009; **23**: 193-198 [PMID: 19255833 DOI: 10.1007/s10557-009-6168-5]

64   **Schömig A**. Catecholamines in myocardial ischemia. Systemic and cardiac release. *Circulation* 1990; **82**: II13-II22 [PMID: 2203558]

65   **Roberts R**, Rogers WJ, Mueller HS, Lambrew CT, Diver DJ, Smith HC, Willerson JT, Knatterud GL, Forman S, Passamani E. Immediate versus deferred beta-blockade following thrombolytic therapy in patients with acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) II-B Study. *Circulation* 1991; **83**: 422-437 [PMID: 1671346 DOI: 10.1161/01.CIR.83.2.422]

66   **Ibanez B**, Macaya C, Sánchez-Brunete V, Pizarro G, Fernández-Friera L, Mateos A, Fernández-Ortiz A, García-Ruiz JM, García-

Álvarez A, Iñiguez A, Jiménez-Borreguero J, López-Romero P, Fernández-Jiménez R, Goicolea J, Ruiz-Mateos B, Bastante T, Arias M, Iglesias-Vázquez JA, Rodriguez MD, Escalera N, Acebal C, Cabrera JA, Valenciano J, Pérez de Prado A, Fernández-Campos MJ, Casado I, García-Rubira JC, García-Prieto J, Sanz-Rosa D, Cuellas C, Hernández-Antolín R, Albarrán A, Fernández-Vázquez F, de la Torre-Hernández JM, Pocock S, Sanz G, Fuster V. 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**: 1495-1503 [PMID: 24002794 DOI: 10.1161/CIRCULATIONAHA.113.003653]

67   **Kolh P**, Windecker S, Alfonso F, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head SJ, Jüni P, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter DJ, Schauerte P, Sousa Uva M, Stefanini GG, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol Ç, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, Sousa Uva M, Achenbach S, Pepper J, Anyanwu A, Badimon L, Bauersachs J, Baumbach A, Beygui F, Bonaros N, De Carlo M, Deaton C, Dobrev D, Dunning J, Eeckhout E, Gielen S, Hasdai D, Kirchhof P, Luckraz H, Mahrholdt H, Montalescot G, Paparella D, Rastan AJ, Sanmartin M, Sergeant P, Silber S, Tamargo J, ten Berg J, Thiele H, van Geuns RJ, Wagner HO, Wassmann S, Wendler O, Zamorano JL. 2014 ESC/EACTS Guidelines on myocardial revascularization: the Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). *Eur J Cardiothorac Surg* 2014; **46**: 517-592 [PMID: 25173601 DOI: 10.1093/eurheartj/ehu278]

68   **Thygesen K**, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Thygesen K, Alpert JS, White HD, Jaffe AS, Katus HA, Apple FS, Lindahl B, Morrow DA, Chaitman BR, Clemmensen PM, Johanson P, Hod H, Underwood R, Bax JJ, Bonow JJ, Pinto F, Gibbons RJ, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasche P, Ravkilde J, Ohman EM, Antman EM, Wallentin LC, Armstrong PW, Simoons ML, Januzzi JL, Nieminen MS, Gheorghiade M, Filippatos G, Luepker RV, Fortmann SP, Rosamond WD, Levy D, Wood D, Smith SC, Hu D, Lopez-Sendon JL, Robertson RM, Weaver D, Tendera M, Bove AA, Parkhomenko AN, Vasilieva EJ, Mendis S, Bax JJ, Baumgartner H, Ceconi C, Dean V, Deaton C, Fagard R, Funck-Brentano C, Hasdai D, Hoes A, Kirchhof P, Knuuti J, Kolh P, McDonagh T, Moulin C, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Torbicki A, Vahanian A, Windecker S, Morais J, Aguiar C, Almahmeed W, Arnar DO, Barili F, Bloch KD, Bolger AF, Botker HE, Bozkurt B, Bugiardini R, Cannon C, de Lemos J, Eberli FR, Escobar E, Hlatky M, James S, Kern KB, Moliterno DJ, Mueller C, Neskovic AN, Pieske BM, Schulman SP, Storey RF, Taubert KA, Vranckx P, Wagner DR. Third universal definition of myocardial infarction. *J Am Coll Cardiol* 2012; **60**: 1581-1598 [PMID: 22958960 DOI: 10.1016/j.jacc.2012.08.001]

69   **Pei H**, Wu Y, Wei Y, Yang Y, Teng S, Zhang H. Remote ischemic preconditioning reduces perioperative cardiac and renal events in patients undergoing elective coronary intervention: a meta-analysis of 11 randomized trials. *PLoS One* 2014; **9**: e115500 [PMID: 25551671 DOI: 10.1371/journal.pone.0115500]

70   **Wang X**, Yan J, Li L, Su Q. The effect of remote ischemic preconditioning in patients undergoing elective percutaneous coronary intervention: a systematic review and meta-analysis of randomized controlled trials. *Exp Clin Cardiol* 2014; **20**: 1411-1435

71   **Zografos TA**, Katritsis GD, Katritsis DG. Remote ischemic preconditioning reduces peri-procedural myocardial injury in elective percutaneous coronary intervention: a meta-analysis. *Int J Cardiol* 2014; **173**: 530-532 [PMID: 24681008 DOI: 10.1016/j.ijcard.2014.03.026]

72   **D'Ascenzo F**, Moretti C, Omedè P, Cerrato E, Cavallero E, Er F, Presutti DG, Colombo F, Crimi G, Conrotto F, Dinicolantonio JJ, Chen S, Prasad A, Biondi Zoccai G, Gaita F. Cardiac remote ischaemic preconditioning reduces periprocedural myocardial infarction for patients undergoing percutaneous coronary interventions: a meta-analysis of randomised clinical trials. *EuroIntervention* 2014; **9**: 1463-1471 [PMID: 24755386 DOI: 10.4244/EIJV9I12A244]

73   **Prasad A**, Gössl M, Hoyt J, Lennon RJ, Polk L, Simari R, Holmes DR, Rihal CS, Lerman A. 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**: 930-936 [PMID: 22517646 DOI: 10.1002/ccd.24443]

74   **Hoole SP**, Heck PM, Sharples L, Khan SN, Duehmke R, Densem CG, Clarke SC, Shapiro LM, Schofield PM, O'Sullivan M, Dutka DP. Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study: a prospective, randomized control trial. *Circulation* 2009; **119**: 820-827 [PMID: 19188504 DOI: 10.1161/CIRCULATIONAHA.108.809723]

75   **Davies WR**, Brown AJ, Watson W, McCormick LM, West NE, Dutka DP, Hoole SP. 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**: 246-251 [PMID: 23696599 DOI: 10.1161/CIRCINTERVENTIONS.112.000184]

76   **Ahmed RM**, Mohamed el-HA, Ashraf M, Maithili S, Nabil F, Rami R, Mohamed TI. Effect of remote ischemic preconditioning on serum troponin T level following elective percutaneous coronary intervention. *Catheter Cardiovasc Interv* 2013; **82**: E647-E653 [PMID: 23404916 DOI: 10.1002/ccd.24825]

77   **Ghaemian A**, Nouraei SM, Abdollahian F, Naghshvar F, Giussani DA, Nouraei SA. Remote ischemic preconditioning in percutaneous coronary revascularization: a double-blind randomized controlled clinical trial. *Asian Cardiovasc Thorac Ann* 2012; **20**: 548-554 [PMID: 23087298 DOI: 10.1177/0218492312439999]

78   **Luo SJ**, Zhou YJ, Shi DM, Ge HL, Wang JL, Liu RF. Remote ischemic preconditioning reduces myocardial injury in patients undergoing coronary stent implantation. *Can J Cardiol* 2013; **29**: 1084-1089 [PMID: 23414904 DOI: 10.1016/j.cjca.2012.11.022]

79   **Xu X**, Zhou Y, Luo S, Zhang W, Zhao Y, Yu M, Ma Q, Gao F, Shen H, Zhang J. Effect of remote ischemic preconditioning in the elderly patients with coronary artery disease with diabetes mellitus undergoing elective drug-eluting stent implantation. *Angiology* 2014; **65**: 660-666 [PMID: 24163121 DOI: 10.1177/0003319713507332]

80   **Zografos T**, Katritsis G, Korovesis S, Giazitzoglou E, Katrit-sis D. Remote ischemic preconditioning is feasible in ad hoc percutaneous coronary interventions. *Eur Heart J* 2013; **34** (Suppl 1)

81   **Bøtker HE**, Kharbanda R, Schmidt MR, Bøttcher M, Kaltoft AK, Terkelsen CJ, Munk K, Andersen NH, Hansen TM, Trautner S, Lassen JF, Christiansen EH, Krusell LR, Kristensen SD, Thuesen L, Nielsen SS, Rehling M, Sørensen HT, Redington AN, Nielsen TT. 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**: 727-734 [PMID: 20189026 DOI: 10.1016/S0140-6736(09)62001-8]

82   **Sloth AD**, Schmidt MR, Munk K, Kharbanda RK, Redington AN, Schmidt M, Pedersen L, Sørensen HT, Bøtker HE. 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**: 168-175 [PMID: 24031025 DOI: 10.1093/eurheartj/eht369]

83   **Carrasco-Chinchilla F**, Muñoz-García AJ, Domínguez-Franco A, Millán-Vázquez G, Guerrero-Molina A, Ortiz-García C, Enguix-Armada A, Alonso-Briales JH, Hernández-García JM, de Teresa-Galván E, Jiménez-Navarro MF. Remote ischaemic postconditioning: does it protect

against ischaemic damage in percutaneous coronary revascularisation? Randomised placebo-controlled clinical trial. *Heart* 2013; **99**: 1431-1437 [PMID: 23850844 DOI: 10.1136/heartjnl-2013-304172]

84 **Lavi S**, D'Alfonso S, Diamantouros P, Camuglia A, Garg P, Teefy P, Jablonsky G, Sridhar K, Lavi R. Remote ischemic postconditioning during percutaneous coronary interventions: remote ischemic postconditioning-percutaneous coronary intervention randomized trial. *Circ Cardiovasc Interv* 2014; **7**: 225-232 [PMID: 24692535 DOI: 10.1161/CIRCINTERVENTIONS.114.001591]

85 **Crimi G**, Ferlini M, Gallo F, Sormani MP, Raineri C, Bramucci E, De Ferrari GM, Pica S, Marinoni B, Repetto A, Raisaro A, Leonardi S, Rubartelli P, Visconti LO, Ferrario M. 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**: 500-502 [PMID: 25183541 DOI: 10.1016/j.ijcard.2014.08.080]

86 **Deftereos S**, Giannopoulos G, Tzalamouras V, Raisakis K, Kossyvakis C, Kaoukis A, Panagopoulou V, Karageorgiou S, Avramides D, Toutouzas K, Hahalis G, Pyrgakis V, Manolis AS, Alexopoulos D, Stefanadis C, Cleman MW. Renoprotective effect of remote ischemic post-conditioning by intermittent balloon inflations in patients undergoing percutaneous coronary intervention. *J Am Coll Cardiol* 2013; **61**: 1949-1955 [PMID: 23500314 DOI: 10.1016/j.jacc.2013.02.023]

87 **Windecker S**, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head SJ, Jüni P, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter DJ, Schauerte P, Sousa Uva M, Stefanini GG, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). *Eur Heart J* 2014; **35**: 2541-2619 [PMID: 25173339]

88 **Hausenloy DJ**, Mwamure PK, Venugopal V, Harris J, Barnard M, Grundy E, Ashley E, Vichare S, Di Salvo C, Kolvekar S, Hayward M, Keogh B, MacAllister RJ, Yellon DM. Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *Lancet* 2007; **370**: 575-579 [PMID: 17707752 DOI: 10.1016/S0140-6736(07)61296-3]

89 **Venugopal V**, Hausenloy DJ, Ludman A, Di Salvo C, Kolvekar S, Yap J, Lawrence D, Bognolo J, Yellon DM. Remote ischaemic preconditioning reduces myocardial injury in patients undergoing cardiac surgery with cold-blood cardioplegia: a randomised controlled trial. *Heart* 2009; **95**: 1567-1571 [PMID: 19508973 DOI: 10.1136/hrt.2008.155770]

90 **Wagner R**, Piler P, Bedanova H, Adamek P, Grodecka L, Freiberger T. 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**: 758-762 [PMID: 20847065 DOI: 10.1510/icvts.2010.243600]

91 **Ali N**, Rizwi F, Iqbal A, Rashid A. Induced remote ischemic pre-conditioning on ischemia-reperfusion injury in patients undergoing coronary artery bypass. *J Coll Physicians Surg Pak* 2010; **20**: 427-431 [PMID: 20642939]

92 **Karuppasamy P**, Chaubey S, Dew T, Musto R, Sherwood R, Desai J, John L, Shah AM, Marber MS, Kunst G. Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and inflammation? *Basic Res Cardiol* 2011; **106**: 511-519 [PMID: 21544683 DOI: 10.1007/s00395-011-0185-9]

93 **Lomivorotov VV**, Shmyrev VA, Nepomnyaschih VA, Ponomarev DN, Knyazkova LG, Lomivorotov VN, Karaskov AM. Remote ischaemic preconditioning does not protect the heart in patients undergoing coronary artery bypass grafting. *Interact Cardiovasc Thorac Surg* 2012; **15**: 18-22 [PMID: 22493101 DOI: 10.1093/icvts/ivs118]

94 **Lucchinetti E**, Bestmann L, Feng J, Freidank H, Clanachan AS, Finegan BA, Zaugg M. 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**: 296-310 [PMID: 22222469 DOI: 10.1097/ALN.0b013e318242349a]

95 **Rahman IA**, Mascaro JG, Steeds RP, Frenneaux MP, Nightingale P, Gosling P, Townsend P, Townend JN, Green D, Bonser RS. Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment? *Circulation* 2010; **122**: S53-S59 [PMID: 20837926 DOI: 10.1161/CIRCULATIONAHA.109.926667]

96 **Ang R**. Highlights in basic autonomic neurosciences: remote ischaemic preconditioning as an autonomic reflex--a question of timing and circumstances? *Auton Neurosci* 2013; **173**: 1-2 [PMID: 23159165 DOI: 10.1016/j.autneu.2012.11.001]

97 **Zhou C**, Liu Y, Yao Y, Zhou S, Fang N, Wang W, Li L. β-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* 2013; **27**: 305-311 [PMID: 23276595 DOI: 10.1053/j.jvca.2012.09.028]

98 **Yu CH**, Beattie WS. The effects of volatile anesthetics on cardiac ischemic complications and mortality in CABG: a meta-analysis. *Can J Anaesth* 2006; **53**: 906-918 [PMID: 16960269 DOI: 10.1007/BF03022834]

99 **D'Ascenzo F**, Cavallero E, Moretti C, Omedè P, Sciuto F, Rahman IA, Bonser RS, Yunseok J, Wagner R, Freiberger T, Kunst G, Marber MS, Thielmann M, Ji B, Amr YM, Modena MG, Zoccai GB, Sheiban I, Gaita F. Remote ischaemic preconditioning in coronary artery bypass surgery: a meta-analysis. *Heart* 2012; **98**: 1267-1271 [PMID: 22875822 DOI: 10.1136/heartjnl-2011-301551]

100 **Spear JF**, Prabu SK, Galati D, Raza H, Anandatheerthavarada HK, Avadhani NG. beta1-Adrenoreceptor activation contributes to ischemia-reperfusion damage as well as playing a role in ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2007; **292**: H2459-H2466 [PMID: 17237252 DOI: 10.1152/ajpheart.00459.2006]

101 **Lange M**, Smul TM, Blomeyer CA, Redel A, Klotz KN, Roewer N, Kehl F. Role of the beta1-adrenergic pathway in anesthetic and ischemic preconditioning against myocardial infarction in the rabbit heart in vivo. *Anesthesiology* 2006; **105**: 503-510 [PMID: 16931983 DOI: 10.1097/00000542-200609000-00014]

102 **Thielmann M**, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, Price V, Tsagakis K, Neuhäuser M, Peters J, Jakob H, Heusch G. 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 [PMID: 23953384 DOI: 10.1016/S0140-6736(13)61450-6]

103 **Haji Mohd Yasin NA**, Herbison P, Saxena P, Praporski S, Konstantinov IE. The role of remote ischemic preconditioning in organ protection after cardiac surgery: a meta-analysis. *J Surg Res* 2014; **186**: 207-216 [PMID: 24135377 DOI: 10.1016/j.jss.2013.09.006]

104 **Yang L**, Wang G, Du Y, Ji B, Zheng Z. Remote ischemic preconditioning reduces cardiac troponin I release in cardiac surgery: a meta-analysis. *J Cardiothorac Vasc Anesth* 2014; **28**: 682-689 [PMID: 24103716 DOI: 10.1053/j.jvca.2013.05.035]

105 **Healy DA**, Khan WA, Wong CS, Moloney MC, Grace PA, Coffey JC, Dunne C, Walsh SR, Sadat U, Gaunt ME, Chen S, Tehrani S, Hausenloy DJ, Yellon DM, Kramer RS, Zimmerman RF, Lomivorotov VV, Shmyrev VA, Ponomarev DN, Rahman IA, Mascaro JG, Bonser RS, Jeon Y, Hong DM, Wagner R, Thielmann M, Heusch G, Zacharowski K, Meybohm P, Bein B, Tang TY. Remote preconditioning and major clinical complications following adult cardiovascular surgery: systematic review and meta-analysis. *Int J Cardiol* 2014; **176**: 20-31 [PMID: 25022819 DOI: 10.1016/j.ijcard.2014.06.018]

106 **Wu Q**, Gui P, Wu J, Ding D, Purusram G, Dong N, Yao S. Effect of limb ischemic preconditioning on myocardial injury in patients undergoing mitral valve replacement surgery. -A randomized controlled trial-. *Circ J* 2011; **75**: 1885-1889 [PMID: 21697609 DOI: 10.1253/circj.CJ-10-1130]

107 **Xie JJ**, Liao XL, Chen WG, Huang DD, Chang FJ, Chen W, Luo ZL, Wang ZP, Ou JS. Remote ischaemic preconditioning reduces myocardial injury in patients undergoing heart valve surgery: randomised controlled trial. *Heart* 2012; **98**: 384-388 [PMID: 22107759 DOI: 10.1136/heartjnl-2011-300860]

108 **Ovize M**, Bonnefoy E. Giving the ischaemic heart a shot in the arm. *Lancet* 2010; **375**: 699-700 [PMID: 20189010 DOI: 10.1016/S0140-6736(09)62156-5]

Baishideng®