

Ischemic conditioning: the challenge of protecting the diabetic heart

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Abstract: The successful clinical translation of novel therapeutic strategies to attenuate lethal myocardial ischemia-reperfusion injury and limit infarct size has been identified as a major unmet need, and is of particular importance in patients with type-2 diabetes. There is a wealth of preclinical evidence that ischemic conditioning (encompassing the three paradigms of preconditioning, postconditioning and remote conditioning) is profoundly cardioprotective and, via up-regulation of endogenous signaling cascades, renders the heart resistant to infarction. However, current phase II trials aimed at exploiting ischemic conditioning for the clinical treatment of myocardial ischemia-reperfusion injury have yielded mixed results, possibly reflecting the emerging concern that the efficacy of conditioning-induced cardioprotection may be compromised in the diabetic heart. Our goal in this review is to provide a summary of our present understanding of the effect of type-2 diabetes on the infarct-sparing effect of ischemic conditioning, and the challenges of limiting ischemia-reperfusion injury in the diabetic heart.

Keywords: Myocardial infarction; diabetes; preconditioning; postconditioning; remote conditioning

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Cardiovascular disease (CVD) remains the leading cause of death in the United States and globally, with acute myocardial infarction (AMI) accounting for a substantial portion of CVD-associated mortality (1). Timely reperfusion is the standard clinical therapy for AMI and, although necessary to rescue ischemic tissue, restoration of blood flow can paradoxically exacerbate cell death (rather than initiate salvage) in populations of ischemic myocytes, a phenomenon termed lethal ischemia-reperfusion (I/R) injury (2-6). The volume of myocardium rendered necrotic following I/R (i.e., myocardial infarct size) is a primary determinant of mortality and morbidity associated with AMI (6-8). Indeed, decades of preclinical and clinical investigation have been devoted to: (I) elucidating the mechanisms of I/R injury, and (II) developing mechanisms-based therapies to augment the benefits of early reperfusion and reduce myocardial infarct size. Despite this substantial investment of time and resources, no advances have, to date, been successfully translated into clinical practice (8-10).

In 1986, Murry *et al.* made the landmark observation

that the heart could be ‘preconditioned’ or rendered resistant to lethal I/R injury, by exposure to a brief and non-lethal, antecedent ischemic insult (11). Subsequent studies expanded the paradigm of myocardial ‘conditioning’ beyond the phenomenon of ischemic preconditioning to encompass postconditioning and remote conditioning (5,12,13). Overwhelming experimental evidence, obtained in multiple models and species, has demonstrated that all three forms of myocardial conditioning induce potent cardioprotection (5,6,9). However, the vast majority (>90%) of these studies have been conducted using healthy, juvenile or adult populations that do not manifest the risk factors and comorbid conditions typically seen in patients with CVD and suffering AMI (14). A major, well-established independent risk factor for CVD and AMI, the prevalence of which has doubled over the past 20 years, is type-2 diabetes (1,15,16), and there is emerging concern that the efficacy of conditioning-induced cardioprotection may be compromised in the diabetic heart (4,14,17-20). Our aim in the current review is to focus on this important issue:

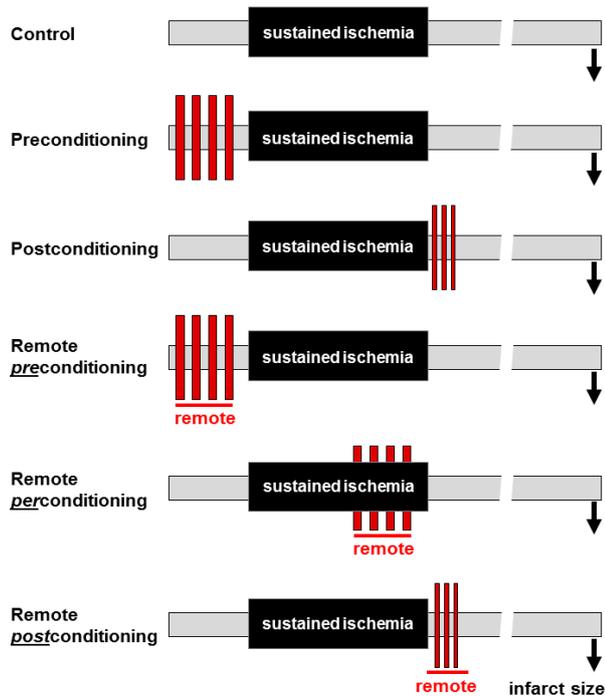


Figure 1 Schematic diagram illustrating the key temporal aspects of preconditioning, postconditioning, and remote pre-, per- and postconditioning. Red boxes denote the conditioning stimulus. Adapted from references (5,14).

we provide a synopsis of our present understanding of the effect of type-2 diabetes on the infarct-sparing effect of ischemic conditioning, and the challenges of limiting I/R injury in the diabetic heart.

What is ischemic conditioning?

Definitions and basic concepts

Ischemic conditioning renders the myocardium resistant to I/R injury via the up-regulation of as-yet incompletely understood, endogenous cardioprotective signaling cascades (5,6). There are three permutations of conditioning that differ in terms of the site and timing of the brief ischemic stimulus (*Figure 1*) (5). Classic ischemic preconditioning, as first described by Murry and colleagues (11), is initiated by subjecting the heart to 2-4 repeated episodes of brief (2-5 minutes) ischemia, interrupted by intervening 5-minute periods of reperfusion, before the onset of a sustained ischemic insult. By contrast, postconditioning is not a pre-ischemic intervention but, rather, as described in the seminal publication by Zhao *et al.* (12), is a form of modified

reperfusion, i.e., blood flow to the ischemic myocardium is restored in a stuttered or staccato manner, with a typical algorithm of 3-6 cycles of (10-30 seconds of reperfusion interspersed with 10-30 seconds periods of re-occlusion), before establishing full and complete reperfusion. For both preconditioning and postconditioning, the protective stimulus (brief antecedent ischemia or stuttered reflow) is applied in heart, at the same site and in the same vascular territory as the sustained period of ischemia. Remote conditioning differs importantly from both of the aforementioned strategies in that cardioprotection is evoked by brief ischemia applied at a distant site, such as a different and distinct myocardial vascular bed [as first reported by Przyklenk *et al.* (13)] or a remote and less vulnerable tissue or organ such as skeletal muscle (21). With regard to timing, protection with remote conditioning can be achieved when the stimulus is applied before sustained coronary occlusion, during the sustained ischemic insult, or at the time of reperfusion (remote pre-, per- or postconditioning, respectively; *Figure 1*) (5).

Reduction of infarct size: the hallmark of ischemic conditioning

Despite these temporal and spatial differences, all three facets of ischemic conditioning share a common and overarching theme: all are profoundly effective in reducing myocardial infarct size beyond that achieved by reperfusion alone (*Figure 2*) (5,6). Conditioning-induced cardioprotection has, with rare exceptions, been documented and confirmed in countless laboratories and in all models and species that have been tested. There are reports that the benefits of ischemic conditioning (in particular, preconditioning and postconditioning) may extend to other pathophysiologic aspects of I/R injury, including postischemic myocardial ‘stunning’, arrhythmias, microvascular damage and endothelial dysfunction (18,23,24). However, there is no question that the most robust endpoint for the assessment of cardioprotection is infarct size, and the established hallmark of ischemic conditioning is its infarct-sparing effect (5,6).

Cellular mechanisms: the major players

In addition to sharing a common primary endpoint—reduction of infarct size—the three facets of ischemic conditioning also appear to share common elements in terms of cellular mechanisms. Not surprisingly, the greatest

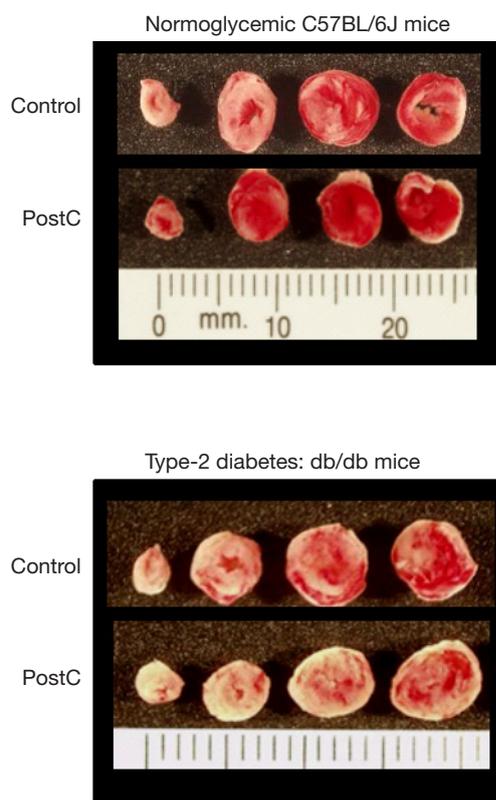


Figure 2 Original images of control and postconditioned (PostC) mouse hearts. Hearts were cut into transverse slices and stained with triphenyltetrazolium chloride; using this method, viable myocardium is stained red, whereas necrotic myocardium remains unstained so appears pale. Infarct size was reduced with PostC in normoglycemic C57BL/6J mice. In contrast, in hearts from db/db mice (model of type-2 diabetes), infarct sizes were comparable in control and PostC groups. Reprinted with permission from reference (22).

insight has been gained into the mechanisms of ischemic preconditioning [comprehensive reviews provided in (4,5,24-27)]. There is a consensus that preconditioning is initiated through ligand-receptor interactions including, most notably, stimulation of G_i -protein coupled receptors. The archetypal trigger for preconditioning, first described in 1991, is release of adenosine from myocardium rendered ischemic during the brief antecedent preconditioning stimulus and binding to adenosine A_1 or A_3 receptors on the cardiomyocyte membranes (28,29). In the ensuing years, redundancies in the ligands capable of triggering preconditioning via binding to their respective receptors were identified, including (but not limited to) bradykinin,

opioids, acetylcholine and $TNF-\alpha$ (30-33). Ligand-receptor binding subsequently activates multiple signaling cascades in a complex, biphasic and possibly redundant manner, following the general paradigm of: (I) initial up-regulation of phosphatidylinositol 3-kinase (PI3 kinase)/Akt, nitric oxide-mediated activation of protein kinase G (PKG) and subsequent activation of the ϵ isoform of protein kinase C (PKC) during the early minutes of sustained ischemia; and (II) receptor re-population and up-regulation of the so-called reperfusion injury salvage kinase (RISK) and/or survival activating factor enhancement (SAFE) pathways during the early seconds-minutes following restoration of blood flow (27,34-36). There appears to be minimal overlap or intersection between these latter two reperfusion-associated signaling cascades: key components of the RISK pathway include PI3 kinase/Akt, extracellular signal regulated kinase (ERK), p70S6 kinase and glycogen synthase kinase 3β (GSK- 3β), while the pivotal constituents of the SAFE pathway are janus activated kinase (JAK) and signal transducer and activator of transcription (STAT) (4,5,24-27,35,36). Nonetheless, both pathways converge on the mitochondria, the proposed end-effector of ischemic preconditioning, with specific molecular targets including the mitochondrial adenosine triphosphate-sensitive potassium (K_{ATP}) channel, mitochondrial connexin 43, and, most notably, the mitochondrial permeability transition pore (mPTP) (25,37-40). Cardioprotection is purportedly conferred by a resultant stabilization of mitochondrial membranes (including suppression of mPTP opening) and better maintenance of mitochondrial integrity (4,5,24-27).

The discovery of infarct size reduction with postconditioning, and observations of a comparable magnitude of cardioprotection with both preconditioning and postconditioning, provided compelling and provocative evidence that pretreatment—and up-regulation of kinase signaling at the onset of ischemia—is not required to render the heart resistant to I/R injury (12). Moreover, the lack of an additive effect of combined administration of preconditioning + postconditioning suggests that common (or redundant) reperfusion-associated mechanisms may underlie the infarct-sparing effect of the two interventions (12). Indeed, receptor-mediated up-regulation of the RISK and/or SAFE pathways, culminating in stabilization of mitochondria (with an emphasis on inhibition of mPTP opening) are hypothesized to play critical roles in the reduction of infarct size achieved with postconditioning (4,5,18,25,27,41-43).

Perhaps not surprisingly, the three common themes of G-protein coupled receptor stimulation on myocyte

membranes, activation of multiple kinases including members of the RISK and/or SAFE pathways, and mitochondria as end-effectors have also been implicated as key mechanistic components of remote conditioning [reviewed in (5,44-47)]. There is, however, an inherently unique aspect of remote conditioning not shared by pre- and postconditioning: the cardioprotective signaling cascades are initiated by communication or transfer of a protective signal from the site of the conditioning stimulus to the heart. Details concerning the identity of the signal(s) and mode of communication remain elusive, but two leading theories are under investigation: remote conditioning may be triggered by blood- or perfusate-borne transport of one or more unknown humoral factors (possibly including a small, <15 kDa hydrophobic molecule) (48-50), and communication via neuronal stimulation (5,44-52). Of note, these two hypotheses are not mutually exclusive, are in all likelihood model-dependent, and, in at least some models, both humoral and neuronal communication may be involved (5,44,45,53,54).

Ancillary and alternative mediators of conditioning-induced cardioprotection

As summarized in the preceding paragraphs, intensive interest and attention has focused on the involvement of RISK and SAFE signaling in the infarct-sparing effect of ischemic pre-, post- and remote conditioning. However, additional and less well-characterized mediators have also been postulated to contribute to conditioning-induced cardioprotection, either in concert with or as possible alternatives to the RISK and SAFE cascades. For example, isoforms of PKC (in particular, PKC ϵ) may play a broader role in conditioning-induced cardioprotection, beyond the well-described early activation following brief preconditioning ischemia: PKC has been implicated as a component of kinase signaling initiated in response to both postconditioning and remote preconditioning (26,55-59). Generation of low, sub-lethal levels of reactive oxygen species (ROS) and signaling via nitric oxide have, similarly, been proposed to integrate with both PKC and the RISK and STAT pathways as mediators of pre- and post-conditioning (27,56,57,59-61).

A long-standing concept that may be relevant to the issue of cardioprotection in diabetic cohorts, particularly for ischemic preconditioning, is that alterations in myocardial metabolism play a causal role. Metabolic hallmarks of cardiac ischemia include the rapid (within seconds-

minutes) shift from aerobic to anaerobic metabolism and the resultant, progressive temporal decline in myocardial ATP concentration as ATP synthesis via anaerobic glycolysis is insufficient to meet the diminished, residual energy consumption of the ischemic tissue (62). The first report of infarct size reduction with preconditioning was accompanied by evidence of an increase in metabolic efficiency: i.e., preconditioning slowed myocardial energy demand during the subsequent period of sustained ischemia, thereby attenuating the rate of ATP utilization and reducing the rate of anaerobic glycolysis (11,63,64). Ensuing studies argued against the concept of a cause-and-effect relationship between reduced energy demand during prolonged ischemia and infarct size reduction with preconditioning (65). Nonetheless, there is evidence for a mechanistic link between metabolism and preconditioning-induced cardioprotection. Ischemic preconditioning is associated with an increase in glucose uptake during sustained ischemia, an effect that has been attributed to: (I) co-activation of Akt and AMP-activated protein kinase (AMPK: the 'metabolic master switch' that responds to ATP depletion and an increase in the ratio of AMP/ATP); (II) translocation of the glucose transporter protein GLUT4 to the cardiomyocyte surface; and (III) subcellular redistribution of hexokinase to mitochondria, where it phosphorylates and facilitates sequestration of glucose (66-73). This metabolic signaling is purportedly required for the infarct-sparing effect of ischemic preconditioning (66-70), and has been implicated to play a secondary role in postconditioning (74).

Finally, there are intriguing but as-yet largely unexplored mediators that (I) have been reported to play a role in myocardial I/R injury; and (II) appear to have mechanistic links with both hyperglycemia/diabetes and ischemic conditioning. For example, p66Shc is a pro-oxidant protein, reportedly associated with the mPTP, which has been proposed to serve as a nexus for the deleterious effects of hyperglycemia and obesity-induced impairment in molecular signaling in multiple cell types including cardiomyocytes (75-77). Moreover, there is evidence that genetic knockout of p66Shc renders the heart resistant to infarction, possibly via attenuation of mitochondrial ROS production (78). A second molecular strategy that appears to mimic the favorable effects of ischemic conditioning—and, indeed, has been implicated to contribute to the infarct-sparing effect of remote preconditioning—is inhibition of arginase 2 signaling and the accompanying increase in nitric oxide production, activation of PKC ϵ and targeting

of the mitochondrial K_{ATP} channel (79-81). Interestingly, recent data have revealed that remote preconditioning fails to inhibit arginase 2 signaling in a rat model of type-1 diabetes (81). Whether a similar response is seen in the setting of type-2 diabetes is, at present, unknown.

Cardiac consequences of diabetes

The big picture: diabetes, CVD and AMI

The successful clinical translation of novel interventions to attenuate myocardial I/R injury has been identified as a major unmet need (8-10). This issue is of particular relevance and importance to patients with type-2 diabetes, as underscored by: (I) the ≥ 2 -fold greater incidence of CVD, acute coronary syndromes and AMI in this patient cohort; (II) evidence of larger infarct sizes and exacerbated necrotic and apoptotic cell death; and thus, perhaps not surprisingly; (III) a 2- to 4-fold greater incidence of CVD-related mortality in diabetic versus non-diabetic subjects (1,82-93). Indeed, CVD is the leading cause of death and disability among diabetics, a poor prognosis that has not been appreciably influenced by the current trend of an overall reduction in mortality associated with AMI (1,16,82,83,92,93). Recent statistics reveal that, at present, Americans have an estimated ~40% lifetime risk for the development of diagnosed diabetes, and a sustained increase in the incidence of type-2 diabetes in the USA and worldwide is predicted for the next 20-30 years. This anticipated escalation in the numbers of patients with diabetes may have the potential to diminish the gains that have been made attenuating the overall prevalence of CVD-related death (1,94-96).

The cellular/molecular perspective

The hallmarks of type-2 diabetes are insulin resistance and accompanying metabolic dysregulation. Reduced insulin sensitivity has both direct effects on glucose uptake and insulin-mediated signaling in cardiac cells, and indirect cellular effects that are secondary to the accompanying hyperglycemia, hyperinsulinemia and hyperlipidemia (97-100). Most importantly in terms of cardioprotection, type-2 diabetes has been associated with impaired PI3 kinase/Akt signaling (components of both insulin signaling and the RISK pathway) and GLUT4 protein expression and/or translocation, as well as defects in AMPK and, indeed, essentially all kinases proposed to contribute to

the infarct-sparing effect of ischemic conditioning. For example, impaired phosphorylation of PKC, PI3 kinase/Akt, ERK, STAT3, and GSK-3 β has been described in diabetic hearts, possibly due to reported increases in activities of multiple phosphatases including phosphatase and tensin homolog (PTEN), MAPK phosphatases (MKPs) and protein phosphatase-2C (PP2C). There is evidence to suggest that downstream targets and proposed end-effectors of conditioning-induced cardioprotection are also modified by type-2 diabetes, including alterations in expression and activity of mitochondrial K_{ATP} channels and increased propensity of mPTP opening in response to increased intracellular Ca^{2+} concentrations in the diabetic myocardium (100-106). Although these insights have, not surprisingly, been largely derived from genetic rodent models of type-2 diabetes, including db/db and ob/ob mice and strains of fatty and lean rats (Zucker fatty, Otsuka Long-Evans-Tokushima fatty and Goto-Kakizaki) (100-104), analysis of myocardial tissue samples collected from diabetic patients at the time of cardiac surgery have yielded corroborative results (105,106).

Diabetes, infarct size and ischemic conditioning

Diabetes and I/R injury

The effect of type-2 diabetes on the infarct-sparing effect of ischemic conditioning has, almost exclusively, been assessed in the aforementioned rodent models. Accordingly, meaningful discussion of conditioning-induced cardioprotection in these models first requires an understanding of the effect of type-2 diabetes on infarct size in untreated control animals.

In contrast to the clinical consensus that diabetes is associated with larger infarct sizes and poor outcomes when compared with non-diabetic patients (1,82-93), data obtained in preclinical models are mixed: diabetes has been reported to increase, decrease or have no effect on cardiomyocyte death (19,107-109). These disparate outcomes have been attributed to multiple factors, including: (I) the duration of the diabetic state at the time of experimentation (with short-term diabetes more typically associated with an apparent reduced sensitivity to I/R injury); (II) differences over time or among models in levels of insulin and fatty acids; and (III) the presence or absence of obesity (19,108,109). The definitions of 'short-term' diabetes, hyperinsulinemia, hyperlipidemia, etc., and precise relationships of these factors with myocardial infarct size,

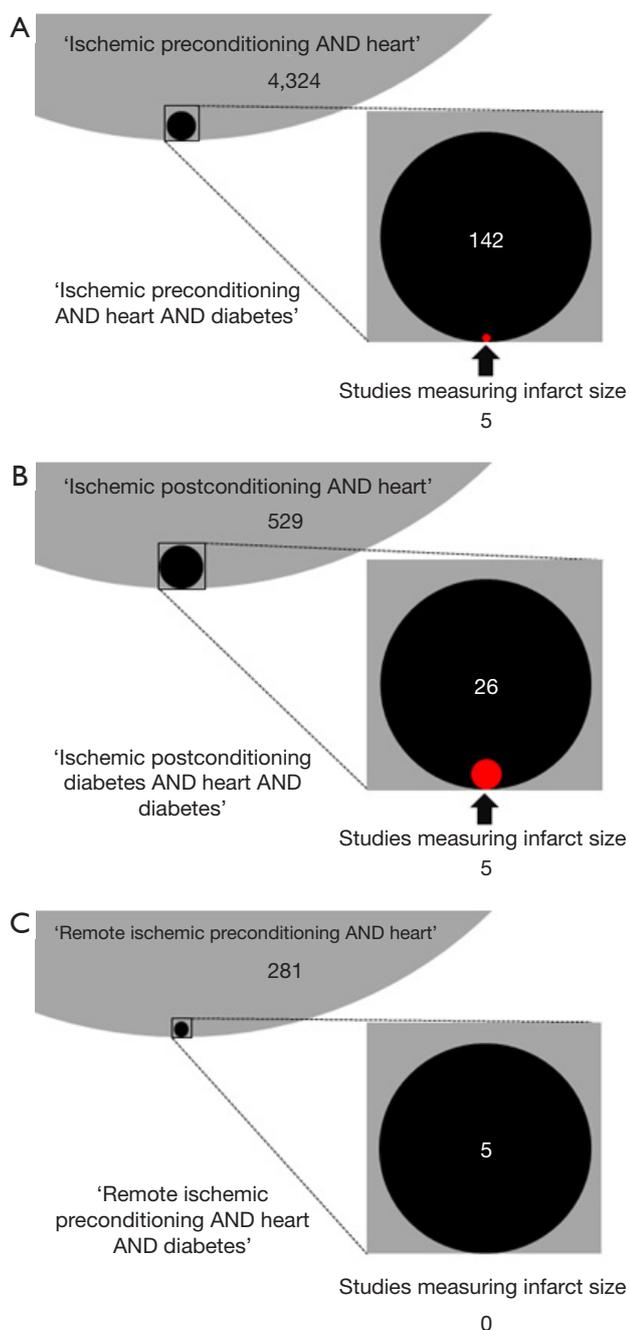


Figure 3 Schematic diagram underscoring the paucity of available data on the efficacy of ischemic conditioning in the setting of diabetes. PubMed queries were performed in September 2014 using the search terms 'ischemic preconditioning and heart' (A), 'postconditioning and heart' (B) and 'remote ischemic preconditioning and heart' (C), with and without the addition of the term 'diabetes'. Studies conducted in diabetic models in which infarct size (the gold standard of ischemic conditioning) was among the primary endpoints are highlighted.

are nebulous. However, it is important to emphasize: the translational relevance of experimental models characterized by a reduced sensitivity of the diabetic heart to I/R injury is considered to be questionable (108).

Ischemic conditioning in models of type-2 diabetes: is efficacy maintained?

A recent (September 2014) PubMed search retrieved >5,000 papers focused on ischemic pre-, post- and remote conditioning in heart (Figure 3). Remarkably, addition of the term 'diabetes' to each search yielded only 173 papers (3.4% of the total number of publications) and, among these, only 10 included myocardial infarct size (the 'gold standard' of conditioning-induced cardioprotection) as one of the endpoints (Figure 3). The fact that <0.2% of currently published studies have specifically investigated the infarct-sparing effect of ischemic conditioning in of type-2 diabetes is extraordinary given the well-documented, profound consequences of diabetes on cardiac pathophysiology.

Among the small number of studies that have addressed this issue, a spectrum of rat and mouse models of type-2 diabetes have been utilized (Table 1). Nonetheless, irrespective of the model used and variations in study design, there is an emerging consensus: both pre- and post-conditioning either fail to reduce infarct size (Figure 2) (22,104,110,112,114-116), or the efficacy of conditioning is attenuated such that an amplified stimulus (i.e., an increased number of episodes of preconditioning ischemia) is required to evoke protection (111,113,117). Loss of conditioning-induced cardioprotection has been described in both lean and obese type-2 diabetic rat models (110) and in obese rats before the onset of significant hyperglycemia (112). Moreover, similar outcomes (a diminished responsiveness to the infarct-sparing effect of ischemic conditioning) have also been reported in models of type-1 diabetes (14,17-19,22,69,79-81,118-126), suggesting that the refractoriness of the diabetic heart to ischemic conditioning is not a simple consequence of hyperglycemia, hyperinsulinemia, hyperlipidemia, obesity or, in all likelihood, any single pathophysiological feature of the disease. Nascent mechanistic insight has, however, been obtained into the cellular mechanisms that may contribute to the compromised efficacy of ischemic conditioning in diabetic models. Perhaps not surprisingly, the complete or partial failure of pre- and postconditioning to limit infarct size in models of type-2 (and type-1) diabetes has largely been attributed to defects in RISK and AMPK signaling, with

Table 1 Effect of type-2 diabetes on infarct size reduction with ischemic conditioning

Author (reference)	Model	Reduction of infarct size?	Comments/mechanistic insights?
Preconditioning			
Kristiansen (110)	Rat: Zucker fatty	No	Protection lost in both lean and obese models.
	Rat: Goto-Kakizaki	No	No mechanism proposed.
Tsang (111)	Rat: Goto-Kakizaki	Attenuated	Efficacy attenuated; amplified preconditioning stimulus required to achieve protection. Impaired Akt phosphorylation.
Katakam (112)	Rat: Zucker fatty	No	Protection lost in before development of hyperglycemia. Impaired activation of mitochondrial K_{ATP} channels.
Hausenloy (113)	Rat: Goto-Kakizaki	Attenuated	Efficacy attenuated; amplified preconditioning stimulus required to achieve protection. Co-administration of glimepimide restored the infarct-sparing effect of preconditioning, possibly by activation of Akt.
Whittington (114)	Rat: Goto-Kakizaki	Attenuated	Amplified preconditioning stimulus was protective in 3 and 8 month old rats; complete loss in efficacy in 12 and 18 month old rats.
		No	Impaired Akt phosphorylation due to chronic up-regulation.
Postconditioning			
Wagner (115)	Rat: WOKW	No	Impaired ERK, GSK-3- β phosphorylation
Bouhidel (104)	Mouse: ob/ob	No	Impaired Akt, ERK, p70S6 kinase, AMPK phosphorylation
Przyklenk (22)	Mouse: db/db	No	Impaired ERK phosphorylation
Zhu (116)	Mouse: db/db	No	Loss of protection associated with differential regulation of mitochondrial proteome
Oosterlinck (117)	Mouse: ob/ob	Attenuated	No mechanism proposed
Remote Conditioning			
No published studies			

desensitization or impaired activation of multiple kinases (including PI3 kinase/Akt, ERK, p70S6 kinase, and/or GSK-3 β), possibly due to augmented activities of MKPs and other phosphatases, all having been implicated to play a role (*Table 1*) (14,18,19,22,104,111,114,115,124). Potential diabetes-associated defects in mitochondrial end-effectors have also been identified, including impaired activation of mitochondrial K_{ATP} channels (112,116).

Finally, there is a notable and fundamental gap in our current knowledge of the efficacy of ischemic conditioning in models of type-2 diabetes. All previous discussion has focused exclusively on pre- and postconditioning; to date, no published studies have utilized remote conditioning as the cardioprotective trigger (*Table 1*). There is, however, one piece of evidence that diabetes may have a complex, confounding effect on the production or release of the as-yet unidentified humoral factor(s) from the site of the

conditioning stimulus (127). A model of ‘transferred’ protection was used, in which the conditioning stimulus (brief repeated episodes of limb ischemia) was applied to diabetic and non-diabetic patient cohorts, serum was collected, dialyzed and administered to a remote target (isolated buffer-perfused rabbit hearts), and the hearts were then subjected to a sustained period of ischemia. For both cohorts, serum collected after the conditioning stimulus rendered the rabbit hearts resistant to infarction: i.e., type-2 diabetes *per se* did not preclude the infarct-sparing effect of remote conditioning. However, in the subset of diabetic subjects with peripheral neuropathy, the transferred serum failed to reduce infarct size in acceptor rabbit hearts, implicating the requisite involvement of a diabetes-sensitive neurogenic component in this model of humorally-mediated remote conditioning (127). The consequences of type-2 diabetes in standard *in vivo* models of remote

conditioning, and the concept that persistent efficacy of remote conditioning in diabetic models may depend on the intact innervation of the effector organ, are topics of ongoing study by our group and others.

Initial insight: ischemic conditioning in diabetic patients

The wealth of preclinical evidence documenting reduction of infarct size with pre-, post- and remote conditioning has provided the groundwork and rationale for ongoing efforts to translate the concept of endogenous conditioning-induced cardioprotection for the clinical treatment of myocardial I/R injury (6,8-10,128). As preconditioning is, by definition, a pretreatment—thereby limiting its potential for clinical use to planned ischemic events such as cardiac surgery and elective percutaneous intervention (PCI)—current attention is focused largely on postconditioning and remote conditioning. Results from ~40 phase II clinical trials have been reported, and large phase III trials are in progress [reviewed in (6,128)]. Overall, the data have been mixed: ~60% of the studies observed significant reductions in cardiac enzyme release and other surrogate endpoints reflecting myocardial infarct size in conditioned cohorts *versus* controls, while the remainder reported either no effect or exacerbated outcomes (6,128). In addition, recent meta-analyses of pooled data from multiple trials underscored the variability among studies and concluded that, at present, there is borderline evidence, or no evidence, for cardioprotection with either postconditioning or remote conditioning (129,130).

In addition to differences in patient demographics and enrollment criteria, protocol logistics (including the number and timing of the conditioning stimuli and duration of sustained ischemia), choice of endpoints, etc., extrapolation of the results obtained in preclinical models of type-2 diabetes suggest that two related factors—the confounding effects of diabetes, together with differing proportions of diabetic patients among studies—may also contribute to the aforementioned variability. Indeed, in an effort to mitigate this concern, some investigators have prospectively excluded the enrollment of diabetic patients (131-134). Initial evidence appears to support of the concept that conditioning-induced cardioprotection may be impaired or lost in patients with diabetes. For example, in two clinical trials in which prospective subset analyses were performed and cardiac enzyme release served as the surrogate for infarct size, preconditioning (triggered by prodromal angina) had no beneficial effect, while postconditioning

tended to exacerbate myocardial injury in diabetic cohorts (135,136). In addition, in a third trial evaluating the efficacy of remote conditioning administered following elective PCI, the incidence of post-procedural MI was significantly increased in patients with *versus* without diabetes (137). Finally, in an *ex vivo* analysis, preconditioning attenuated hypoxia-reoxygenation-induced cell death in human atrial samples harvested from non-diabetic patients at the time of cardiac surgery, but failed to render atrial tissue resistant to injury in samples obtained from patients with type-2 diabetes (138).

Summary and future directions

Despite the paucity of studies conducted in preclinical models of type-2 diabetes in which myocardial infarct size was among the primary endpoints (*Table 1* and *Figure 3*), a consensus is emerging: the diabetic rodent heart is refractory to the profound infarct-sparing effect of preconditioning, postconditioning and, possibly, remote conditioning. It could be argued these data may be of limited clinical relevance, given the overt simplicity of the mouse and rat models: the duration of diabetes is comparatively acute (on the order of weeks, rather than months-years), and, with few exceptions (22,113,114), the models (I) do not mimic the multiple comorbid conditions seen in substantial subsets of patients; and (II) do not include groups treated with the battery of pharmacologic agents that would be administered to patients as standard clinical care for both AMI and the management of hyperglycemia. Nonetheless, although far from conclusive, the initial clinical data appear to corroborate the preclinical results.

Does ischemic conditioning have the potential to achieve the as-yet unmet clinical challenge of attenuating myocardial I/R injury, reducing infarct size and improving outcome in patients post-MI? And, if so: is the efficacy of conditioning-induced cardioprotection compromised in diabetic patients? Definitive resolution of these issues awaits the completion of Phase III clinical trials that are prospectively designed with sufficient statistical power to discern the presence versus absence of an infarct-sparing effect of ischemic conditioning in stratified subgroups of patients with and without type-2 diabetes.

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and proof-read/approved the final version. KP provided assistance and confirmed the accuracy of the literature review, edited the manuscript and proof-read the final version.

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