Effect of hyperglycaemia and diabetes on acute myocardial ischaemia–reperfusion injury and cardioprotection by ischaemic conditioning protocols

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This article is part of a themed issue entitled “Risk factors, comorbidities, and comedications in cardioprotection” co-edited by Rainer Schulz, Ioanna Andreadou, Derek Hausenloy and Peter FerdinandyDiabetic patients are at increased risk of developing coronary artery disease and experience worse clinical outcomes following acute myocardial infarction (AMI). As such, novel therapeutic strategies are required to protect the myocardium against the detrimental effects of acute ischaemia/reperfusion injury (IRI). In this regard, a number of strategies for protecting the myocardium against acute IRI have been described. These strategies include one or more brief cycles of non-lethal ischaemia and reperfusion prior to the index ischaemic event (ischaemic preconditioning, IPC) or at the onset of reperfusion (ischaemic postconditioning, IPost) either to the heart itself or to an extracardiac organ/tissue (remote ischaemic conditioning, RIC). Experimental studies suggest that the diabetic heart is resistant to these cardioprotective strategies although clinical evidence for this are lacking. In this article, we provide an overview of the available animal models of diabetes for investigating acute myocardial IRI and cardioprotection. Next, we perform a detalied review of experimental studies investigating the effects of hyperglycaemia on susceptibility to acute myocardial IRI. We then review the response of the diabetic heart to cardioprotective strategies such as IPC, IPost and RIC. Finally, we highlight the effects of anti-hyperglycaemic agents on susceptibility to acute myocardial IRI and cardioprotection.

Keywords: animal models, anti-hyperglycaemic medications, cardioprotection, hyperglycaemia, Type 1 diabetes mellitus, Type 2 diabetes mellitus, ischaemic preconditioning, ischaemic postconditioning, remote ischaemic conditioning.

Abbreviations

AMI: acute myocardial infarction

AMPK: AMP-activated protein kinase

DPP-4: Dipeptidyl peptidase-4

DM: diabetes mellitus

FFA: free fatty acid

eNOS: endothelial nitric oxide synthase

GLP-1RAs: Glucagon-like peptide-1 receptor agonists

HF: heart failure

HKII: hexokinase II

iNOS: inducible nitric oxide synthase

IPC: ischaemic preconditioning

IPost: postconditioning

IRI: ischaemia/reperfusion injury

IS: infarct size

MI: myocardial infarct

mPTP: mitochondrial permeability transition pore

PPAR-γ: peroxisome proliferator-activated receptor-γ

PPCI: percutaneous coronary intervention

RCT: randomized clinical trial

RIC: remote ischaemic conditioning

RISK: Reperfusion Injury Salvage Kinase

SAFE: Survivor Activating Factor Enhancement

SGLT2: sodium–glucose co-transporter 2

STAT3: signal transducer and activator of transcription 3

STEMI: ST-elevation myocardial infarction

T1DM: type 1 diabetes mellitus

T2DM: type 2 diabetes mellitus

1. Introduction

Diabetes mellitus (DM) affects 430 million adults globally (8.8% of the world's population), and is a major cause of morbidity and mortality. The major pathological consequences of DM arise from the effects of chronic hyperglycaemia on the macrovasculature (resulting in coronary artery disease, peripheral artery disease, and cerebrovascular disease), and microvasculature (resulting in diabetic retinopathy, nephropathy and neuropathy). In DM patients the risk of developing cardiovascular disease is increased 2 to 4 fold when compared to non-DM patients (Bertoluci and Rocha 2017). Furthermore, patients with DM experience worse clinical outcomes in a number of clinical settings of acute myocardial ischaemia/reperfusion injury (IRI) including AMI (Donahoe *et al.,* 2007), coronary angioplasty (Mathew *et al.,* 2004), and cardiac bypass surgery (Alserius *et al.,* 2006), suggesting that the diabetic heart may be more susceptible to acute IRI. In contrast, animal studies have been inconclusive with experimental studies suggesting that the diabetic heart may be more, equally or even less susceptible to acute IRI (Whittington *et al.,* 2013). However, one major reason for the disparity between the clinical and animal data may be due to the choice of acute myocardial IRI models and diabetic animal models used in the experimental studies (Whittington *et al.,* 2013). Indeed, standardisation, reproducibility and rigour are mandatory in animal and clinical studies to achieve clinical translation in cardioprotection (Jones *et al.,* 2015; Bøtker *et al.,* 2018).

Given the worse clinical outcomes in diabetic patients with coronary artery disease, novel therapeutic strategies, that are effective in the diabetic heart, are required to protect the myocardium against the detrimental effects of acute IRI. A number of strategies exist for protecting the heart against acute IRI. These are based on applying one or more brief cycles of non-lethal ischaemia and reperfusion prior to the index ischaemic event (ischaemic preconditioning, IPC) or at the onset of reperfusion (ischaemic postconditioning, IPost) either to the heart itself or an organ/tissue away from the heart (remote ischaemic conditioning, RIC) (Fig. 1). The latter has relevant therapeutic potential in the clinical scenario (Pickard *et al.,* 2015). In order to translate ischaemic conditioning into the clinical arena for the benefit of diabetic patients, it is important to first determine in animal studies whether the diabetic heart

is amenable to endogenous cardioprotection. In experimental animal studies, it appears that the diabetic heart is resistant to endogenous cardioprotection (Ferdinandy *et al.,* 2014), but clinical evidence for this is lacking. Pharmacological agents which recruit the signalling pathways underlying ischaemic conditioning, can recapitulate cardioprotection – termed 'pharmacological conditioning'. Interestingly, by targeting these signalling pathways many anti-diabetic agents can either mimic or confound cardioprotection, further complicating the study of cardioprotection in the diabetic heart.

In this article, firstly we provide an overview of the commonly used rodent and pig models of diabetes for investigating acute myocardial IRI and cardioprotection. Next, we perform a comprehensive review of experimental studies investigating the effects of hyperglycaemia on susceptibility to acute myocardial IRI. Then, we review the response of the diabetic heart to cardioprotective strategies such as IPC, IPost and RIC. Finally, we highlight the effects of anti-hyperglycaemic agents on susceptibility to acute myocardial IRI and cardioprotection.

2. Experimental animal models of diabetes

Animal models of DM are crucial to understanding the pathophysiological effects of diabetes on the cardiovascular system, and identifying and validating novel therapeutic targets and signalling pathways. DM animal models can be subdivided into 4 groups: surgical, pharmacological, diet and genetic/selective inbreeding-induced DM (summarized in Table 1). Surgical (pancreatectomy) and pharmacological models usually result in pancreatic mass reduction, insulin deficiency, hyperglycaemia, and thus represent Type 1 DM (T1DM) models. Pharmacological models include injection of drugs such as streptozotocin or alloxan, which are selectively toxic to pancreatic β-cells, and induce DM as early as 24-48h post-injection (Rerup and Tarding, 1969). Selective in-breeding has produced several rodent models of Type 2 DM (T2DM), usually associated with a panoply of risk factors. The most common genetic rodent models of T2DM include Zucker Diabetic Fatty and obese ZSF1 rats, db/db, and ob/ob mice. All of these models display dysfunctional or absent leptin homeostasis and insulin

resistance at different time points. T2DM can also be induced by diets with high fat and/or high carbohydrate content (Table 1) (Maioli *et al.,* 2016). Diet-induced DM requires months to achieve the full T2DM spectrum and no standard protocol has been established. This prolonged onset of T2DM might be closer to the human scenario, providing several opportunities to perform acute myocardial IRI studies according to the stage of the disease. Variations in diet compositions are particularly important considering the vast amount of studies reporting that the type of fat in the diet can affect cardioprotection or pathology (Stanley *et al.,* 2012). Thus, diet formulation should be taken into account (Heydemann 2016). Many of these rodent models share many features with human DM cardiomyopathy (Bugger and Abel, 2008) as well as higher incidence of acute myocardial IRI (Greer *et al.,* 2006).

There are several limitations of DM animal models that need to be taken into consideration: (1) rodent models present with sudden and uncontrolled hyperglycaemia or insulin resistance while in the clinical setting the onset of diabetes is often gradual and the hyperglycaemia is usually well-controlled with anti-diabetic medication; (2) pancreatic islets architecture is distinct from humans; (3) monogenic models are not representative of human DM; (4) DM develops at varying stages in rodent models, which has an impact on the timing of the acute myocardial IRI study: in the initial stages, IRI may reflect changes that are secondary to damaging circulatory metabolic milieu and the underlying obesity and insulin resistance, whereas in the later stages, IRI may reflect the added effects of hyperglycaemia of different durations; (5) in genetic models metabolic dysregulation appears at very early developmental stages. Finally, the lack of spontaneous ischaemia and atherosclerosis in rodents (Boudina and Abel, 2007), could be considered either a disadvantage or an advantage since the impact of obesity, insulin resistance and diabetes can be studied independently of coronary artery disease (Ishibashi *et al.,* 1994).

Although there is no animal model that fully recapitulates human pathology of diabetes, large animal models are available that closely mimic human cardiac physiology and anatomy. In particular, the minipig and pig heart models with regional myocardial IRI is of paramaount translational value (reviewed in Elmadhun *et al.,* 2013). Pig models of diet-induced metabolic

syndrome and T2DM, streptozocin- or alloxan-induced T1DM, or genetically engineered pigs can be used in IRI studies (Diemar *et al.,* 2015; Jones *et al.,* 2015). Although the cardioprotective signalling is in part different from that in rodent hearts, all cardioprotective phenomena described above have been demonstrated in pigs (Skyschally *et al.,* 2018).

3. Effects of hyperglycaemia and diabetes on infarct size

In clinical studies, perturbations of blood glucose levels at the time of acute myocardial IRI, either hyper- or hypoglycaemia, are known to be associated with poor cardiovascular outcomes. This observation was supported in one of the largest epidemiological studies of its type, the Cooperative Cardiovascular Project (Kosiborod *et al.,* 2005). This retrospective study of 141,680 patients found that hyperglycaemia was deleterious in diabetic patients and particularly in those without recognized diabetes. In fact, clinical outcomes in non-diabetic patients were significantly worse when compared to diabetic individuals, with a markedly steeper relationship between presentation glucose levels and 30-day and 1-year mortality (Kosiborod *et al.,* 2005). As summarised elsewhere, this has been observed in a number of clinical studies (Deedwania *et al.,* 2008), but the challenge has been to demonstrate causality between hyperglycaemia and clinical outcomes. Interestingly, myocardial infarct (MI) size, as quantified by late gadolinium enhancement cardiovascular magnetic resonance (CMR), correlated with glucose levels at the time of presentation, with greater infarct sizes observed in non-diabetic than in diabetic patients presenting with similar blood glucose levels (Eitel *et al.,* 2012). In addition to glucose levels, insulin resistance and altered metabolism is important in determining the cardiac damaging effects of diabetes (Ishibashi et al., 1994; Giblett *et al.,* 2016).

In order to better understand the relationship between glucose levels and MI size in the experimental setting, we undertook a literature search in Pubmed using the terms “diabetes, hyperglycemia, ischemia-reperfusion injury, infarct size, and heart” of studies published between January 2012 and February 2019. From the obtained 512 articles, we identified 84 original articles that reported on infarct size for both control and hyperglycaemic

or diabetic hearts. For studies older than 2012, we made use of articles analysed by a previous review article on this topic (Miki *et al.,* 2012) – this provided another 46 articles. Figure 2 provides a summary of these 130 articles which have been classified into acute hyperglycaemic conditions, early phase (≤ 2 wks) of T1DM, late phase of T1DM (>2 weeks), and T2DM. Each condition was additionally split into *ex-vivo* (isolated heart) and *in-vivo* models. This allowed us to separate pathologic effects of glucose that could be attributed to the heart itself (intrinsic properties) or to changes in the metabolic milieu of the circulatory system and the heart.

Acute hyperglycaemia: In the isolated heart perfused in the absence of insulin most studies reported increased IS with hyperglycemia, although some studies also reported decreased IS (Fig. 2A). Increased IS was commonly observed with glucose levels > 30 mM, whereas reduced IS was associated with glucose around 8 mM; IS was unaltered with glucose levels between 11-22 mM. The *in-vivo* models (Fig. 2B), most studies compared normoglycaemia, 5-10 mM, with hyperglycaemic levels between 15-20 mM demonstrating, for unclear reasons, either no effects on IS, or increased vulnerability to acute IRI. It therefore seems that at 20 mM glucose *in-vivo* hearts show vulnerability to acute IRI as compared to *ex-vivo* hearts. This seems counter-intuitive knowing that, *in-vivo*, hyperglycaemia increases insulin plasma level, whereby insulin can act as a cardioprotective agent against acute IRI (Zuurbier *et al.,* 2005;) through activation the Akt/hexokinase II (HKII) pathway. This could be explained by the fact that hyperglycaemia directly impairs insulin signalling (Yu *et al.,* 2014). Although for most *in-vivo* studies only hyperglycaemic conditions of 15-20 mM were examined, one study showed that IS increased when raising glucose from 16 to 30 mM (Kersten *et al.,* 1998). For both the *ex-vivo* and the *in-vivo* condition, hyperglycaemia above 10 mM never reduced IS of the heart. In summary, acute hyperglycaemia increases IS in the isolated heart when glucose >30 mM, whereas increases in IS are already present *in-vivo* at glucose levels of 20 mM.

Early phase of T1DM (≤2 weeks): interestingly, the early phase of T1DM is often associated with reduced IS in the *ex-vivo* heart (Fig. 2C). However, it should be noted that in

all these isolated heart studies, the hearts were actually perfused at normoglycaemia (5-11 mM), which deviates from their hyperglycaemic metabolic milieu *in vivo*. Various mechanisms explaining this intrinsic protected state of early T1DM heart have been proposed, such as increased expression and/or phosphorylation of Akt, eNOS, PKC, ERK, heat-shock proteins or maintenance of end-ischaemic mitochondrial HKII (Gurel *et al.,* 2013). Keeping HKII at the mitochondria during ischemia is known to confer protection against cardiac ischaemia-reperfusion injury (Smeele et al., 2011). In contrast, *in-vivo*, the early phase of T1DM was associated with an increase in susceptibility to acute IRI (Fig. 2D). This is likely due to the fact that in the *in-vivo* setting hearts are subjected to acute IRI at higher glucose levels >20mM. In summary, in the early T1DM condition, there appears to be intrinsic protection in the isolated heart, whereas there is increased vulnerability of the *in-vivo* heart to acute IRI, and this is likely due to the hyperglycaemic conditions. This offers the therapeutic option of targeting these extra-cardiac factors of the metabolic milieu, e.g. with exogenous insulin and drugs which lower blood glucose, to reduce acute IRI of the early T1DM heart.

Late phase of T1DM (>2 weeks): after prolonged T1DM, the isolated heart appears to lose its protected state, showing either similar or increased IS (Fig. 2E). It is unknown why protection is lost - this may be due to chronic low insulin signalling, prolonged hyperglycaemia and/or dyslipidaemia. In *in-vivo* condition (Fig. 2F) the susceptibility to acute IRI was also increased.

T2DM: the isolated heart of T2DM animals shows a mixed response to acute IRI experiments performed using the isolated heart of T2DM animals show mixed results, with most studies reporting either increased IS or no change in IS (Fig. 2G), and a minority showing reduced IS. However, T2DM in the *in-vivo* setting was mainly associated with increased IS (Fig. 2H), probably due to the fact that all the isolated hearts were perfused with normal (5-7 mM) levels of glucose, whereas *in-vivo* hearts are subjected to much higher glucose (>20 mM) and FFA levels. These hearts are insulin-resistant, rendering the protective reperfusion injury salvage kinase (RISK) pathway to be less responsive to acute IRI. The RISK pathway concerns pro-survival kinase signalling cascades, such as phosphatidylinositol-3-OH kinase

(PI3K)–Akt and p42/p44 extra-cellular signal-regulated kinase Erk 1/2. Activation of these kinase pathways confers protection against ischemia-reperfusion injury (Hausenloy and Yellon, 2004).

Interestingly, in the *in-vivo* setting a few studies report hearts to have reduced IS. This could be related to the obesity paradox, and early T2DM, where insulin signalling may be still effective and cellular protective signalling pathways are initially activated, similar to that observed in the early T1DM setting.

In summary, it appears that hyperglycaemia and diabetes increase the susceptibility to acute myocardial IRI, and observed differences arise due to the IRI models used and the duration of diabetes.

4. Effects of hyperglycaemia and diabetes on IPC

In order to protect the diabetic heart against the detrimental effects of acute IRI, it is important to ascertain whether the diabetic heart is amenable to cardioprotective strategies such as IPC, IPost and RIC. Here, we review the effects of hyperglycaemia and diabetes on cardioprotection elicited by IPC. The potent infarct size-limiting effects of IPC have been confimed in all species tested including man, and has also been shown to be effective in the multi-centre network of experimental research centres that made up the Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR) (Jones *et al.,* 2015). However, there is substantial experimental evidence that the infarct-limiting effects of IPC are attenuated in the presence of co-morbidities including DM (Ferdinandy *et al.,* 2014). IPC cardioprotective mechanisms have been extensively described, and include RISK, SAFE and NO/PKG pathways which converge on mitochondria (Hausenloy *et al.,* 2016; Penna *et al.,* 2015).

Several studies have evaluated the effect of IPC on cardiac IRI in animal models of diabetes. A reduced cardioprotective effect of IPC has been reported in many studies (Ebel *et al.,* 2003; Kersten *et al.,* 1998). No effects or worsening of acute IRI have also been reported as a consequence of either IPC or pharmacological preconditioning (Kristiansen *et al.,* 2004;

del Valle *et al.,* 2003). Recent representative examples of ischaemic and pharmacological preconditioning studies are summarised in Table 2.

It appears that *hyperglycaemia per se* is responsible for the attenuation of the protective efficacy of IPC. Indeed, acute hyperglycaemia may blunt infarct size reduction by IPC, as well as the protection induced by mitochondrial K_{ATP} channel opener and anaesthetics (Kehl *et al.,* 2002; Kersten *et al.,* 1998). The blunting may be overcome by increasing the dose of protectants or the numbers/duration of PC cycles. Indeed, in animal models, several authors (Hausenloy *et al.,* 2013b; Tsang *et al.,* 2005) reported that cardioprotection by IPC against ischaemic injury requires an increased preconditioning stimulus in diabetic hearts. This finding was confirmed by Hjortbak *et al* (2018) who reported that a strong IPC stimulus may protect diabetic heart in pre-diabetic, early and late-stage T2DM in a Zucker diabetic fatty rat model. Drugs that affect glycaemia or improve the cardioprotective pathways may restore IPC cardioprotection (see Table 2). Yet, studies emphasise that hypoglycaemia and glucose fluctuations, obtained with insulin or sulphonylureas, can aggravate the cardiac susceptibility to acute IRI and the response to cardioprotective manoeuvers to a greater extent in a non-diabetic when compared to a diabetic model (Pælestik *et al.,* 2017; Saito *et al.,* 2016) (see also later section on the effects of anti-hyperglycaemic medications).

Contradictory results observed in animal models have also been reported in patients with diabetes, where the picture is complicated by the large inter-individual variability of the methods used to assess infarct size, so that a large number of patients is necessary to define the efficacy of new cardioprotective approaches in humans (Reinstadler *et al.,* 2017). Moreover, in the clinical scenario, IPC is not so feasible to investigate. For example, pre-infarct angina has been studied as an endogenous IPC stimulus and has generally associated with better clinical outcomes in non-diabetic patients. However, in patients with diabetes, this beneficial effect was not observed (Ishihara *et al.,* 2001). Diabetes-induced impairment of IPC protection in human hearts has also been indicated by studies in which myocardial damage was assessed during percutaneous coronary revascularization (Lee and Chou, 2003) and during the warm-up phenomenon elicited by a treadmill exercise test (Ovünç 2000). Moreover,

preconditioning protected trabeculae from non-diabetic patients but not trabeculae from diabetic patients (Hassouna *et al.,* 2006; Sivaraman *et al.,* 2010). The limited possibilities to study IPC in humans and the fact that patients with diabetes are increasingly well controlled by drugs makes it more challenging to study the influences of diabetes on IPC cardioprotection. Nevertheless, it is likely that also in humans an elevation of preconditioning threshold occurs (Sivaraman *et al.,* 2010). This has been confirmed in IPC studies in other tissues and organs in which contradictory results are obtained in diabetic conditions (Altintas *et al.,* 2016; Thomaz Neto *et al.,* 2013). In many of these studies only an augmented preconditioning protocol achieves protection.

Dysfunctions in sarcolemmal and mitochondrial K_{ATP} channels (del Valle et al, 2003; Kersten *et al.,* 2001) as well as glycogen synthase kinase-3β downregulation (Yadav *et al.,* 2010) have been proposed as possible mechanisms mediating diabetic attenuation of the protective effect of IPC. Nevertheless, to protect the diabetic myocardium, it appears necessary to increase the IPC stimulus to achieve a critical level of Akt phosphorylation to confer protection (Tsang *et al.,* 2005) (Fig. 3). Glimepiride, an activator of Akt, may lower the threshold for IPC; thus both 1 and 3 cycles of IPC (5/10 min ischaemia/reperfusion) may induce a cardioprotective effect in diabetic rat hearts treated with glimepiride (Hausenloy *et al.,* 2013b).

In summary, in experimental animal and human *ex vivo* heart tissue studies, the presence of hyperglyaemia and DM appear to attenuate the cardioprotective efficacy of IPC, and this appears to be mediated by interference with signalling pathways underlying IPC. However, the confounding effects of hyperglycaemia and DM on cardioprotection, can be overcome by increasing the IPC stimulus. Evidence for this phenomenon are lacking in clinical studies. The disadvantage of IPC as a cardioprotective strategy is that it needs to be applied prior to the index ischaemic event, which is not possible to predict in the setting of AMI – as such, IPost, which is applied at the onset of reperfusion, may be more effective in the setting of AMI.

5. Effects of hyperglycaemia and diabetes on IPost

Since IPost can be applied at the onset of reperfusion, it can be easly applied to AMI at the time of PPCI through the inflation and deflation of the angioplasty balloon (Staat *et al.,* 2005). The cardioprotective effect of IPost has been confirmed in several different animal models using varying protocols according to gender, age, species, number of cycles and duration of ischaemia/reperfusion, precluding the possibility of defining a single IPost algorithm (for review see Pagliaro *et al.,* 2011; Skyschally *et al.,* 2009). IPost has been reported to confer cardioprotection via the production of several different autacoids (such as bradykinin, adenosine and opioids), that recruit known cardioprotective signalling pathways (such as the survivor activating factor enhancement [SAFE], NO/PKG and RISK cascades), and which converge on the mitochondrial permeability transition pore (Bell *et al.,* 2016; Boengler *et al.,* 2011; Cohen and Downey, 2011; Lacerda *et al.,* 2012; Oosterlinck *et al.,* 2013; Pagliaro *et al.,* 2011, Pagliaro and Penna, 2015; Penna *et al.,* 2015).

The clinical studies of IPost in STEMI patients have mixed results with IPost limiting MI size (assessed by cardiac biomarkers and cardiac MRI) in most (Staat *et al.,* 2015; Thibault *et al.,* 2007; Xue et al, 2010), but not all studies (Freixa *et al.,* 2012; Hahn *et al.,* 2013; Sörensson *et al.,* 2010). In addition, the DANAMI-3 study failed to demonstrate a beneficial effect of IPost on clinical outcomes in STEMI patients treated by PPCI, although the study was underpowered given the lower than expected event rate (Lønborg *et al.,* 2017). The reasons for lack of efficacy of IPost in these studies are not clear but have been attributed to prior preconditioning by pre-infarct angina, lack of direct stenting, the presence of comorbidities (such as diabetes), and co-medications (such as platelet P2Y12 inhibitors). Here, we will focus on the experimental data reporting the effect of diabetes on the cardioprotective efficacy of IPost (Table 3).

A number of experimental studies have demonstrated that the cardioprotective effects of IPost are blunted in both T1DM and T2DM animal models (Drenger *et al.,* 2011; Przyklenk *et al.,* 2011; Ren *et al.,* 2011). Also, in an *in vitro* cell study, it was found that hyperglycaemia blunted IPost-induced protection (Chen *et al.,* 2016). Przyklenk *et al* (2011) found that IPost

was ineffective in type 1 and 2 DM murine models, and cardioprotection was restored in the presence of insulin treatment. However, this finding was in contrast with anaesthetic-induced postconditioning protection, where insulin treatment failed to restore cardioprotection in diabetic animals (Drenger *et al.,* 2011). This was attributed to marked inhibition of the SAFE (JAK-STAT3) and RISK (PI3K/Akt/eNOS) signalling cascades in the presence of diabetes (Drenger *et al.,* 2011; Raphael *et al.,* 2015) (Fig 3). It has been suggested that PTEN/Akt signalling is altered in the presence of diabetes (Mocanu and Yellon, 2007; Xue *et al.,* 2016). It has been reported that the diabetic heart may be refractory to protection by Jak2-activating ligands because of angiotensin-II type 1 (AT1)-mediated upregulation of calcineurin activity; however, it is not clear how calcineurin activity interferes with protection by Jak2 (Hotta *et al.,* 2010). Recently, also in a hyperglycemic experimental model a reduced level of Akt phosphorylation has been observed, a condition which has been associated with the loss of the cardioprotective effects of insulin in the isolated rat heart (Nakadate *et al.,* 2017). Moreover, increased susceptibility to acute myocardial IRI in the aged, diabetic heart has been shown to be a consequence of impaired RISK signalling due to chronic Akt phosphorylation (Whittington *et al.,* 2013). In the leptin receptor-deficient db/db mice model of T2DM, the failure of IPost to confer cardioprotection was attributed to the dysregulation of proteins involved with the production of cellular ATP (such as F(1)-ATPase (γ and Echs1)), and heat shock proteins (Zhu *et al.,* 2012).

In summary, the presence of hyperglycaemia/diabetes appears to blunt IPost via the downregulation of known cardioprotective signalling pathways (such as SAFE and RISK), and the addition of pharmacological postconditioning agents can restore cardioprotection.

6. Effects of hyperglycaemia and diabetes on RIC

The major disadvantage of both IPC and IPost are that they require the intervention to be applied directy to the heart, thereby hampering their clinical translation to AMI patients. Therefore, the phenomenon of RIC, in which the conditioning episodes of ischaemia and reperfusion are applied to an organ or tissue away from the heart, has greater therapeutic

potential in the clinical setting (Cabrera-Fuentes *et al.,* 2016; Giannopoulos *et al.,* 2017; Pickard *et al.,* 2015). RIC has further advantages including the ability to confer systemic protection against acute IRI in other non-cardiac organs or tissues, and the ability to confer protection when applied either prior to, during, or at the end of the index ischaemic event, further aiding its clinical translation. The discovery that the RIC stimulus can be applied to the limb by simply restricting and restoring blood flow using either a tourniquet or pneumatic cuff to induce intermittent limb ischaemia and reperfusion, has greatly facilitated the translation of RIC into the clinical setting. Limb RIC has been shown to reduce peri-operative myocardial injury in patients undergoing cardiac bypass surgery but it failed to improve clinical outcomes in this setting (ERICCA/RIPHeart). In STEMI patients, limb RIC applied in the ambulance or on arrival at the hospital prior to PPCI, has been reported to improve myocardial salvage and/or reduce MI size (Hausenloy *et al., 2015*). However, the recently published large multicentre 5401 STEMI patients CONDI2/-ERIC-PPCI trial, failed to show any clinical benefit of limb RIC, with no differences in rates of cardiac death or hospitalisation for heart failure when compared to control, regardless of diabetes present in 11.9% of patients subjected to RIC (Hausenloy *et al.,* 2019).

The reasons for the neutral results of limb RIC in the clinical setting are not clear but could relate to the presence of co-morbidities (such as age or DM) and co-medications (such as P2Y12 platelet inhibitors) acting as confounders of cardioprotection. In this regard, experimental studies have shown that acute hyperglycaemia was able to abrogate cardioprotection elicited by limb RIC in a rat AMI model, and this effect was associated with increased incidence and duration of arrhythmias and an increase in nitrosative stress and activation of the mTOR pathway (Baranyai *et al.,* 2015). In the clinical setting evidence for hyperglycaemia or diabetes interfering with RIC cardioprotection is lacking, although clinical studies in CABG and STEMI patients have reported cardioprotection with RIC despite including 20% diabetic patients (Eitel *et al.,* 2015). Interestingly, Kottenberg *et al* (2014) have reported that cardioprotection by RIC was abrogated in sulphonylurea-treated diabetic patients undergoing cardiac surgery, data that is consistent with this agent antagonising the

ATP-dependant potassium channel, which is known to mediate cardioprotection. Recently, a review by Tyagi *et al* (2019) summarized the possible mechanisms that can explain how diabetes abolishes cardioprotective effects of RIC. It has been reported that protection conferred by RIC may involve the attenuation of the sympathetic nervous system response to ischaemia, in healthy humans (Lambert *et al.,* 2016). We can speculate that the inefficacy of RIC in diabetes may also be in part explained by the autonomic dysfunction that is getting worse in T2DM patients (Istenes *et al.,* 2014). Indeed, the metaboreflex (the reflex response stimulated by metabolite accumulation during limb exercise and/or ischaemia) is abnormal in T2DM patients and it is characterised by an exaggerated vasoconstriction (perhaps due to sympathetic over-stimulation) not accompanied by a concomitant increase in heart performance (Roberto *et al.,* 2019). This speculation is in line with a study, where the plasma dialysate collected from patients with diabetes after RIC triggered cardioprotection only in the absence of diabetic neuropathy of the upper limbs (Jensen *et al.,* 2012). However, additional studies are necessary (especially multicentric RCT in patients with AMI for RIC with clinical outcome as the primary endpoint) to understand the role of hyperglycaemic and diabetes on the loss of cardioprotective effects by RIC and whether combined approaches (e.g. RIC plus IPost) may be necessary to overcome the protective blinding induced by diabetes in post AMI patients.

In summary, there is initial experimental evidence that acute hyperglycaemia blunts limb RIC cardioprotection, but evidence in the clinical setting is lacking. Therefore, further large clinical cardioprotection studies are needed to determine whether DM is actually a confounder of limb RIC cardioprotection.

7. Effects of anti-hyperglycaemic medications on acute myocardial IRI and cardioprotection

The majority of diabetic patients are on anti-hyperglycaemic medications to control their blood glucose levels, and there is experimental and clinical data suggesting that these medications can themselves either confer cardioprotection or interfere with cardioprotection elicited by IPC,

IPost and RIC. It must be noted that some of these anti-hyperglycaemic agents confer cardiovascular protection which may be unrelated to cardioprotection against acute myocardial IRI. These issues make it challenging to determine whether the presence of diabetes actually confounds cardioprotection in clinical studies. In this section, we provide an overview highlighting the effects of older and newer anti-hyperglycaemic medications on acute myocardial IRI and cardioprotection.

Sulphonylureas: this class of anti-hyperglycaemic agents act by binding to a subunit of the β cell K_{ATP} channel complex, leading to the closure of the channel, thus stimulating/potentiating insulin secretion and lowering blood glucose levels (Brunton *et al.,* 2006). By also binding to cardiac K_{ATP} channels, sulphonylureas such as glibenclamide have been shown in experimental studies to interfere with IPC cardioprotection, since K_{ATP} channel opening has been shown to contribute to IPC cardioprotection (Yumei *et al.,* 2011). It appears that the newer sulphonylureas such as glimepiride (Mocanu *et al.,* 2001) and gliclazide (Maddock *et al.,* 2004) do not interfere with IPC cardioprotection, and this is possibly related to their greater specificity for pancreatic compared to myocardial K_{ATP} channels (Gribble *et al.,* 1999). In the clinical setting, diabetic patients undergoing cardiac bypass surgery who were on treatment with sulphonylureas were not protected by RIC (Kottenberg *et al.,* 2014), and in another study glibenclamide was shown to abolish endothelial protection induced by RIC (Loukogeorgakis *et al.,* 2007).

Metformin: this agent is a biguanide whose effects are mediated by the activation of the AMP-activated protein kinase (AMPK) and which lowers blood glucose levels by reducing liver production of glucose and increasing insulin sensitivity (Cho *et al.,* 2015). There is extensive experimental animal data showing that treatment with metformin either prior to ischaemia or at onset of reperfusion can reduce MI size (reviewed in Yumei *et al.,* 2011). The mechanisms underlying metformin cardioprotection are diverse and include activation of adenosine receptors, recruitment of the RISK pathway, AMPK activation, modulation of complex I and inhibition of mitochondrial permeability transition pore (mPTP) opening at reperfusion (Bromage and Yellon, 2015; Mohsin *et al.,* 2019). In the clinical setting, most meta-analyses

have supported the cardiovascular safety of metformin and have shown it to reduce the risk of re-infarction and all-cause mortality in the long-term in patients with coronary artery disease and chronic heart failure, independent of its glucose lowering effects (Varjabedian *et al.,* 2018). However, no acute protection by metformin administration during CABG was observed (El Messaoudi *et al.,* 2015), questioning the translatability of metformin for protection against acute I/R conditions in the clinical setting.

Thiazolidinediones: these agents act as selective agonists for nuclear PPAR-γ and lower blood glucose levels by reducing insulin resistance. Experimental animal studies have reported cardioprotection with these agents administered either prior to ischaemia and at onset of reperfusion, (Ye *et al.,* 2008; Zhang *et al.,* 2010) with potential mechanisms including decreased expression of microRNA-29a and 29c (Ye *et al.,* 2010), activation of the RISK pathway (Wynne *et al.,* 2005), and alternative pathways including Src family kinase- and matrix metalloproteinase-dependent transactivation of EGF and PDGF receptors (Ichiki *et al.,* 2004). Clinical studies and a meta-analysis have suggested that pioglitazone reduces cardiovascular complications in patients with T2DM (Nissen *et al.,* 2008), whereas in contrast, rosiglitazone has been associated with worsened adverse cardiovascular outcomes (Lincoff *et al.,* 2007).

Glucagon-like peptide-1 receptor agonists (GLP-1RAs): this class of anti-hyperglycaemic agents lower blood glucose levels by an insulin incretin effect (Hui Peng *et al.,* 2016). Several studies have shown that GLP-1 or GLP-1 analogues administered either as pre- and/or postconditioning agents limit MI size in small animal models (Matsubara *et al.,* 2009; Sonne *et al.,* 2008). However, studies in pigs have shown divergent results: GLP-1 and liraglutide do not limit infarct size (Kavianipour *et al.,* 2003; Kristensen *et al.,* 2009), whereas exenatide reduces infarct size (Timmers *et al.,* 2009). Proposed mechanisms of actions include activation of the GLP receptor, PKA and RISK pathways, and eNOS phosphorylation. Indeed, the mechanisms through which the cardioprotection occurs is not fully defined but may include activation of the subcellular pathways of IPC, and modulation of myocardial metabolism (reviewed in Giblett *et al.,* 2016). GLP-1RA therapy can also modulate innate immune-

mediated inflammation (Hogan *et al.,* 2014). Limited data suggest that GLP-1 RAs may be effective for the treatment of cardiac disorders in patients with and without diabetes mellitus. These studies suggest that GLP-1 RAs may have potential pleiotropic beneficial effects in patients with cardiovascular disease beyond their role in managing diabetes. These medications may be cardioprotective after an AMI but are less promising in heart failure (HF) (Marso *et al.,* 2016; reviewed in Wroge and Williams, 2016).

Dipeptidyl peptidase-4 (DPP-4) inhibitors: this is a new class of drugs for treating T2DM lowering blood glucose levels by augmenting endogenous levels of GLP-1 through the inhibition of DPP-4 (Pauly *et al.,* 1996). Experimental studies in small animals and in pigs have shown that sitagliptin and vildagliptin limited MI size when they were administered before ischaemia or at reperfusion (Hausenloy *et al.,* 2013a; Theiss *et al.,* 2013). The mechanism of action includes augmentation of the effects of endogenous incretins, activation of the GLP-1 receptor leading to generation of cAMP with downstream activation of PKA (reviewed in Yoon *et al.,* 2014). Clinical trials evaluating the overall cardiovascular risks and benefits after administration of DPP-4 inhibitors have shown that hospitalisation for HF was increased in saxagliptin-treated patients (Scirica *et al.,* 2013), whereas the rates of major adverse cardiovascular events were not increased with the alogliptin and sitagliptin as compared with placebo (Green *et al.,* 2015; White *et al.,* 2013). Nevertheless, the relationship between DPP-4 inhibitors and HF is complex (reviewed in Ziff *et al.,* 2018).

Sodium–glucose co-transporter 2 (SGLT2) inhibitors: this new class of approved anti-hyperglycaemic agents lower blood glucose by inhibiting glucose reasorption in the kidney (Majewski and Bakris, 2015; Wanner *et al.,* 2016). Recently published landmark cardiovascular outcome trials [EMPA-REG OUTCOME (Fitchett *et al.,* 2016), CANVAS (Neal *et al.,* 2017), and DECLARE-TIMI 58 trial (Wiviott *et al.,* 2019)] have shown that the SGLT2 inhibitors (empagliflozin, canagliflozin and dapagliflozin) reduced rates of cardiovascular death and hospitalisation for HF in T2DM patients at risk of cardiovascular disease. However, the mechanisms underlying these protective cardiovascular effects remain unclear.

Experimental studies have investigated whether SGLT2 inhibitors are able to exert cardioprotective effects against acute myocardial IRI. Chronic therapy with empagliflozin (Andreadou *et al.,* 2017), canagliflozin (Lim *et al.,* 2019) or dapagliflozin (Tanajak *et al.,* 2018) have been reported to reduce MI size in both DM and non-DM rodent models of acute myocardial IRI. The cardioprotective mechanisms have been attributed to a variety of factors including increased STAT3 phosphorylation, reduced myocardial IL-6 and iNOS expression, inhibition of mitochondrial fission, preservation of mitochondrial function and regulation of redox signalling in the ischaemic myocardium (Andreadou *et al.,* 2017; Mizuno *et al.,* 2018; Ng *et al.,* 2018; Tanajak *et al.,* 2018). However, acute administration of SGLT2 inhibitors failed to reduce MI size in the isolated mouse/rat heart, suggesting that the infarct size-reducing effects of SGLT2 inhibitors may require long-term treatment (Uthman *et al.,* 2019; Lim *et al.,* 2019). However, SGLT2 inhibitors have shown acute functional protective effects, improving cardiac performance during ischaemia (Uthman *et al.*, 2019; Baker *et al.*, 2019). This may suggest that some of the beneficial effects of SGLT2 inhibitors are acutely and directly on the myocardium, despite the fact that SGLT2 is mainly expressed in the kidney and only minimally in the heart. In this regard, interesting studies have suggested that the SGLT2 inhibitors may have off-target inhibitory effects on the cardiac sodium hydrogen exchanger which would be expected to prevent sodium and calcium overload and may in part explain their cardioprotective effects (Uthman *et al.,* 2018, 2019).

Whether the observed cardioprotective effects of chronic SGLT2 inhibitor therapy can explain the cardiovascular outcome benefits observed in the large clinical outcomes studies is not known, and other benefits on cardiac metabolism, cardiac hypertrophy, and heart function have been proposed (Baker *et al.,* 2019; García-Ropero *et al.,* 2019; Habibi *et al.,* 2017; Oshima *et al.,* 2019; Santos-Gallego *et al.,* 2019; Verma *et al.,* 2019; Xue *et al.,* 2019; Yurista *et al.,* 2019).

In summary, investigating the confounding effects of anti-hyperglycaemic agents on cardioprotective strategies such as IPC, IPost and RIC is challenging given that many of these therapies (such as metformin, thiazolidinediones, GLP-1 agonists, DPP-4 inhibitors, SGLT2

inhibitors) appear to exert cardioprotective effects against acute IRI in experimental animal studies. However, whether these cardioprotective effects can explain their beneficial effects on cardiovascular outcomes in diabetic patients is not clear. Further studies are needed to determine the mechanisms underlying the cardioprotective effects of the newer anti-hyperglycaemic agents such as GLP-1 agonists, DPP-4 inhibitors and SGLT2 inhibitors.

8. Summary and conclusions

In summary, there is convincing data that hyperglycaemia and diabetes may attenuate the cardioprotective effects of IPC, IPost, and RIC, but whether this is true in the clinical setting, has not been demonstrated (Kleinbongard *et al.,* 2019). At least in the experimental setting, a stronger 'conditioning' stimulus or use of certain drugs can target hyperglycaemia/DM-induced downregulated signalling pathways, in order to restore cardioprotection. The picture is further complicated by the heterogeneity of animal models used, and this may explain some of the diverse results reported. Further experimental studies are needed to elucidate the potential mechanisms underlying the confounding effects of hyperglycaemia/DM on endogenous cardioprotection. Importantly, additional clinical studies are needed to confirm whether the confounding effects of hyperglycaemia/DM on endogenous cardioprotection are replicated in diabetic patients. The latter is difficult to investigate given that most DM patients are on anti-hyperglycaemic agents (such as GLP-1 agonists, DPP-4 inhibitors and SGLT2 inhibitors) which in themselves are known to be cardioprotective. Importantly, novel cardioprotective strategies for inducing ischaemia tolerance and to reduce IRI in patients with diabetes are needed to improve clinical outcomes in this high-risk group.

Nomenclature Statement

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al*., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al*., 2017a; Alexander *et al*., 2017b).

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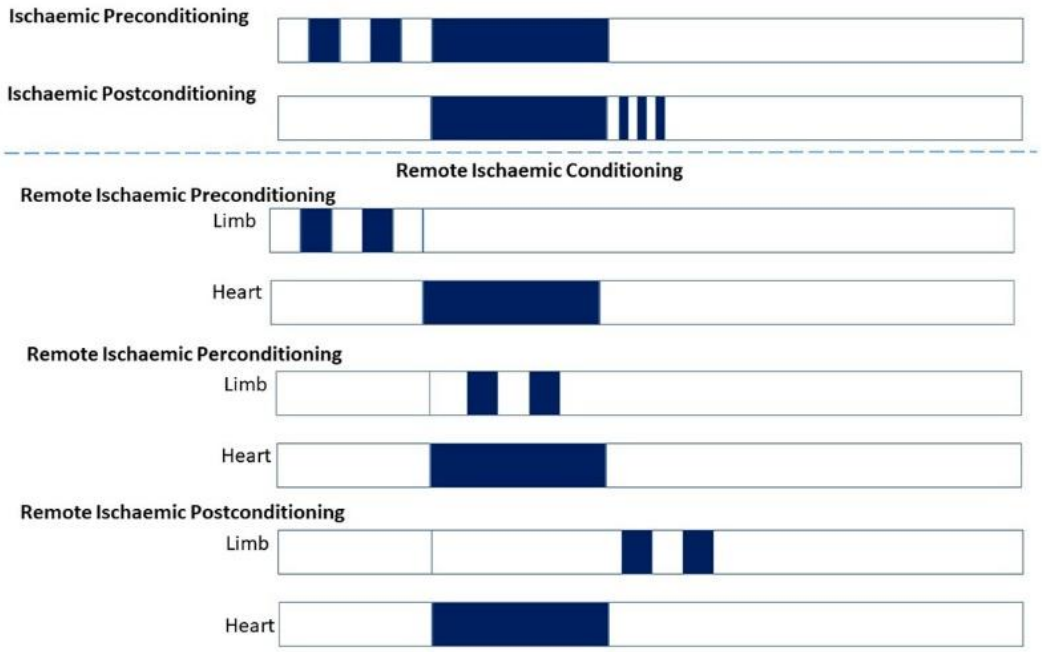

Figure 1: Schematic representation of the various protocols of ischaemic conditioning. White boxes represent time of normal perfusion or reperfusion; dark blue boxes are periods of ischaemia. The number and duration of cycles used can vary with different experiments.

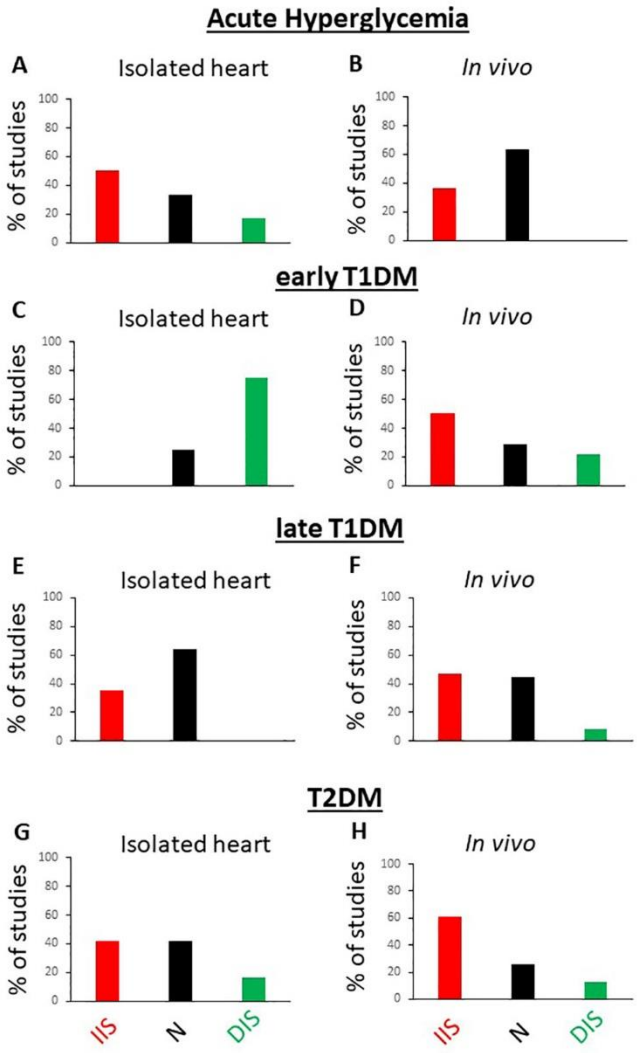

Figure 2: Schematic summary of 130 studies reporting effects on cardiac sensitivity to IRI of acute hyperglycaemia (A, B), early type 1 Diabetes Mellitus (C, D), late type 1 Diabetes Mellitus (E, F) and Type 2 Diabetes Mellitus (G, H) for the isolated heart (A, C, E and G) or the *in vivo* condition (B, D, F and H), respectively. Within each of the 8 categories, to obtain the percentage, the number of studies reporting increased IS (IIS), neutral (N) or decreased IS (DIS) is divided by the total number of studies in that category. The following references were used (given by their PubMed Identifier number, PMID):

A (***IIS:*** 24286628, 12189448, 20206553; ***N:*** 20206553, 24908083; ***DIS:*** 26491698);

B (***IIS:*** 30596897, 9683464, 10749717, 29506687, 29169909, 17519283, 26014921, 24371503, 27753144; ***N***: 20124983, 23666677, 26581389, 26359322, 12739155, 18362595, 30596897, 18305078, 22436066, 25446919, 11753019, 9683464, 11247788, 22239823, 20125034, 25812079, 18655783, 24845581; ***DIS:*** none);

C (***IIS:*** none; ***N:*** 20578962; ***DIS:*** 30682388, 26674282, 27418904);

D (***IIS:*** 27217295, 28500760, 24286628, 12189448, 15331549, 15054118; **N**: 24466263, 23922853, 22881281, 17192474; ***DIS:*** 22347376, 16955284, 12956412);

E (***IIS:*** 26487889, 23262803, 29035826, 22948709, 17664136 ***N:*** 22826102, 26504753, 20981553, 26423303, 10615421, 25436201, 21182845, 23262803, 20950993 ***DIS:*** none);

F (***IIS:*** 8287395, 26973173, 24923878, 23777472, 24041262, 28851567, 15734856, 22829582, 24303086, 28038474, 29062465, 29605032, 29477090, 29533954, 30797815, 28714516, 28765969; ***N***: 23342967, 21368653, 12739155, 10749717, 11247788, 25140754, 24041262, 26783539, 16955284, 12148084, 23591995, 12956412, 22482760, 28183205, 3359580, 14738870; ***DIS:*** 17505800, 9769241, 14738870);

G (***IIS:*** 27398138, 25911189, 23106693, 2338503, 29121919; ***N***: 18080084, 28335529, 20578962, 26582369, 16046302; ***DIS:*** 15480537).

H (***IIS:*** 26763290, 18689499, 18083782, 26489513, 25068621, 29474385, 18436235, 19910577, 14534360, 17008456, 18178726, 19755525, 23201226, 29912977, 22853195, 23432808, 30062222, 15677515, 26885264; ***N:*** 19151258, 19244549, 19597978, 24346177, 23507122, 26861496, 29524006, 25432364; ***DIS:*** 26229969, 25953257, 25432364.

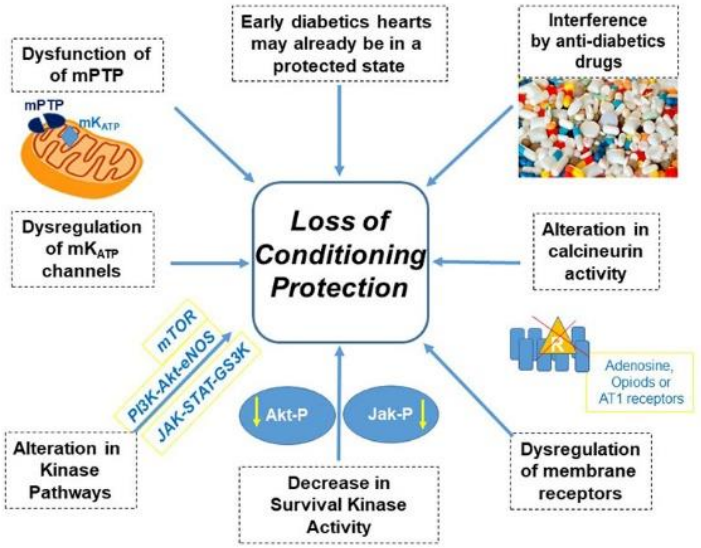

Figure 3: Mechanisms of loss of conditioning protection in diabetic hearts. In the early stages of diabetes, the heart is in a paradoxical state of protection. Subsequently, diabetic hearts may have an increased threshold for conditioning protection, the reasons for which are multifactorial. These include downregulation and alteration of the prosurvival kinase pathways, dysregulation of the mPTP, dysfunction of the mitochondrial K_{ATP} channel in the mitochondria and increased calcineurin activity. Furthermore, anti-diabetic drugs can either confer cardioprotection or interfere with endogenous cardioprotection.

Table 1: Brief overview of the most frequently used rodent models of diabetes mellitus

		Pathophysiological changes									
Model (species) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemia	Hyperlipidaemia	Hypertrophy	Hypertension	Other features	Advantages	Disadvantages
Pancreatectomy (all species) (Mering JV, 1889)	Surg	1			✓			✓	• Type 1 DM due to pancreatic mass reduction, deficient insulin production and hyperglycaemia.	• Useful for pancreatic regeneration studies. • Can be used in all animal model species. • Avoids pharmacologic toxicity of DM-induction drugs. • Similar to type-2 DM due to pancreatic degeneration.	• Risk of infection and haemorrhage. • Post-operative precautions. • Digestive complications.
Alloxan (mouse, rat) (Rerup, 1970)	Phar	1			✓			✓	• Significant hyperglycaemia. • Ketosis and/or ketoacidosis. • Glycosuria, hyperlipidaemia, polyphagia, polydipsia. • Neuropathy and cardiomyopathy.	• Fast, economic and consistent.	• High mortality rate. • Alloxan has a very short half-life (<1min). • Hyperglycaemia frequently reverts by pancreatic regeneration.
Streptozotocin (mouse, rat) (Rakieten *et al.,* 1963)	Phar	1			✓			✓	• Significant hyperglycaemia. • Polyuria, polydipsia. • Muscular atrophy. • Neuropathy.	• Fast, economic and consistent.	• High mortality rate. • Very severe model. • Muscle cachexia. • Ketoacidosis
OLETF - Otsuka Long-Evans Tokushima Fatty Rat (♂) (Kawano *et al.,* 1992)	Gen	2	✓	✓	✓	✓	✓	✓	• Polyuria, polydipsia. • Mild obesity. • Diastolic dysfunction. • Diabetic nephropathy with nodular glomerulosclerosis (30 wks).	• Good model to test antidiabetic and anti-hypertensive drugs. • Progressive DM: 1. Prediabetic phase (0-9 wks): pancreatic islet hyperplasia and lymphocytes' infiltration. 2. Type-2 DM phase: Intermediate phase (10-40 wks): pancreatic islet fibrosis. 3. Type-1 DM: (>40 wks): pancreatic islet atrophy.	• Late DM development. • Only males develop DM.
ZDF - Zucker Diabetic Fatty Rat	Gen	2	✓	✓	✓	✓	✓	✓	• Dysfunctional leptin receptor. • Hyperphagia. • 25-55% decreased GLUT4 expression.	• Widely used. • Mild hyperglycaemia (similar to humans)	• Hydronephrosis. • Due to different genetic backgrounds, no comparisons can

(Janssen *et al.,* 1999)									• Impaired cardiac contractility and diastolic function (~20 wks). • Increased fatty acid oxidation. • Progressing hepatic steatosis	• Good model to test insulin-resistance, insulin sensitizers or insulinotropic drugs. • ZDF were selectively bred from Zucker fatty rat which are similar but without hyperglycaemia.	be made between ZDF and Zucker fatty rats. • Special diet requirements. • Some degree of infertility.
		Pathophysiological changes									
Model (specie) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemia	Hyperlipidaemia	Hypertrophy	Hypertension	Other features	Advantages	Disadvantages
ZSF1 obese – Zucker Spontaneous Fatty Rat (Hamdani *et al.,* 2013)	Gen	2	✓	✓	✓	✓	✓	✓	• Dysfunctional leptin receptor. • Hyperphagia. • Metabolic syndrome. • Progressive nephropathy (~40 wks) • Liver steatosis (~20 wks) without steatohepatitis. • ♀ ZSF X ♂ SHHF rats.	• Widely used. • Robust animal model heart failure with preserved ejection fraction.	• Nephropathy. • Expensive. • Special diet requirements.
Goto-kakizaki Rat (Goto *et al.,* 1988)	Gen	2		✓	✓		✓		• Decreased insulin production. • Retinopathy, microangiopathy, neuropathy and nephropathy. • Mild hyperglycaemia at an early stage of life.	• Stable degree of glucose intolerance. • Useful for studying advanced diabetic nephropathy. • Wistar rats are the control group.	• Non obese. • Nephropathy.
Wistar Fatty Rat (Kazumi *et al.,* 1997)	Gen	1/2	✓	✓	✓	✓	✓	✓	• Hyperglycaemia, hyperlipidaemia e hyperinsulinemia (12 wks). • WKY x Zucker selective breeding.	• Wistar rats are the control group.	• Only males develop type-2 DM. • Not commercially available. • Infertile.
Db/db or Lepr^{db} Mice (Chen *et al.,* 1996)	Gen	2	✓	✓	✓	✓	✓	✓	• Leptin receptor deficiency. • Peripheral neuropathy. • Diabetic cardiomyopathy. • Impaired diastolic function, mitochondrial energetic, Ca^{2+} homeostasis and cardiac efficiency. • Increased LV mass, fatty acid oxidation and RAAS activation.	• Advantages associated with mice reduced size.	• Glucose levels progressively increase until the 16th week.

Ob/ob or **Lep^{ob} Mice** (Ingalls *et al.,* 1950)	Gen	2	✓	✓	✓	✓	✓		• Leptin deficiency. • Hyperphagia and obesity (4 wks). • Hyperglycaemia and hyperinsulinemia (15 wks, following obesity). • Impaired diastolic function, mitochondrial energetic, Ca^{2+} homeostasis and cardiac efficiency. • Increased LV mass, fatty acid oxidation and lipid content.	• Advantages associated with mice reduced size. • Allows the evaluation of the early effects of obesity and insulin resistance on cardiac function and the effects of additional hyperglycaemia at older ages. • Good model to test anti-obesity treatments.	• Certain degree of infertility. • Reduced metabolism and hypothermia. • Impaired wound healing. • Transient hyperglycaemia • Increased hormone production from both pituitary and adrenal glands.
		Pathophysiological changes									
Model (specie) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemia	Hyperlipidaemia	Hypertrophy	Hypertension	Other features	Advantages	Disadvantages
High fat diet C57BL/6J Mice (Surwit *et al.,* 1995)	Diet	2	✓	✓	✓	✓			• Leptin and insulin resistance. • Hyperphagia and obesity. • Glucose intolerance. • Cardiac dysfunction (20 wks).	• Advantages associated with mice reduced size. • Present many genetic and environmental features of the human disease. • High fat diet C57BL/6J mice changes myocardial substrate utilization prior to obesity and severe insulin resistance. • Useful for pharmacologic tests. • Similar to the onset of type-2 DM in humans	• Reduced metabolism and hypothermia. • Long period of high-fat diet intake. • Mild hyperglycaemia.
Diet-induced-obesity (DIO)-sensitive Sprague Dawley Rat (Levin *et al.,* 1997)	Diet	2	✓	✓	✓	✓			• Hyperleptinaemia.	• Similar to the onset of type-2 DM in humans	• Long period of high-fat diet intake. • The obesity prone/resistant phenotype is inheritable, making it possible to generate stable prone/resistant substrains. This reproduces many of the features of polygenic human obesity (which can be considered an advantage). • Mild hyperglycaemia.

Animal models are subdivided into 4 types: surgical (Surg), pharmacological (Phar), genetic (Gen) and diet models. LV, left ventricle; RAAS, rennin-angiotensin-aldosterone system; wks, weeks; WKY, Wistar Kyoto rats.

Table 2 – Ischaemic and Pharmacological Preconditioning in Diabetic and Hyperglycaemic Models (recent and representative articles)

Model	Ischaemia/ Reperfusion protocol	Preconditioning protocol	Results	PMID:
Ischaemic Preconditioning (IPC)				
Acute hyperglycaemia **Mice**: C57BL/6. (Acute hyperglycaemia induced by i.p. 20% dextrose 50 mins prior to LAD occlusion)	**Ex vivo**, 40 min ischaemia/1 hr reperfusion	2 cycles of 5 min ischaemia/ 5 min reperfusion	Acute hyperglycaemia exacerbates IRI and abolished IPC	24371503
Acute hyperglycaemia **Rats**: Male Wistar - (Hyperglycaemia induced by 22 mmol/l, ex vivo)	**Ex vivo**, 30 min ischaemia/2 hr reperfusion	2 cycles of 5 min ischaemia/ 5 min reperfusion	Acute hyperglycaemia did not affect IRI. IPC enhanced IRI	27959577 24908083
T1DM **Rats:** STZ, 50 mg/kg i.p	**Ex vivo**, 30 min ischaemia/2 hr reperfusion (6 wks after STZ)	2 cycles of 5 min ischaemia/5 min reperfusion	IPC was attenuated but was restored by zinc chloride and zinc ionophore pyrithione	26423303
T1DM **Rats**: Male Sprague-Dawley; STZ, 70 mg/kg	**In vivo**, 30 min ischaemia/3 hr reperfusion (7 days after STZ**)**	2 cycles of 5 min ischaemia/5 min reperfusion	T1DM did not affect IRI but IPC was abrogated Exogenous insulin supplementation restored IPC cardioprotection	23922853
TD2M **Rats**: Male Zucker diabetic fatty rats (homozygote (fa/fa)) at ages 6- (prediabetic), 12- (early TD2M) and 24-weeks of age (Late T2DM)	**Ex vivo**, 40 min ischaemia/2 hr reperfusion.	2 cycles of 5 min ischaemia/5 min reperfusion	T2DM increased vulnerability to IRI but the cardioprotective effect of IPC was preserved in in pre-diabetic, early and late stage T2DM models	29474385
TD2M **Rats**: Cohen diabetes-sensitive (CDs) rats fed high-sucrose/low-copper diet (HSD)	**Ex vivo**, 35 min ischaemia/2 hr reperfusion	3 cycles of 2 min ischaemia/3 min reperfusion	CDs-HSD hearts failed to show IPC-associated protection.	27458721
TD2M **Rats:** Diabetic Goto-Kakizaki rats, 3, 8, 12, or 18 months of age	**Ex vivo**, 35 min ischaemia/2 hr reperfusion	3 cycles of 5 min ischaemia/10 min reperfusion	T2DM was associated with increased susceptibility to IRI in the aged, diabetic heart and IPC was attenuated	23723063
T2DM **Rats:** Diabetic ZDF (fa/fa) and non-diabetic (fa/+)	**Ex vivo**, 40 min ischaemia/2 hr reperfusion; Hypoglycaemia (Hypo; glucose 3 mmol/l)	2 cycles of 5 min ischaemia/5 min reperfusion;	IPC was effective in both diabetic and non-diabetic hearts. Hypoglycaemia worsened IRI in both models and IPC in non-diabetic only.	29121919
T2DM **Rats**: Goto-Kakizaki rats (type II lean model of diabetes)	**Ex vivo**, 35 min ischaemia/2 hr reperfusion	1 or 3 cycles of 5 min ischaemia/10 min reperfusion;	3-IPC cycles were required for cardioprotection in T2DM. Pre-treatment with glimepiride lowered the threshold for IPC and both 1 and 3 cycles of IPC limited IRI.	2326338
Pharmacological Preconditioning (PPC)				
Acute hyperglycaemia **Rats**: Male Wistar, Infusion of modified Krebs–Henseleit (600 mg/dL glucose)	**Ex vivo**, 15 min ischaemia/20 min reperfusion	Insulin (0.5 U/L)	Acute hyperglycaemia blunts the cardioprotective effects of pre-ischemic insulin PPC	28376800

T1DM **Rats**: Sprague-Dawley, Diabetes injected with 1% streptozotocin (55 mg/kg)	**In vivo** 30 min ischaemia/4 hr reperfusion (5 wks after STZ)	Geniposide, intragastric administration (100 mg/kg) before, once a day for 7 days.	Geniposide PPC reduced IRI in T1DM	30797815
T1DM **Rat**: Wistar either sex, a single dose of alloxan monohydrate (120 mg/kg)	**Ex vivo**, 30-min ischaemia/2 hr reperfusion (5 wks after alloxan)	Atrial natriuretic peptide (ANP) 0.1 μM/L	ANP PPC reduced IRI in T1DM	27020807
T1DM/T2DM **Rats**: Wistar male. STZ injection at the age of 4 week (35 mg/kg, i.p).	**Ex vivo**, 30 min ischaemia/1 hr reperfusion (3 mos after STZ)	NaHS (20 μM) for 15 min prior to I/R	H_2S PPC reduced IRI in both models	30682388
T2DM **Rats**: Wistar, STZ (35 mg/kg, i.p., once) and feeding a high fat diet (HFD) for 6 weeks	**Ex vivo**, 30 min ischaemia/2 hr reperfusion	Sphingosine-1-phosphate agonist FTY720 (0.6 μmol/L) before ischaemia for 20 min	PPC by S1P agonist FTY720 reduces IRI in T2DM	26582369
T2DM **Mice**: Male nondiabetic (C57BLKS/J) and diabetic (BKS.Cg-ock7M+/+Leprdb/J mice;	**In vivo**, 30 min ischaemia/ 2 h of reperfusion	Na_2S either 24 hr before ischaemia or as a daily injection for 7 days	Na_2S PPC attenuates myocardial IRI in T2DM	23479260

PubMed Identifier number, PMID; Krebs-Henseleit, KH; Streptozotocin, STZ.

Table 3 –Ischaemic and Pharmacological Postconditioning in DM and Hyperglycaemic Models (recent and representative articles)

Model	Ischaemia/ Reperfusion protocol	Postconditioning protocol	Results	PMID:
Ischaemic Postconditioning (IPost)				
T1DM Mice: Hyperglycaemia/DM induced by a single i.p. injection of STZ (180 mg/kg). Obesity induced by high-carbohydrate diet (11 weeks) both in wildtype and TNFα knockout mice.	**Ex vivo**, 35 min ischaemia/ 45 min reperfusion (5-10 days after STZ)	6 cycles 10 s reperfusion/10 s ischaemia at the onset of reperfusion	Obese or DM mice were protected with IPost in wildtype animals but not TNFα-/- mice	23125848
T1DM **Rats**: Male Sprague–Dawley, Hyperglycaemia/DM induced by a single injection of STZ (60 mg/kg)	**Ex vivo**, 30 min global ischaemia/40 min reperfusion (8 wks after STZ)	5 cycles 10 s of reperfusion /10 s of ischaemia at the onset of reperfusion, or calcitonin gene-related peptide (CGRP) or substance P (SP)	IPost ineffective in DM hearts CGRP- or SP-induced PPC improved post-Ischaemic cardiac function and lowered CK and cTnI release	21554904
T1DM/T2DM **Mice:** Wildtype C57BL/6J; db/db mice (T2DM); C57BL/6J mice injected with STZ (150 mg/kg i.p.; T1DM), Normoglycaemia was re-established by islet cell transplantation in STZ-injected mice	**Ex vivo**, 30 min global ischaemia/2 hr reperfusion (2-4 wks after STZ)	3 cycles or 6 cycles 10 s of reperfusion/10 s of ischaemia at the onset of reperfusion	3 cycles IPost ineffective in reducing MI size in DM conditions. 6 cycles IPost worsened MI size in DM conditions Therapeutic control of insulin and blood glucose levels reestablished the infarct-sparing effect of IPost	20578962
T2DM **Mice:** Adult male C57BL/6J wild-type (WT) and type 2 DM obese db/db	**In vivo**: 30 min ischaemia/24 hr reperfusion.	6 cycles 10 s reperfusion/10 s ischaemia at the onset of reperfusion	IPost ineffective in DM	21722304
T2DM **Mice:** Wildtype C57BL/6J, Ob/Ob (DM model), and DKO (Metabolic syndrome model)	In vivo, 30 min ischaemia/1 hr reperfusion	3 cycles 10 s reperfusion/10 s ischaemia at the onset of reperfusion	DM and metabolic syndrome attenuated IPost	23507122
Pharmacological Postconditioning (PPC)				
Hyperglycaemia **Rabbits:** New Zealand white Hyperglycaemia induced by 15% dextrose for 60 min, starting 10 min before the ischaemia and continued until 10 min after the starting of reperfusion	**In vivo**, 40 min ischaemia/3 hr reperfusion	Isoflurane (1-MAC)	Hyperglycaemia inhibited isoflurane PPost Diazoxide restored isoflurane PPost	25812079
Hyperglycaemia **Rats**: Wistar. Glucose 50% was administered i.v. over 35 min starting 5 min before ischaemia and was continued until 5 min of reperfusion	**In vivo**, 25 min ischaemia/2 hr rreperfusion	Sevoflurane (1-MAC) for 5 min starting 1 min prior to the onset of reperfusion	Hyperglycaemia inhibited Sevoflurane PPost Sevoflurane PPost restored by CsA	18305078
Hyperglycaemia **Rats**: Wistar, Hyperglycaemia induced by 50% glucose starting 5 min before ischaemia and lasting until 60 min after reperfusion	**In vivo**, 30 min ischamia/2 hr reperfusion	Milrinone (a phosphodiesterase 3 inhibitor; 30 μg/kg) or Levosimendan (a calcium sensitizer; 10-	Normal dose of milrinone and high dose of levosimendan, were protective in hyperglycaemic conditions.	22239823

		100 μg/kg) given 5 min before reperfusion		
Hyperglycaemia Cultured primary neonatal rat cardiomyocytes incubated in a high glucose concentration medium (D-glucose final concentration 35 mM) for 48 h	**In vitro**, 3 hr of hypoxia/3 hr reoxygenation	Sevoflurane (2.4%) to the cells at the beginning of reoxygenation for 15 min	Sevoflurane PPost ineffective dynamin-related protein 1 inhibitor restored protective effects	27684054
T1DM **Rat**: Sprague–Dawley Hyperglycaemia/DM induced by a single intravenous injection of STZ (65 mg/kg)	**In vivo**, 30 min ischaemia/3 hr reperfusion (4-5 wks after STZ)	Sevoflurane 2.4% given by inhalation for 5 min at the end of ischaemia; 3 cycles of 20 s occlusion/20 s reperfusion after myocardial ischaemia	IPost and sevoflurane PPost ineffective in DM model	21368653
T1DM **Rats**: Sprague-Dawley Male Hyperglycaemia/DM induced by a single injection of STZ (50 mg/kg i.p.),	**Ex vivo**, 35 min global ischaemia/1 hr reperfusion (4 wks after STZ)	Sevoflurane (2.4% and 3.6%) for 15 min, at the end of ischaemia bubbled at a rate of 1.5 Litre/min into the perfusion buffer	Sevoflurane PPost ineffective in DM model, even when pre-treated with simvastatin	30682140
T1DM **Rats**: male Sprague-Dawley, DM induced by a single injection of STZ (55 mg/kg) Normoglycaemia was re-established by 2-days or 2-wks insulin treatment in STZ-injected animals	**In vivo**, 30 min ischaemia/2 hr reperfusion (2 days or 2wks after STZ)	Sufentanil PPost (1 μg/kg, i.v.) 5 min before the onset of reperfusion	Sufentanil PPC restored by long-term insulin treatment.	26748398
T1DM **Rats**: Sprague-Dawley male, DM induced by high-fat diet after 4 wks, STZ (45 mg/kg)	**In vivo**, 40 min ischaemia/ 3 hr reperfusion (4 wks after STZ)	Atorvastatin	Atorvastatin PPC effective	28672889
T1DM **Rats**: Male Sprague-Dawley, DM induced by a single injection of STZ (65 mg/kg, i.p.)	**In vivo**, 45 min ischaemia/ 90 min reperfusion (5-8 wks after STZ)	Sevoflurane 2% for 15 min in the second half of ischaemia	N-Acetylcysteine co-administered with STZ restored sevoflurane PPost cardioprotection	26783539
T1DM **Rats**: Male Sprague-Dawley, Hyperglycaemia/DM induced by a single injection of STZ (65 mg/kg, i.p.)	**In vivo**, 30 min ischaemia/2 hr reperfusion (2 wks after STZ)	Sevoflurane (1-MAC) first 5 min after the onset of reperfusion.	DM blocked sevoflurane PPost	22482760
T1DM/T2DM **Patients:** right atrial trabeculae obtained from patients with T1 or T2DM	**In vitro**, 30 min hypoxia/1 hr reoxygenation	Desflurane (3, 6 and 9%) administered during the first 5 min of reoxygenation.	Only PPost with desflurane 6 or 9% improved isometric force of contraction in both types of DM myocardium	19945895 21862498
T2DM **Mice**: Male C57BL/6, DM induced by STZ (40 mg/kg, i.p.) for five consecutive day	**In vivo**, 45 min ischaemia/2 hr reperfusion.	Sevoflurane inhalation of 2% during the first 15 min of coronary reperfusion period.	DM blocked sevoflurane PPost.	26973173
T2DM **Rats**: Sprague-Dawley, DM induced by high-fat and high-sugar diet for 6 wks and a single injection of STZ (40 mg/kg, i.p.)	**In vivo**, 40 min ischaemia/2 hr reperfusion (1 wk after STZ)	Sevoflurane (2.4%; 1-MAC) for 15 min at onset of reperfusion	Sevoflurane PPost ineffective in DM model. Deferoxamine-activated hypoxia-inducible factor-1 restored sevoflurane PPost	28316125

T2DM **Rats**: Zucker Obese (preDM /normoglycaemic model)	**In vivo**, 25 min ischaemia/2 hr reperfusion	Sevoflurane (1-MAC) for 5 min starting 1 min prior to the onset of reperfusion	Sevoflurane PPost ineffective in pre-DM model, regardless of pre-treatment with CsA	19819119

PubMed Identifier number, PMID; Diabetes Mellitus, DM; Minimal Alveolar Concentration, 1-MAC; Streptozotocin, STZ.