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

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Abbreviations: AMI, acute myocardial infarction; DPP-4, dipeptidyl peptidase-4; DM, diabetes mellitus; eNOS, endothelial NOS; HF, heart failure; HKII, hexokinase II; IPC, ischaemic preconditioning; IPost, ischaemic postconditioning; IRI, ischaemia–reperfusion injury; IS, infarct size; MI, myocardial infarct; PPCI, percutaneous coronary intervention; RIC, remote ischaemic conditioning; RISK, reperfusion injury salvage kinase; SAFE, survivor activating factor enhancement; SGLT2, sodium–glucose co-transporter 2; STEMI, ST-elevation myocardial infarction; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Abstract
Diabetic patients are at increased risk of developing coronary artery disease and experience worse clinical outcomes following acute myocardial infarction. Novel therapeutic strategies are required to protect the myocardium against the effects of acute ischaemia–reperfusion injury (IRI). These include one or more brief cycles of nonlethal ischaemia and reperfusion prior to the ischaemic event (ischaemic preconditioning [IPC]) or at the onset of reperfusion (ischaemic postconditioning [IPost]) either to the heart or to extracardiac organs (remote ischaemic conditioning [RIC]). Studies suggest that the diabetic heart is resistant to cardioprotective strategies, although clinical evidence is lacking. We overview the available animal models of diabetes, investigating acute myocardial IRI and cardioprotection, experiments investigating the effects of hyperglycaemia on susceptibility to acute myocardial IRI, the response of the diabetic heart to cardioprotective strategies e.g. IPC, IPost and RI. Finally highlighting the effects of anti-hyperglycaemic agents on susceptibility to acute myocardial IRI and cardioprotection.

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 diabetes mellitus 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 diabetes mellitus patients, the risk of developing cardiovascular disease is increased twofold to fourfold, when compared with non-diabetes mellitus patients (Bertoluci & Rocha, 2017). Furthermore, patients with experience worse clinical outcomes in a number of clinical settings of acute myocardial ischaemia–reperfusion injury (IRI), including acute myocardial infarction (AMI; Donahoe et al., 2007), coronary angioplasty (Mathew et al., 2004) and cardiac bypass surgery (Alserius, Hammar, Nordqvist, & Ivert, 2006), suggesting that the diabetic heart may be more susceptible to acute ischaemia–reperfusion injury. 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 ischaemia–reperfusion injury (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 ischaemia–reperfusion injury 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 (Bøtker et al., 2018; Jones et al., 2015).

Given the worse clinical outcomes in diabetic patients with coronary artery disease, novel therapeutic strategies, which are effective in the diabetic heart, are required to protect the myocardium against the detrimental effects of acute ischaemia–reperfusion injury. A number of strategies exist for protecting the heart against acute. 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 to an organ/tissue F1 away from the heart (remote ischaemic conditioning [RIC]; Figure 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, Hausenloy, Heusch, Baxter, & Schulz, 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 ischaemia–reperfusion injury and cardioprotection. Next, we perform a comprehensive review of experimental studies investigating the effects of hyperglycaemia on susceptibility to acute myocardial ischaemia–reperfusion injury. 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 ischaemia–reperfusion injury and cardioprotection.

2 | EXPERIMENTAL ANIMAL MODELS OF DIABETES

Animal models of diabetes mellitus are crucial to understanding the pathophysiological effects of diabetes on the cardiovascular system, identifying and validating novel therapeutic targets and signalling pathways. Diabetes mellitus animal models can be subdivided into four groups: surgical, pharmacological, diet and genetic/selective inbreeding-induced diabetes mellitus (summarised in Table 1). Surgical (pancreatectomy) and pharmacological models usually result in pancreatic mass reduction, insulin deficiency and hyperglycaemia and thus represent type 1 diabetes mellitus (T1DM) models. Pharmacological models include injection of drugs such as streptozotocin or alloxan, which are selectively toxic to pancreatic β-cells, and induce diabetes mellitus as early as 24–48 hr post-injection (Rerup & Tarding, 1969). Selective in-breeding has produced several rodent models of type 2 diabetes mellitus (T2DM), usually associated with a panoply of risk factors. The most common genetic rodent models of type 2 diabetes mellitus include Zucker diabetic fatty, obese ZSF1 rats, and db/db and ob/ob mice. All of these models display dysfunctional or absent **leptin** homeostasis and insulin resistance at different time points. Type 2 diabetes mellitus can also be induced by diets with high-fat and/or high-carbohydrate content (Table 1; Maioli et al., 2016). Diet-induced diabetes mellitus requires months to achieve the full type 2 diabetes mellitus spectrum and no standard protocol has been established. This prolonged onset of type 2 diabetes mellitus might be closer to the human scenario, providing several opportunities to perform acute myocardial ischaemia–reperfusion injury 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, Dabkowski, Ribeiro, & O'Connell, 2012). Thus, diet formulation should be taken into account (Heydemann, 2016). Many of these rodent models share many features with human diabetes mellitus cardiomyopathy (Bugger & Abel, 2008) as well as higher incidence of acute myocardial ischaemia–reperfusion injury (Greer, Ware, & Lefer, 2006). There are several limitations of diabetes mellitus animal models that need to be taken into consideration: (a) 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; (b) pancreatic islets architecture is distinct from humans; (c) monogenic models are not representative of human diabetes mellitus; (d) diabetes mellitus develops at varying stages in rodent models, which has an impact on the timing of the acute myocardial ischaemia–reperfusion injury study. In the initial stages, ischaemia–reperfusion injury may reflect changes that are secondary to damaging circulatory metabolic milieu and the underlying obesity and insulin resistance, whereas in the later stages, ischaemia–reperfusion injury may reflect the added effects of hyperglycaemia of different durations and (e) in genetic models, metabolic dysregulation appears at very early developmental stages. Finally, the lack of spontaneous ischaemia and atherosclerosis in rodents (Boudina & 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, Goldstein, Brown, Herz, & Burns, 1994).

Although there is no animal model that fully represents the 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 ischaemia–reperfusion injury is of paramount translational value (reviewed in Elmadhun et al., 2013). Pig models of diet-induced metabolic syndrome and type 2 diabetes mellitus, streptozocin- or alloxan-induced type 1 diabetes mellitus or genetically engineered pigs can be used in ischaemia–reperfusion injury 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 ischaemia–reperfusion injury, either hyperglycaemia 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 recognised diabetes. In fact, clinical outcomes in non-diabetic patients were significantly worse when compared with 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, correlated with glucose levels at the time of presentation, with greater infarct sizes (ISs) 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 are important in determining the cardiac damaging effects of diabetes (Giblett, Clarke, Dutka, & Hoole, 2016; Ishibashi et al., 1994). 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, hyperglycaemia, 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, Itoh, Sunaga, & Miura, 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 weeks) of type 1 diabetes mellitus, late phase of type 1 diabetes mellitus (>2 weeks) and type 2 diabetes mellitus. Each condition was additionally split into *ex vivo* (isolated heart) and *in vivo* models. This allowed us to separate pathological 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.

3.1 | Acute hyperglycaemia

In the isolated heart perfused in the absence of insulin, most studies reported increased infarct size with hyperglycaemia, although some studies also reported decreased infarct size (Figure 2a). Increased infarct size was commonly observed with glucose levels >30 mM, whereas reduced infarct size was associated with glucose around 8 mM; infarct size was unaltered with glucose levels between 11 and 22 mM. In the *in vivo* models (Figure 2b), most studies compared normoglycaemia, 5–10 mM, with hyperglycaemic levels between 15 and 20 mM demonstrating, for unclear reasons, either no effects on infarct size or increased vulnerability to acute ischaemia–reperfusion injury. It therefore seems that at 20-mM glucose, *in vivo* hearts show vulnerability to acute ischaemia–reperfusion injury as compared with *ex vivo* hearts. This seems counterintuitive knowing that, *in vivo*, hyperglycaemia increases insulin plasma level, whereby insulin can act as a cardioprotective agent against acute ischaemia–reperfusion injury (Zuurbier, Eerbeek, & Meijer, 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 infarct size increased when raising glucose from 16 to 30 mM (Kersten, Schmeling, Orth, Pagel, & Warltier, 1998). For both the *ex vivo* and *in vivo* conditions, hyperglycaemia above 10 mM never reduced infarct size of the heart. In summary, acute hyperglycaemia increases infarct size in the isolated heart when glucose >30 mM, whereas increases in infarct size are already present *in vivo* at glucose levels of 20 mM.

3.2 | Early phase of type 1 diabetes mellitus (≤2 weeks)

Interestingly, the early phase of type 1 diabetes mellitus is often associated with reduced infarct size in the *ex vivo* heart (Figure 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 type 1 diabetes mellitus heart have been proposed, such as increased expression and/or phosphorylation of **Akt**, **endothelial NOS** (eNOS), PKC, **ERK** and **heat shock proteins** or maintenance of end-ischaemic mitochondrial hexokinase II (HKII; Gurel et al., 2013). Keeping HKII at the mitochondria during ischaemia is known to confer protection against cardiac ischaemia–reperfusion injury (Smeele et al., 2011). In contrast, *in v*ivo, the early phase of type 1 diabetes mellitus was associated with an increase in susceptibility to acute ischaemia–reperfusion injury (Figure 2d). This is likely due to the fact that in the *in vivo* setting, hearts are subjected to acute ischaemia–reperfusion injury at higher glucose levels >20 mM. In summary, in the early type 1 diabetes mellitus condition, there appears to be intrinsic protection in the isolated heart, whereas there is increased vulnerability of the *in vivo* heart to acute ischaemia–reperfusion injury and this is likely due to the hyperglycaemic conditions. This offers the therapeutic option of targeting these extracardiac factors of the metabolic milieu, for example, with exogenous insulin and drugs that lower blood glucose, to reduce acute ischaemia–reperfusion injury of the early type 1 diabetes mellitus heart.

3.3 | Late phase of type 1 diabetes mellitus (>2 weeks)

After prolonged type 1 diabetes mellitus, the isolated heart appears to lose its protected state, showing either similar or increased infarct size (Figure 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 (Figure 2f), the susceptibility to acute ischaemia–reperfusion injury was also increased.

3.4 | Type 2 diabetes mellitus

The isolated heart of type 2 diabetes mellitus animals shows a mixed response to acute ischaemia–reperfusion injury experiments performed using the isolated heart of type 2 diabetes mellitus animals that show mixed results, with most studies reporting either increased infarct size or no change in infarct size (Figure 2g) and a minority showing reduced infarct size. However, type 2 diabetes mellitus in the *in vivo* setting was mainly associated with increased infarct size (Figure 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 free fatty acid levels. These hearts are insulin resistant, rendering the protective reperfusion injury salvage kinase (RISK) pathway to be less responsive to acute ischaemia–reperfusion injury. The RISK pathway concerns pro-survival kinase signalling cascades, such as PI3K–Akt and p42/p44 ERK Erk 1/2. Activation of these kinase pathways confers protection against ischaemia–reperfusion injury (Hausenloy & Yellon, 2004).

Interestingly, in the *in vivo* setting, a few studies report hearts to have reduced infarct size. This could be related to the obesity paradox and early type 2 diabetes mellitus, where insulin signalling may be still effective and cellular protective signalling pathways are initially activated, similar to that observed in the early type 1 diabetes mellitus setting.

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

4 | EFFECTS OF HYPERGLYCAEMIA AND DIABETES ON ISCHAEMIC PRECONDITIONING (IPC)

In order to protect the diabetic heart against the detrimental effects of acute ischaemia–reperfusion injury, 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 confirmed in all species tested including man and have also been shown to be effective in the multicentre network of experimental research centres that made up the Consortium for preclinicAl assESsment of cARdioprotective therapies (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 diabetes mellitus (Ferdinandy et al., 2014). IPC cardioprotective mechanisms have been extensively described and include RISK, survivor activating factor enhancement (SAFE), and NO/PKG pathways that converge on mitochondria (Hausenloy et al., 2016; Penna et al., 2015).

Several studies have evaluated the effect of IPC on cardiac ischaemia–reperfusion injury 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 ischaemia–reperfusion injury have also been reported as a consequence of either IPC or pharmacological preconditioning (del Valle, Lascano, Negroni, & Crottogini, 2003; Kristiansen et al., 2004). 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, Wynne, Mocanu, & Yellon, 2013; Tsang, Hausenloy, Mocanu, Carr, & Yellon, 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 prediabetic and early- and late-stage type 2 diabetes mellitus 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 ischaemia–reperfusion injury and the response to cardioprotective manoeuvres to a greater extent in a non-diabetic when compared with 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 interindividual 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 revascularisation (Lee & 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, Hausenloy, Wynne, & Yellon, 2010). The limited possibilities to study IPC in humans and the fact that patients with diabetes are increasingly well controlled by drugs make 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, Ozgen Altintas, Kumas, & Asil, 2019; 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 down-regulation (Yadav, Singh, & Sharma, 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; Figure 3). **Glimepiride**, an activator of Akt, may lower the threshold for IPC. Thus, both 1 and 3 cycles of IPC (5/10 min of ischaemia/reperfusion) may induce a cardioprotective effect in diabetic rat hearts treated with glimepiride (Hausenloy, Wynne, et al., 2013).

In summary, in experimental animal and human *ex vivo* heart tissue studies, the presence of hyperglycaemia and diabetes mellitus appears 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 diabetes mellitus 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 acute myocardial infarction as such, IPost, which is applied at the onset of reperfusion, may be more effective in the setting of acute myocardial infarction.

5 | EFFECTS OF HYPERGLYCAEMIA AND DIABETES ON ISCHAEMIC POSTCONDITIONING (IPost)

Since IPost can be applied at the onset of reperfusion, it can be easily applied to acute myocardial infarction at the time of percutaneous coronary intervention (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, Moro, Tullio, Perrelli, & Penna, 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), which recruit known cardioprotective signalling pathways (such as the SAFE, NO/PKG and RISK cascades) and which converge on the mitochondrial permeability transition pore (Bell et al., 2016; Boengler, Heusch, & Schulz, 2011; Cohen & Downey, 2011; Lacerda, Opie, & Lecour, 2012; Oosterlinck et al., 2013; Pagliaro et al., 2011; Pagliaro & Penna, 2015; Penna et al., 2015).

The clinical studies of IPost in ST-elevation myocardial infarction (STEMI) patients have mixed results with IPost limiting MI size (assessed by cardiac biomarkers and cardiac MRI) in most (Staat et al., 2015; Thibault, Piot, & Ovize, 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 percutaneous coronary intervention, 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 preinfarct angina, lack of direct stenting, the presence of co-morbidities (such as diabetes) and co-medications (such as platelet **P2Y12** inhibitors). Here, we will focus on the experimental data reporting the effect **T3** 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 type 1 diabetes mellitus and type 2 diabetes mellitus animal models (Drenger et al.,2011; Przyklenk, Maynard, Greiner, & Whittaker, 2011; Ren, Song, Lu, & Chen, 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 diabetes mellitus and type 2 diabetes mellitus 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, Gozal, Navot, & Zuo, 2015; Figure 3). It has been suggested that PTEN/Akt signalling is altered in the presence of diabetes (Mocanu & 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-mediated up-regulation of calcineurin activity, however it is not clear how calcineurin activity interferes with protection by Jak2 (Hotta et al., 2010). Recently, also in a hyperglycaemic experimental model, a reduced level of Akt phosphorylation has been observed, a condition that 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 ischaemia–reperfusion injury 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 type 2 diabetes mellitus, 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, Xi, & Kukreja, 2012). In summary, the presence of hyperglycaemia/diabetes appears to blunt IPost via the down-regulation 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 REMOTE ISCHAEMIC CONDITIONING (RIC)

The major disadvantage of both IPC and IPost is that they require the intervention to be applied directly to the heart, thereby hamperingtheir clinical translation to acute myocardial infarction 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 ischaemia–reperfusion injury 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 percutaneous coronary intervention has been reported to improve myocardial salvage and/or reduce MI size (Hausenloy et al., 2015). However, the recently published large multicentre 5,401 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 (HF) when compared with 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 diabetes mellitus) 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 acute myocardial infarction model. 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 are consistent with this agent antagonising the ATP-dependant potassium channel, which is known to mediate cardioprotection. Recently, a review by Tyagi, Singh, Virdi, and Jaggi (2019) summarised 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 type 2 diabetes mellitus patients (Istenes et al., 2014). Indeed, the metaboreflex (the reflex response stimulated by metabolite accumulation during limb exercise and/or ischaemia) is abnormal in type 2 diabetes mellitus patients and it is characterised by an exaggerated vasoconstriction (perhaps due to sympathetic overstimulation) 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, Støttrup, Kristiansen, & Bøtker, 2012). However, additional studies are necessary (especially multicentric randomised clinical trial in patients with acute myocardial infarction 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-acute myocardial infarction 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 diabetes mellitus is actually a confounder of limb RIC cardioprotection.

7 | EFFECTS OF ANTI-HYPERGLYCAEMIC MEDICATIONS ON ACUTE MYOCARDIAL ISCHAEMIA–REPERFUSION INJURY 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 that may be unrelated to cardioprotection against acute myocardial ischaemia–reperfusion injury. 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 antihyperglycaemic medications on acute myocardial ischaemia–reperfusion injury and cardioprotection.

7.1 | 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, Lazo, & Parker, 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 (Ye et al., 2011). It appears that the newer sulphonylureas such as **glimepiride** (Mocanu et al., 2001) and gliclazide (Maddock, Siedlecka, & Yellon, 2004) do not interfere with IPC cardioprotection, and this is possibly related to their greater specificity for pancreatic compared with myocardial K_{ATP} channels (Gribble & Ashcroft, 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).

7.2 | Metformin

This agent is a biguanide whose effects are mediated by the activation of the AMP-activated protein kinase and 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 Ye et al., 2011). The mechanisms underlying metformin cardioprotection are diverse and include activation of adenosine receptors, recruitment of the RISK pathway, AMP-activated protein kinase activation, modulation of complex I and inhibition of mitochondrial permeability transition pore opening at reperfusion (Bromage & 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 glucoselowering effects (Varjabedian, Bourji, Pourafkari, & Nader, 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.

7.3 | 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, Hu, Lin, Zhang, & Perez-Polo, 2010), activation of the RISK pathway (Wynne, Mocanu, & Yellon, 2005) and alternative pathways including Src family kinase- and MMP-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 type 2 diabetes mellitus (Nissen et al., 2008), whereas in contrast, **rosiglitazone** has been associated with worsened adverse cardiovascular outcomes (Lincoff, Wolski, Nicholls, & Nissen, 2007).

7.4 | Glucagon-like peptide-1 receptor agonists

This class of anti-hyperglycaemic agents lower blood glucose levels by an insulin incretin effect (Peng, Want, & Aroda, 2016). Several studies have shown that **GLP-1** or GLP-1 analogues administered as either preconditioning or postconditioning agents limit MI size in small animal models (Matsubara et al., 2009; Sonne, Engstrom, & Treiman, 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). Glucagonlike peptide-1 receptor agonist therapy can also modulate innate immune-mediated inflammation (Hogan et al., 2014). Limited data suggest that GLP-1 receptor agonists may be effective for the treatment of cardiac disorders in patients with and without diabetes mellitus. These studies suggest that GLP-1 receptor agonists may have potential pleiotropic beneficial effects in patients with cardiovascular disease beyond their role in managing diabetes. These medications may be cardioprotective after an acute myocardial infarction but are less promising in heart failure (Marso et al., 2016; reviewed in Wroge & Williams, 2016).

7.5 | Dipeptidyl peptidase-4 inhibitors

This is a new class of drugs for treating type 2 diabetes mellitus lowering blood glucose levels by augmenting endogenous levels of GLP-1 through the inhibition of dipeptidyl peptidase-4 (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, Whittington, et al., 2013; Theiss, Gross, & Vallaster, 2013). The mechanism of action includes augmentation of the effects of endogenous incretins and activation of the GLP-1 receptor leading to generation of cAMP with downstream activation of PKA (reviewed in Yoon, Ye, & Birnbaum, 2014). Clinical trials evaluating the overall cardiovascular risks and benefits after administration of dipeptidyl peptidase-4 inhibitors have shown that hospitalisation for heart failure 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 dipeptidyl peptidase-4 inhibitors and heart failure is complex (reviewed in Ziff, Bromage, Yellon, & Davidson, 2018).

7.6 | Sodium–glucose co-transporter 2 inhibitors

This new class of approved anti-hyperglycaemic agents lower blood glucose by inhibiting glucose reabsorption in the kidney (Majewski & 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 DECLARETIMI 58 trial, Wiviott et al., 2019) have shown that the sodium– glucose co-transporter 2 (SGLT2) inhibitors (**empagliflozin**, **canagliflozin** and **dapagliflozin**) reduced rates of cardiovascular death and hospitalisation for heart

failure in type 2 diabetes mellitus 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 ischaemia–reperfusion injury. 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 diabetes mellitus and non-diabetes mellitus rodent models of acute myocardial ischaemia–reperfusion injury. The cardioprotective mechanisms have been attributed to a variety of factors including increased **STAT3** phosphorylation, reduced myocardial **IL-6** and **inducible NOS** 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 longterm treatment (Lim et al., 2019; Uthman et al., 2019). However, SGLT2 inhibitors have shown acute functional protective effects, improving cardiac performance during ischaemia (Baker et al., 2019; Uthman 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, Vargas-Delgado, Santos-Gallego, & Badimon, 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 antihyperglycaemic 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, dipeptidyl peptidase-4 inhibitors and SGLT2 inhibitors) appear to exert cardioprotective effects against acute ischaemia–reperfusion injury 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, dipeptidyl peptidase-4 inhibitors, and SGLT2 inhibitors.

8 | SUMMARY AND CONCLUSIONS

In summary, there are 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, Bøtker, Ovize, Hausenloy, & Heusch, 2019). At least in the experimental setting, a stronger "conditioning" stimulus or use of certain drugs can target hyperglycaemia/diabetes mellitus-induced down-regulated 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/diabetes mellitus on endogenous cardioprotection. Importantly, additional clinical studies are needed to confirm whether the confounding effects of hyperglycaemia/diabetes mellitus on endogenous cardioprotection are replicated in diabetic patients. The latter is difficult to investigate given that most diabetes mellitus patients are on anti-hyperglycaemic agents (such as GLP-1 agonists, dipeptidyl peptidase-4 inhibitors and SGLT2 inhibitors), which in themselves are known to be cardioprotective. Importantly, novel cardioprotective strategies for inducing ischaemia tolerance and to reduce ischaemia–reperfusion injury in patients with diabetes are needed to improve clinical outcomes in this high-risk group.

8.1 | Nomenclature of targets and ligands

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 Q31 Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Fabbro, et al., 2019; Alexander, Kelly, et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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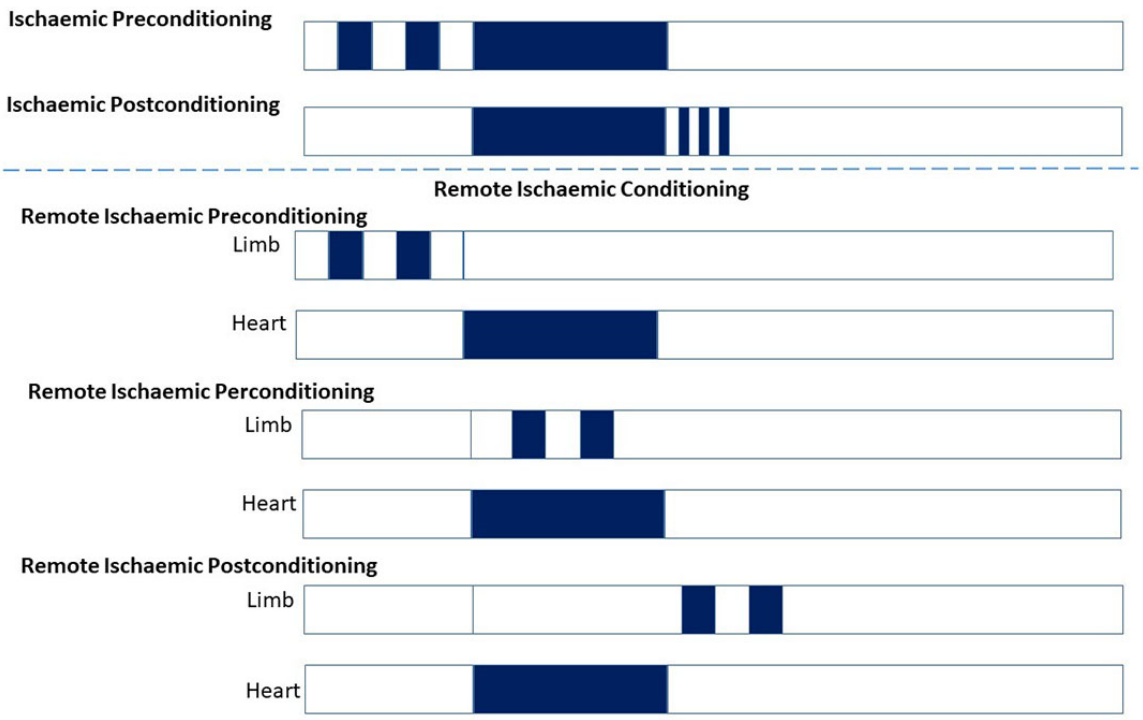

Figure 1: Schematic representation of the various protocols of ischaemic conditioning. White boxes represent time of normal perfusion or reperfusion; dark blue boxes are periods of ischaemia. The number and duration of cycles used can vary with different experiments.

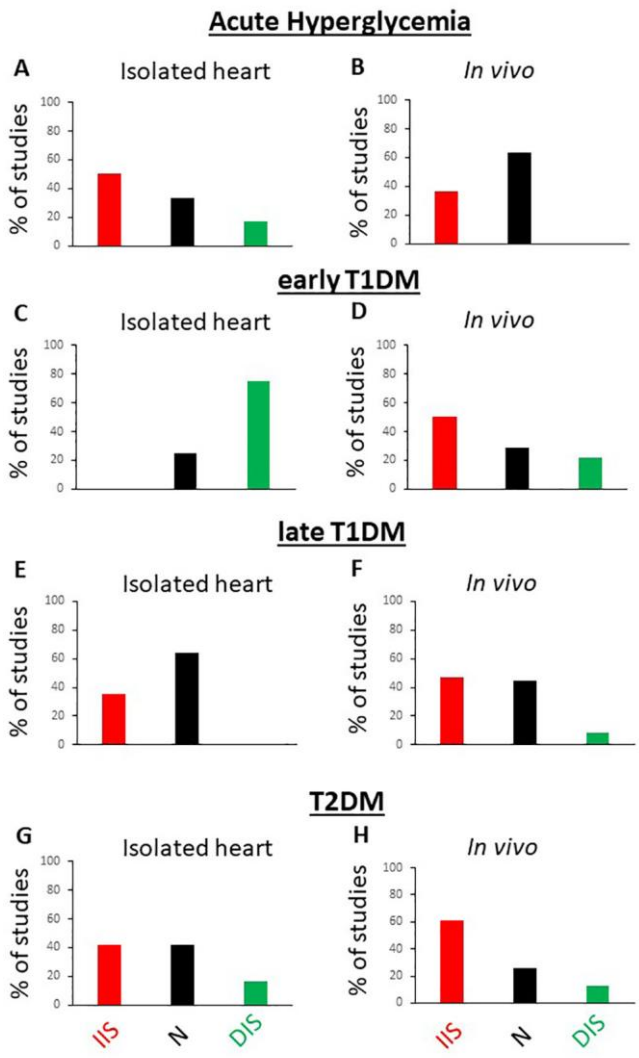

Figure 2: Schematic summary of 130 studies reporting effects on cardiac sensitivity to IRI of acute hyperglycaemia (A, B), early type 1 Diabetes Mellitus (C, D), late type 1 Diabetes Mellitus (E, F) and Type 2 Diabetes Mellitus (G, H) for the isolated heart (A, C, E and G) or the *in vivo* condition (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.

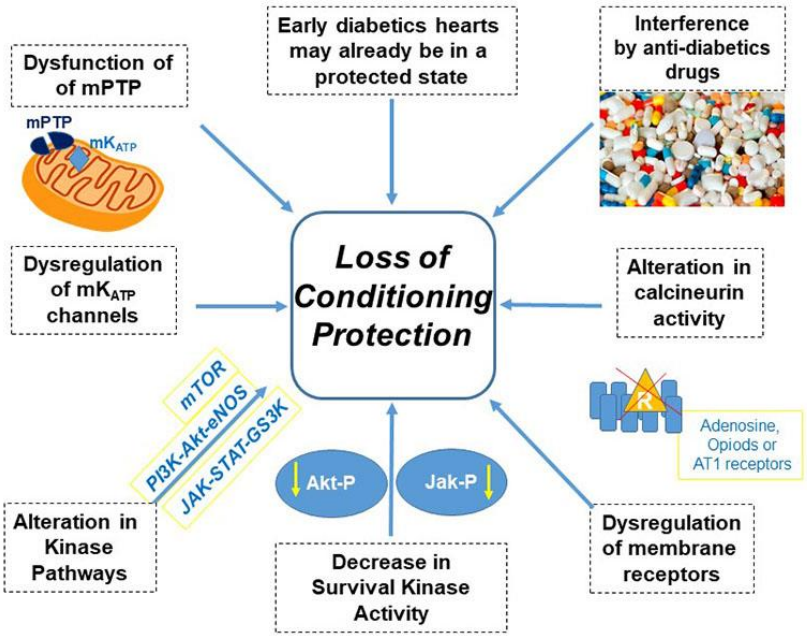

Figure 3: Mechanisms of loss of conditioning protection in diabetic hearts. In the early stages of diabetes, the heart is in a paradoxical state of protection. Subsequently, diabetic hearts may have an increased threshold for conditioning protection, the reasons for which are multifactorial. These include 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
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.
Alloxan (mouse, rat) (Rerup, 1970)	Phar	1			✓			✓	• Significant hyperglycaemia. • Ketosis and/or ketoacidosis. • Glycosuria, hyperlipidaemia, polyphagia, polydipsia. • Neuropathy and cardiomyopathy.	• Fast, economic and consistent.
Streptozotocin (mouse, rat) (Rakieten *et al.,* 1963)	Phar	1			✓			✓	• Significant hyperglycaemia. • Polyuria, polydipsia. • Muscular atrophy. • Neuropathy.	• Fast, economic and consistent.
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 hype lymphocytes' infiltration. 2. Type-2 DM phase: Intermediate phase (10-40 wks): pa fibrosis. 3. Type-1 DM: (>40 wks): pancreatic islet atrophy.
ZDF - Zucker Diabetic Fatty Rat (Janssen *et al.,* 1999)	Gen	2	✓	✓	✓	✓	✓	✓	• Dysfunctional leptin receptor. • Hyperphagia. • 25-55% decreased GLUT4 expression. • Impaired cardiac contractility and diastolic function (~20 wks). • Increased fatty acid oxidation. • Progressing hepatic steatosis	• Widely used. • Mild hyperglycaemia (similar to humans) • Good model to test insulin-resistance, insulin sensitizers or in drugs. • ZDF were selectively bred from Zucker fatty rat which are sim without hyperglycaemia.
		Pathophysiological changes								

Model (specie) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemia	Hyperlipidaemia	Hypertrophy	Hypertension	Other features	Advantages
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 fra
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.
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.
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.
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+}	• Advantages associated with mice reduced size. • Allows the evaluation of the early effects of obesity and insul on cardiac function and the effects of additional hyperglycaer ages. • Good model to test anti-obesity treatments.

									homeostasis and cardiac efficiency. • Increased LV mass, fatty acid oxidation and lipid content.	
		Pathophysiological changes								
Model (specie) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemia	Hyperlipidaemia	Hypertrophy	Hypertension	Other features	Advantages
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 hum • High fat diet C57BL/6J mice changes myocardial substrate uti to obesity and severe insulin resistance. • Useful for pharmacologic tests. • Similar to the onset of type-2 DM in humans
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

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

Abbreviations: KH, Krebs–Henseleit; LAD, left anterior descending artery; PMID, PubMed identifier number; STZ, streptozotocin; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.