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

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Abstract

Diabetic 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 endogenous 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 endogenous 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 systematic 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 endogenous 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.

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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

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

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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 3 fold when compared to non-DM patients. 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; Haffner *et al.,* 1998; Malmberg *et al.,* 2000), coronary angioplasty (Mathew *et al.,* 2004), and cardiac bypass surgery (Alserius *et al.,* 2006; Calafionre *et al.,* 2003; Thourani *et al.,* 1999), 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 (Li *et al.,* 2013b; 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, which are effective in the diabetic heart, are required to protect the myocardium against the detrimental effects of acute IRI. A number of endogenous 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) (Murry *et al.,* 1986) or at the onset of reperfusion (ischaemic postconditioning, IPost) (Zhao *et al.,* 2003) 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

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amenable to endogenous cardioprotection. In experimental animal studies, it appears that the diabetic heart is resistant to endogenous cardioprotection (Andreadou *et al.,* 2015; Ferdinandy *et al.,* 2014; Heusch 2015a), but clinical evidence for this is lacking. Pharmacological agents which recruit the signaling 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 available animal models of diabetes for investigating acute myocardial IRI and cardioprotection. Next, we perform a systematic 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 endogenous 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

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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; Van den Bergh *et al.,* 2006) as well as propensity to 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 – if it is performed before the onset of DM, it may reflect changes that are secondary to damaging circulatory metabolic milieu and the underlying obesity and insulin-resistance, whereas if it is performed after the onset of DM, it may reflect the added effects of hyperglycaemia of different durations; (5) finally, the lack of spontaneous ischaemia and atherosclerosis in rodents (Boudina and Abel, 2007), can be considered 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, large animal models are available that closely mimic human physiology and anatomy. In particular, the minipig and pig heart models with regional myocardial IRI is of paramaount translational value (reviwed in Elmadhun *et al.,* 2013; Heusch *et al.,* 2011; Spurlock and Gabler, 2008). Pig models of diet-induced metabolic syndrome and T2DM, streptozocin- or alloxan-induced T1DM, or genetically engineered pigs can be used in coronoropathies and IRI studies (Diemar *et al.,* 2015; Jones *et al.,* 2015; McKenney-Drake *et al.,* 2016; Neeb *et al.,* 2010; Trask *et al.,* 2012; Wolf *et al.,* 2014). Although the cardioprotective

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signalling is in part different from that in rodent hearts, all cardioprotective phenomena have been demonstrated in pigs (Skyschally *et al.,* 2018; Heusch *et al.,* 2011).

3. Effects of hyperglycaemia and diabetes on the susceptibility to acute myocardial ischaemia/reperfusion injury

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 order to better understand the relationship between glucose levels and MI size in the experimental setting, we undertook a systemic review of all studies published between January 2012 and February 2019, investigating the effect of hyperglycaemia on MI size. We identified 84 articles that fulfilled these selection criteria. 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

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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 (Fig. 2A) there was a biphasic phase response to cardioprotection according to perfused glucose levels, with cardioprotection at 8 mM, no effects on cardioprotection at 11-22 mM, and increased susceptibility to acute IRI at >30 mM. For the *in-vivo* models (Fig. 2B), most studies compared normoglycaemia, 5-10 mM, with hyperglycaemic levels between 15-20 mM demonstrating either no effects on cardioprotection, 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 the *in-vivo* condition, hyperglycaemia will induce increases in insulin plasma level, whereby insulin can act as a cardioprotective agent against acute IRI (Jonassen *et al.,* 2001; 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 vulnerability 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 induced a protected state of the heart. In summary, acute hyperglycaemia increases susceptibility to acute IRI in the isolated heart when glucose >30 mM, whereas increased vulnerability to acute IRI is already present *in-vivo* at glucose levels of 20 mM.

Early phase of T1DM (≤2 weeks): interestingly, the early phase of T1DM can cause a protective state of the heart 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 the hyperglycaemic metabolic milieu the hearts are subjected to in the *in-vivo* setting. Various mechanisms explaining this intrinsic protected state of early T1DM heart have been proposed, such as increases in Akt, eNOS, PKC, ERK, heat-shock proteins or end-ischaemic mitochondrial HKII (Balakumar and Sharma, 2012; Gurel *et al.,* 2013). 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

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>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): in the prolonged state of T1DM, the isolated heart appears to lose its protected state, showing either no effect on cardioprotection or increased vulnerability to acute IRI (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, with most studies reporting either increased vulnerability or no change (Fig. 2G), and a minority showing protection. However, T2DM in the *in-vivo* setting was mainly associated with increased vulnerability to IRI (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. Interestingly, in the *in-vivo* setting a few studies report hearts to be in a protected state. 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 with 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 endogenous 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

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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; Heusch 2015a, 2015b; Penna *et al.,* 2008, 2015; Wider and Przyklenk, 2014).

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; Katakam *et al.,* 2007; Kersten *et al.,* 1998, 2000). No effects or worsening of acute IRI have also been reported as a consequence of IPC (Kristiansen *et al.,* 2004; del Valle *et al.,* 2003). Also pharmacological preconditioning with several agents has produced contradictory results. Recent examples of ischaemic and pharmacological preconditioning studies which confirmed contradictory results in terms of IPC effectiveness in diabetic models 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 anesthetics (Kehl *et al.,* 2002; Kersten *et al.,* 1998; Kersten *et al.,* 2000). The blunting may be overcome by increasing the dose of protectants or the numbers/duration of PC cycles. Indeed, in animal models, several authors (Bouchard and Lamontagne, 1998; 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 adjust 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

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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 (Koka *et al.* 2013; Reinstadler *et al.,* 2017; Sun *et al.,* 2013). 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 (Ishihara *et al.,* 1997; Kloner *et al.,* 1998). 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; Badaut *et al.,* 2005; Fernandez *et al.,* 2012; 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

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(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 signaling 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; Penna *et al.,* 2008; 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 pathays (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; Hausenloy *et al.,* 2011; Inserte *et al.,* 2011; Lacerda *et al.,* 2009, 2012; Oosterlinck *et al.,* 2013; Pagliaro *et al.,* 2011, Pagliaro and Penna, 2015; Penna *et al.,* 2006, 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

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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 (Bouhidel *et al.,* 2008; Ferdinandy *et al.,* 2014) (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; Raphael *et al.,* 2010). 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.,* 2010, 2015) (Fig 3). It has been suggested that PTEN/Akt alteration is altered in the presence of diabetes (Mocanu and Yellon, 2007). Diabetic heart may be refractory to protection by Jak2-activating ligands because of angiotensin-II type 1 (AT1)-mediated upregulation of calcineurin activity (Hotta *et al.,* 2010). Recently, also in a hyperglycemic experimental model a reduced level of Akt phosphorylation has been observed, a condition which reduced 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).

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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; Hausenloy and Yellon, 2008; Heusch *et al.,* 2015; Pickard *et al.,* 2015; Sivaraman *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 (Bøtker *et al.,* 2010; 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 (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

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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 remote ischaemic conditioning. It has been reported that protection conferred by RIC may involve the attenuation of the sympathetic nervous system, 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 compared to metabolic syndrome 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.

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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 (Gross, 1995; Heusch 2015a; Yumei *et al.,* 2011). It appears that the new 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

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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, (Wayman *et al.,* 2002; Ye *et al.,* 2008; Zhang *et al.,* 2010) with potential mechanisms including decreased expression of microRNA-29a and 29c (Ye *et al.,* 2010a), 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 (Lovshin and Drucker, 2009; 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 (Ban *et al.,* 2008; 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 reduced infarct size (Timmers *et al.,* 2009). Proposed mechanisms of actions include activation of the GLP receptor, PKA and RISK pathways, and eNOS phosphorylation (Inserte *et al.,* 2004). In general, 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

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(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.,* 2016a, 2016b; Pfeffer *et al.,* 2015; 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 are administered before ischaemia or at reperfusion (Chinda *et al.,* 2013; Hausenloy *et al.,* 2013a; Huisamen *et al.,* 2004; Theiss *et al.,* 2013; Ye *et al.,* 2010b). 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).

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.

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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 (Baartscheer *et al.,* 2017; 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 endogenous 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

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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. Further experimental studies are needed to elucidate the potential mechanisms underlying the confounding effects of hyperglycaemia/DM on endogenous cardioprotection. Furthermore, 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 in patients with diabetes are needed to improve clinical outcomes in this high-risk group.

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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, Fabbro *et al*., 2017; Alexander, Kelly *et al*., 2017).

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Conflict of interest: none.

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Figure Legends

Figure 1: Schematic representation of the various protocols of ischaemic conditioning.

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 the number of studies reporting increased vulnerability, neutral or protective effects is divided by the total number of studies in that category. The following references were used, going from vulnerable (***V***), neutral (***N***) and protected (***P***):

A (***V:*** Mapanga *et al.,* 2014; Marfella *et al.,* 2002; Wong *et al.*; ***N:*** Wong *et al.,* 2011; Zálešák *et al.,* 2014; ***P:*** Yao *et al.,* 2015),

B (***V:*** Ham *et al.,* 2018; Kersten *et al.,* 1998; Kersten *et al.,* 2000; Liu *et al.,* 2017a; Soh *et al.,* 2018; Su *et al.,* 2007; Su *et al.,* 2007; Tian *et al.,* 2015; Yang *et al.,* 2013; Yu *et al.,* 2017; ***N:*** Amour *et al.,* 2010; Baotic *et al.,* 2013; Baranyai *et al.,* 2015; Diemar *et al.,* 2015; Ebel *et al.,* 2003; Gu *et al.,* 2008; Ham *et al.,* 2018; Huhn *et al.,* 2008; Ichinomiya *et al.,* 2012; Jun *et al.,* 2014; Kehl *et al.,* 2002; Kersten *et al.,* 1998; Kersten *et al.,* 2001; Matsumoto *et al.,* 2012; Raphael *et al.,* 2010; Raphael *et al.,* 2015; Weber *et al.,* 2008; Yu *et al.,* 2014; ***P:*** none),

C (***V:*** none; ***N:*** Przyklenk *et al.,* 2011; ***P:*** Ansari *et al.,* 2019; Ferko *et al.,* 2015; Pourkhalili *et al.,* 2012),

D (***V:*** Li *et al.,* 2016; Liu *et al.,* 2017b; Mapanga *et al.,* 2014; Marfella *et al.,* 2002; Marfella *et al.,* 2004; Xiao *et al.,* 2004; ***N:*** Han *et al.,* 2014; Ji *et al.,* 2013; Chen *et al.,* 2013; Gross *et al.,* 2007; ***P:*** Eguchi *et al.,* 2012; Ma *et al.,* 2006; Ravingerová *et al.,* 2003),

E (***V:*** Janahmadi *et al.,* 2015; Pei *et al.,* 2013; Ramezani-Aliakbari *et al.,* 2017; Sharma *et al.,* 2013; Thirunavukkarasu *et al.,* 2007; ***N:*** Babiker *et al.,* 2012; Badalzadeh *et al.,* 2015; Ghaboura *et al.,* 2011; Jamwal *et al.,* 2016; Joyeux *et al.,* 1999; Najafi *et al.,* 2014; Okazaki *et al.,* 2011; Pei *et al.,* 2013; Shi-Ting *et al.,* 2011; ***P:*** none),

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F (***V:*** Forrat *et al.,* 1993; Gao *et al.,* 2016; Ho *et al.,* 2014; Li *et al.,* 2013a; Li *et al.,* 2013b; Li *et al.,* 2017; Liu *et al.,* 2005; Liu *et al.,* 2012; Liu *et al.,* 2013; Liu *et al.,* 2017a; Qiu *et al.,* 2017; Qiu *et al.,* 2018; Ranjbar *et al.,* 2018; Wang *et al.,* 2018a; Wang *et al.,* 2019; Wu *et al.,* 2017; Zhou *et al.,* 2017; ***N:*** Ananthakrishnan *et al.,* 2013; Drenger *et al.,* 2011; Ebel *et al.,* 2003; Kersten *et al.,* 2000; Kersten *et al.,* 2001; Kiss *et al.,* 2014; Li *et al.,* 2013b; Lin *et al.,* 2016; Ma *et al.,* 2006; Nieszner *et al.,* 2002; Potier *et al.,* 2013; Ravingerová *et al.,* 2003; Tai *et al.,* 2012; Tratsiakovich *et al.,* 2017; Vogel and Apstein, 1988; Xu *et al.,* 2004; ***P:*** Galagudza *et al.,* 2007; Hadour *et al.,* 1998; Xu *et al.,* 2004), **G** (***V:*** Chen *et al.,* 2016; Das *et al.,* 2015; Fan *et al.,* 2012; Koka *et al.,* 2013; Pælestik *et al.,* 2017; ***N***: Bhamra *et al.,* 2008; Czompa *et al.,* 2017; Przyklenk *et al.,* 2011; Rana *et al.,* 2016; Tsang *et al.,* 2005; ***P:*** Kristiansen *et al.,* 2004), **H** (***V:*** Baumgardt *et al.,* 2016; Bouhidel *et al.,* 2008; Calvert *et al.,* 2008; Ding *et al.,* 2015; Guo *et al.,* 2014; Hjortbak *et al.,* 2018; Honda *et al.,* 2008; Hotta *et al.,* 2010; Jordan *et al.,* 2003; Katakam *et al.,* 2007, La Bonte *et al.,* 2008, Miki *et al.,* 2009; Pons *et al.,* 2013; Qi *et al.,* 2018; Van der Mieren *et al.,* 2012; Van der Mieren *et al.,* 2013; Wang *et al.,* 2018b; Yue *et al.,* 2005; Zhu *et al.,* 2015; ***N:*** Bulhak *et al.,* 2009; Huhn *et al.,* 2009; Matsumoto *et al.,* 2009; Muravyeva *et al.,* 2014; Oosterlinck *et al.,* 2013; Qian *et al.,* 2016; Wider *et al.,* 2018; Zuurbier *et al.,* 2014; ***P:*** Korkmaz-Icöz *et al.,* 2015; Poncelas *et al.,* 2015; Zuurbier *et al.,* 2014).

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.

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References

Alexander SP, Fabbro D, Kelly E, Marrion N, Peters JA, Faccenda E, *et al.* (2017). THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Enzymes. British Journal of Pharmacology, 174: S272–S359.

Alexander SP, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD *et al.* (2017). THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Overview. British Journal of Pharmacology, 174: S1-S16.

Alserius T, Hammar N, Nordqvist T, Ivert T (2006). Risk of death or acute myocardial infarction 10 years after coronary artery bypass surgery in relation to type of diabetes. Am Heart J 152:599-605.

Altintas O, Ozgen Altintas M, Kumas M, Asil T (2016). Neuroprotective effect of ischemic preconditioning via modulating the expression of cerebral miRNAs against transient cerebral ischemia in diabetic rats. Neurol Res 38:1003-1011.

Amour J, Brzezinska AK, Jager Z, Sullivan C, Weihrauch D, Du J, *et al.* (2010). Hyperglycemia adversely modulates endothelial nitric oxide synthase during anesthetic preconditioning through tetrahydrobiopterin- and heat shock protein 90-mediated mechanisms. Anesthesiology 112:576-585.

Ananthakrishnan R, Li Q, O'Shea KM, Quadri N, Wang L, Abuchowski A, *et al.* (2013). Carbon monoxide form of PEGylated hemoglobin protects myocardium against ischemia/reperfusion injury in diabetic and normal mice. Artif Cells Nanomed Biotechnol 41:428-436.

Andreadou I, Efentakis P, Balafas E, Togliatto G, Davos CH, Varela A, *et al.* (2017). Empagliflozin limits myocardial infarction in vivo and cell death in vitro: role of STAT3, mitochondria, and redox aspects. Frontiers in Physiology 8:1077.

Andreadou I, Iliodromitis EK, Rassaf T, Schulz R, Papapetropoulos A, Ferdinandy P (2015). The role of gasotransmitters NO, H2S and CO in myocardial ischaemia/reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. Br J Pharmacol 172:1587-1606.

Ansari M, Kurian GA (2019). Hydrogen sulfide preconditioning could ameliorate reperfusion associated injury in diabetic cardiomyopathy rat heart through preservation of mitochondria. Biochimie 158:208-216.

Baartscheer A, Schumacher CA, Wüst RC, Fiolet JW, Stienen GJ, Coronel R, *et al.* (2017). Empagliflozin decreases myocardial cytoplasmic Na(+) through inhibition of the cardiac Na(+)/H(+) exchanger in rats and rabbits. Diabetologia 60:568-573.

Babiker FA, van Golde J, Vanagt WY, Prinzen FW (2012). Pacing postconditioning: impact of pacing algorithm, gender, and diabetes on its myocardial protective effects. J Cardiovasc Transl Res 5:727-734.

Badalzadeh R, Mohammadi M, Yousefi B, Farajnia S, Najafi M, Mohammadi S (2015). Involvement of Glycogen Synthase Kinase-3β and Oxidation Status in the Loss of Cardioprotection by Postconditioning in Chronic Diabetic Male Rats. Adv Pharm Bull 5:321-327.

Badaut J, Hirt L, Price M, de Castro Ribeiro M, Magistretti PJ, Regli L (2005). Hypoxia/hypoglycemia preconditioning prevents the loss of functional electrical activity in organotypic slice cultuRes Brain Res 1051:117-122.

For Peer Review

Baker HE, Kiel AM, Luebbe ST, Simon BR, Earl CC, Regmi A, *et al.* (2019). Inhibition of sodium-glucose cotransporter-2 preserves cardiac function during regional myocardial ischemia independent of alterations in myocardial substrate utilization. Basic Res Cardiol 114:25.

Balakumar P, Sharma NK. (2012). Healing the diabetic heart: does myocardial preconditioning work? Cell Signal 24: 53–59.

Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M (2008). Cardioprotective and vasodilatory actions of glucagonlike peptide 1 receptor are mediated through both glucagonlike peptide 1 receptor-dependent and -independent pathways. Circulation 117:2340–2350.

Banks RE (1995). The 4th R of research. Contemp Top Lab Anim Sci 34:50-51.

Baotic I, Ge ZD, Sedlic F, Coon A, Weihrauch D, Warltier DC, *et al.* (2013). Apolipoprotein A-1 mimetic D-4F enhances isoflurane-induced eNOS signaling and cardioprotection during acute hyperglycemia. Am J Physiol Heart Circ Physiol 305:219-227.

Baranyai T, Nagy CT, Koncsos G, Onódi Z, Károlyi-Szabó M, Makkos A, *et al.* (2015). Acute hyperglycemia abolishes cardioprotection by remote ischemic perconditioning. Cardiovasc Diabetol 14:151.

Baumgardt SL, Paterson M, Leucker TM, Fang J, Zhang DX, Bosnjak ZJ, *et al.* (2016). Chronic Co-Administration of Sepiapterin and L-Citrulline Ameliorates Diabetic Cardiomyopathy and Myocardial Ischemia/Reperfusion Injury in Obese Type 2 Diabetic Mice. Circ Heart Fail 2016 9:002424.

Bell RM, Bøtker HE, Carr RD, Davidson SM, Downey JM, Dutka DP, *et al.* (2016) 9th Hatter Biannual Meeting: position document on ischaemia/reperfusion injury, conditioning and the ten commandments of cardioprotection. Basic Res Cardiol 111:41.

Bhamra GS, Hausenloy DJ, Davidson SM, Carr RD, Paiva M, Wynne AM, *et al.* (2008). Metformin protects the ischemic heart by the Akt-mediated inhibition of mitochondrial permeability transition pore opening. Basic Res Cardiol 103:274-284.

Boengler K, Heusch G, Schulz R (2011). Mitochondria in postconditioning. Antioxid Redox Signal 14:863-880.

Bøtker HE, Hausenloy D, Andreadou I, Antonucci S, Boengler K, Davidson SM, *et al.* (2018). Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. Basic Res Cardiol 2018 113:39.

Bøtker HE, Kharbanda R, Schmidt MR, Bøttcher M, Kaltoft AK, Terkelsen CJ, *et al.* (2010). Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. Lancet 375:727-734.

Bouchard JF, Lamontagne D (1998). Protection afforded by preconditioning to the diabetic heart against ischaemic injury. Cardiovasc Res 37:82-90.

Boudina S, Abel ED (2007). Diabetic cardiomyopathy revisited. Circulation 115: 3213-3223.

Bouhidel O, Pons S, Souktani R, Zini R, Berdeaux A, Ghaleh B (2008). Myocardial ischemic postconditioning against ischemia-reperfusion is impaired in ob/ob mice. Am J Physiol Heart Circ Physiol 295:1580-1586.

For Peer Review

Bromage DI, Yellon DM (2015). The pleiotropic effects of metformin: time for prospective studies. Cardiovasc Diabetol 14:109.

Brunton LL, Lazo JS, Parker KL (2006). Goodman and Gilman's the pharmacological basis of therapeutics, 11th edn. McGrawHill, New York.

Bugger H, Abel ED (2008). Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. Clin Sci (Lond) 114: 195-210.

Bulhak AA, Jung C, Ostenson CG, Lundberg JO, Sjöquist PO, Pernow J (2009). PPAR-alpha activation protects the type 2 diabetic myocardium against ischemia-reperfusion injury: involvement of the PI3-Kinase/Akt and NO pathway. Am J Physiol Heart Circ Physiol 296:719-727.

Cabrera-Fuentes HA, Alba-Alba C, Aragones J, Bernhagen J, Boisvert WA, Bøtker HE, *et al.* (2016). Meeting report from the 2nd International Symposium on New Frontiers in Cardiovascular Research. Protecting the cardiovascular system from ischemia: between bench and bedside. Basic Res Cardiol 111:7.

Calafiore AM, Di Mauro M, Di Giammarco G, Contini M, Vitolla G, Iaco AL, *et al.* (2003). Effect of diabetes on early and late survival after isolated first coronary bypass surgery in multivessel disease. J Thorac Cardiovasc Surg 125:144-154.

Calvert JW, Gundewar S, Jha S, Greer JJ, Bestermann WH, Tian R, *et al.* (2008). Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling. Diabetes 57:696-705.

Charan K, Goyal A, Gupta JK, Yadav HN (2016). Role of atrial natriuretic peptide in ischemic preconditioning-induced cardioprotection in the diabetic rat heart. J Surg Res 201:272-278.

Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, *et al.* (1996). Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 84:491-495.

Chen L, Cai P, Cheng Z, Zhang Z, Fang J (2017). Pharmacological postconditioning with atorvastatin calcium attenuates myocardial ischemia/reperfusion injury in diabetic rats by phosphorylating GSK3β. Exp Ther Med 14:25-34.

Chen L, Chen M, Du J, Wan L, Zhang L, Gu E (2016). Hyperglycemia attenuates remifentanil postconditioning-induced cardioprotection against hypoxia/reoxygenation injury in H9c2 cardiomyoblasts. J Surg Res 203:483-490.

Chen Q, Xu T, Li D, Pan D, Wu P, Luo Y, *et al.* (2016). JNK/PI3K/Akt signaling pathway is involved in myocardial ischemia/reperfusion injury in diabetic rats: effects of salvianolic acid A intervention. Am J Transl Res 8:2534-2548.

Chen QL, Gu EW, Zhang L, Cao YY, Zhu Y, Fang WP (2013). Diabetes mellitus abrogates the cardioprotection of sufentanil against ischaemia/reperfusion injury by altering glycogen synthase kinase-3β. Acta Anaesthesiol Scand 57:236-242.

Chinda, K, Palee, S, Surinkaew, S, Phornphutkul, M, Chattipakorn, S, Chattipakorn, N (2013). Cardioprotective effect of dipeptidyl peptidase-4 inhibitor during ischemia–reperfusion injury. Int J Cardiol 167:451–457.

Cho K, Chung JY, Cho SK, *et al.* (2015). Antihyperglycemic mechanism of metformin occurs via the AMPK/LXRa/POMC pathway. Sci Rep 5:8145.

For Peer Review

Cohen MV, Downey JM (2011). Ischemic postconditioning: from receptor to end-effector. Antioxid Redox Signal 14:821-831.

Czompa A, Gyongyosi A, Szoke K, Bak I, Csepanyi E, Haines DD, *et al.* (2017). Effects of Momordica charantia (Bitter Melon) on Ischemic Diabetic Myocardium. Molecules 22:488.

Das A, Salloum FN, Filippone SM, Durrant DE, Rokosh G, Bolli R, *et al.* (2015). Inhibition of mammalian target of rapamycin protects against reperfusion injury in diabetic heart through STAT3 signaling. Basic Res Cardiol 110:31.

Deedwania P, Kosiborod M, Barrett E, Ceriello A, Isley W, Mazzone T, *et al.* (2008). American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity, and Metabolism. Hyperglycemia and acute coronary syndrome: a scientific statement from the American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 117:1610-1619.

del Valle HF, Lascano EC, Negroni JA, Crottogini AJ (2003). Absence of ischemic preconditioning protection in diabetic sheep hearts: role of sarcolemmal KATP channel dysfunction. Mol Cell Biochem 249:21-30.

Diemar SS, Sejling AS, Iversen KK, Engstrøm T, Honge JL, Tønder N, *et al.* (2015). Influence of acute glycaemic level on measures of myocardial infarction in non-diabetic pigs. Scand Cardiovasc J 49:376-382.

Ding M, Lei J, Han H, Li W, Qu Y, Fu E, *et al.* (2015). SIRT1 protects against myocardial ischemia-reperfusion injury via activating eNOS in diabetic rats. Cardiovasc Diabetol 14:143.

Donahoe SM, Stewart GC, McCabe CH, Mohanavelu S, Murphy SA, Cannon CP, *et al.* (2007). Diabetes and mortality following acute coronary syndromes. JAMA 298:765-775.

Drenger B, Ostrovsky IA, Barak M, Nechemia-Arbely Y, Ziv E, Axelrod JH (2011). Diabetes blockade of sevoflurane postconditioning is not restored by insulin in the rat heart: phosphorylated signal transducer and activator of transcription 3- and phosphatidylinositol 3-kinase-mediated inhibition. Anesthesiology 114:1364-1372.

Ebel D, Müllenheim J, Frässdorf J, Heinen A, Huhn R, Bohlen T, *et al.* (2003). Effect of acute hyperglycaemia and diabetes mellitus with and without short-term insulin treatment on myocardial ischaemic late preconditioning in the rabbit heart in vivo. Pflugers Arch 446:175-182.

Eguchi M, Kim YH, Kang KW, Shim CY, Jang Y, Dorval T, *et al.* (2012). Ischemia-reperfusion injury leads to distinct temporal cardiac remodeling in normal versus diabetic mice. PLoS One 7:30450.

Eitel I, Hintze S, de Waha S, Fuernau G, Lurz P, Desch S, *et al.* (2012). Prognostic impact of hyperglycemia in nondiabetic and diabetic patients with ST-elevation myocardial infarction: insights from contrast-enhanced magnetic resonance imaging. Circ Cardiovasc Imaging 5:708-718.

Eitel I, Stiermaier T, Rommel KP, Fuernau G, Sandri M, Mangner N, *et al.* (2015). Cardioprotection by combined intrahospital remote ischaemic perconditioning and postconditioning in ST-elevation myocardial infarction: the randomized LIPSIA CONDITIONING trial. Eur Heart J 36:3049-3057.

El Messaoudi S, Nederlof R, Zuurbier CJ, van Swieten HA, Pickkers P, Noyez L, *et al*. (2015). Effect of metformin pretreatment on myocardial injury during coronary artery bypass surgery in

For Peer Review

patients without diabetes (MetCAB): a double-blind, randomised controlled trial. Lancet Diabetes Endocrinol 3:615-23.

Elmadhun NY, Sabe AA, Robich MP, Chu LM, Lassaletta AD, Sellke FW (2013). The pig as a valuable model for testing the effect of resveratrol to prevent cardiovascular disease. Ann N Y Acad Sci 1290:130-135.

Fan Y, Yang S, Zhang X, Cao Y, Huang Y (2012). Comparison of cardioprotective efficacy resulting from a combination of atorvastatin and ischaemic post-conditioning in diabetic and non-diabetic rats. Clin Exp Pharmacol Physiol 39:938-943.

Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R (2014). Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. Pharmacol Rev 66:1142-1174.

Ferko M, Kancirová I, Jašová M, Waczulíková I, Čarnická S, Kucharská J, *et al.* (2015). Participation of heart mitochondria in myocardial protection against ischemia/reperfusion injury: benefit effects of short-term adaptation processes. Physiol Res 5:617-625.

Fernandez DC, Pasquini LA, Dorfman D, Aldana Marcos HJ, Rosenstein RE (2012). Ischemic conditioning protects from axoglial alterations of the optic pathway induced by experimental diabetes in rats. PLoS One 7:e51966.

Fitchett D, Zinman B, Wanner C, Lachin JM, Hantel S, Salsali A, *et al.* (2016). Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME® trial. Eur Heart J 37:1526-1534.

Forrat R, Sebbag L, Wiernsperger N, Guidollet J, Renaud S, de Lorgeril M (1993). Acute myocardial infarction in dogs with experimental diabetes. Cardiovasc Res 27:1908-1912.

Freixa X, Bellera N, Ortiz-Pérez JT, Jiménez M, Paré C, *et al.* (2012). Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. Eur Heart J 33:103-112.

Galagudza MM, Nekrasova MK, Syrenskii AV, Nifontov EM (2007). Resistance of the myocardium to ischemia and the efficacy of ischemic preconditioning in experimental diabetes mellitus. Neurosci Behav Physiol 37:489-493.

Gao S, Yang Z, Shi R, Xu D, Li H, Xia Z, *et al.* (2016). Diabetes blocks the cardioprotective effects of sevoflurane postconditioning by impairing Nrf2/Brg1/HO-1 signaling. Eur J Pharmacol 779:111-21.

García-Ropero Á, Vargas-Delgado AP, Santos-Gallego CG, Badimon JJ (2019). Inhibition of Sodium Glucose Cotransporters Improves Cardiac Performance. Int J Mol Sci 20. pii: E3289.

Ghaboura N, Tamareille S, Ducluzeau PH, Grimaud L, Loufrani L, Croué A, *et al.* (2011). Diabetes mellitus abrogates erythropoietin-induced cardioprotection against ischemic-reperfusion injury by alteration of the RISK/GSK-3β signaling. Basic Res Cardiol 106:147-162.

Giannopoulos G, Vrachatis DA, Panagopoulou V, Vavuranakis M, Cleman MW, Deftereos S (2017). Remote Ischemic Conditioning and Renal Protection. J Cardiovasc Pharmacol Ther 22:321–329.

Giblett JP, Clarke SJ, Dutka DP, Hoole SP (2016). Glucagon-Like Peptide-1: A Promising Agent for Cardioprotection During Myocardial Ischemia. JACC Basic Transl Sci 1:267-276.

Goto Y, Suzuki K, Ono T, Sasaki M, Toyota T (1988). Development of diabetes in the non-obese NIDDM rat (GK rat). Adv Exp Med Biol 246: 29-31.

For Peer Review

Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, *et al.* (2015). Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 373:232-242.

Greer JJ, Ware DP, Lefer DJ (2006). Myocardial infarction and heart failure in the db/db diabetic mouse. Am J Physiol Heart Circ Physiol 290:146-153.

Gribble FM, Ashcroft FM (1999). Differential sensitivity of beta-cell and extrapancreatic K(ATP) channels to gliclazide. Diabetologia 42:845-848.

Grievink H, Kuzmina N, Chevion M, Drenger B (2019). Sevoflurane postconditioning is not mediated by ferritin accumulation and cannot be rescued by simvastatin in isolated streptozotocin-induced diabetic rat hearts. PLoS One 14:0211238.

Gross ER, Hsu AK, Gross GJ (2007). Diabetes abolishes morphine-induced cardioprotection via multiple pathways upstream of glycogen synthase kinase-3beta. Diabetes 56:127-136.

Gross GJ (1995). ATP-sensitive potassium channels and myocardial preconditioning. Basic Res Cardiol 90:85-88.

Gu W, Kehl F, Krolikowski JG, Pagel PS, Warltier DC, Kersten JR (2008). Simvastatin restores ischemic preconditioning in the presence of hyperglycemia through a nitric oxide-mediated mechanism. Anesthesiology 108:634-642.

Guo W, Jiang T, Lian C, Wang H, Zheng Q, Ma H (2014). QKI deficiency promotes FoxO1 mediated nitrosative stress and endoplasmic reticulum stress contributing to increased vulnerability to ischemic injury in diabetic heart. J Mol Cell Cardiol 75:131-140.

Gurel E, Ustunova S, Kapucu A, Yilmazer N, Eerbeek O, Nederlof R, *et al.* (2013). Hexokinase cellular trafficking in ischemia-reperfusion and ischemic preconditioning is altered in type I diabetic heart. Mol Biol Rep 40:4153-60.

Habibi J, Aroor AR, Sowers JR, Jia G, Hayden MR, Garro M, *et al.* (2017). Sodium glucose transporter 2 (SGLT2) inhibition with empagliflozin improves cardiac diastolic function in a female rodent model of diabetes. Cardiovasc Diabetol 16:9.

Hadour G, Ferrera R, Sebbag L, Forrat R, Delaye J, de Lorgeril M (1998). Improved myocardial tolerance to ischaemia in the diabetic rabbit. J Mol Cell Cardiol 30:1869-1875.

Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M (1998). Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 339:229-234.

Hahn JY, Song YB, Kim EK, Yu CW, Bae JW, Chung WY, *et al.* (2013). Ischemic postconditioning during primary percutaneous coronary intervention: the effects of postconditioning on myocardial reperfusion in patients with ST-segment elevation myocardial infarction (POST) randomized trial. Circulation 128:1889-1896.

Ham SY, Nam SB, Kwak YL, Kim TL, Park JK, Shim YH (2018). Age-related difference in the effect of acute hyperglycemia on myocardial ischemia-reperfusion injury. J Gerontol A Biol Sci Med Sci DOI: 10.1093/gerona/gly292.

Hamdani N, Franssen C, Lourenco A, Falcao-Pires I, Fontoura D, Leite S, *et al.* (2013). Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model. Circ Heart Fail 6: 1239-1249.

For Peer Review

Han Z, Cao J, Song D, Tian L, Chen K, Wang Y, *et al.* (2014). Autophagy is involved in the cardioprotection effect of remote limb ischemic postconditioning on myocardial ischemia/reperfusion injury in normal mice, but not diabetic mice. PLoS One 9:86838.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S, *et al.* (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucleic Acids Research 46(D1): D1091–D1106.

Hassouna A, Loubani M, Matata BM, Fowler A, Standen NB, Galinanes M (2006). Mitochondrial dysfunction as the cause of the failure to precondition the diabetic human myocardium. Cardiovasc Res 69:450–458.

Hausenloy DJ, Barrabes JA, Bøtker HE, Davidson SM, Di Lisa F, Downey J, *et al.* (2016). Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery. Basic Res Cardiol 111:70.

Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, *et al.* (2015). ERICCA Trial Investigators. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. N Engl J Med 373:1408-1417.

Hausenloy DJ, Kharbanda RK, Møller UK, Ramlall M, Aarøe J, Butler R, *et al.* (2019). Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. Lancet DOI: 10.1016/S0140-6736(19)32039-2.

Hausenloy DJ, Lecour S, Yellon DM (2011). Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. Antioxid Redox Signal 14:893-907.

Hausenloy DJ, Whittington HJ, Wynne AM, Begum SS, Theodorou L, Riksen N, *et al.* (2013a). Dipeptidyl peptidase-4 inhibitors and GLP-1 reduce myocardial infarct size in a glucose-dependent

Hausenloy DJ, Wynne AM, Mocanu MM, Yellon DM (2013b). Glimepiride treatment facilitates ischemic preconditioning in the diabetic heart. J Cardiovasc Pharmacol Ther 18:263–269.

Hausenloy DJ, Yellon DM (2008). Remote ischaemic preconditioning: underlying mechanisms and clinical application. Cardiovasc Res 79:377–386.

Heusch G (2015a). Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. Circ Res 116:674–699.

Heusch G (2015b). Treatment of Myocardial Ischemia/Reperfusion Injury by Ischemic and Pharmacological Postconditioning. Compr Physiol 5:1123-1145.

Heusch G, Bøtker HE, Przyklenk K, Redington A, Yellon D (2015). Remote ischemic conditioning. J Am Coll Cardiol 65:177–195.

Heusch G, Skyschally A, Schulz R (2011) The in-situ pig heart with regional ischemia/reperfusion - ready for translation. J Mol Cell Cardiol 50:951-963.

Heydemann A (2016). An Overview of Murine High Fat Diet as a Model for Type 2 Diabetes Mellitus. J Diabetes Res 2016:2902351.

Hjortbak MV, Hjort J, Povlsen JA, Jensen RV, Støttrup NB, Laursen MR, *et al.* (2018). Influence of diabetes mellitus duration on the efficacy of ischemic preconditioning in a Zucker diabetic fatty rat model. PLoS One 13:0192981.

For Peer Review

Ho YJ, Lee AS, Chen WP, Chang WL, Tsai YK, Chiu HL, *et al.* (2014). Caffeic acid phenethyl amide ameliorates ischemia/reperfusion injury and cardiac dysfunction in streptozotocin-induced diabetic rats. Cardiovasc Diabetol 13:98.

Hogan AE, Gaoatswe G, Lynch L, Corrigan MA, Woods C, O'Connell J, *et al.* (2014). Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. Diabetologia 57:781–784.

Honda T, Kaikita K, Tsujita K, Hayasaki T, Matsukawa M, Fuchigami S, *et al.* (2008). Sugiyama S, Sakashita N, Ogawa H, Takeya M Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia-reperfusion injury in mice with metabolic disorders. J Mol Cell Cardiol 44:915-926.

Hotta H, Miura T, Miki T, Togashi N, Maeda T, Kim SJ, *et al.* (2010). Angiotensin II type 1 receptor-mediated upregulation of calcineurin activity underlies impairment of cardioprotective signaling in diabetic hearts. Circ Res 106:129-132.

Huhn R, Heinen A, Hollmann MW, Schlack W, Preckel B, Weber NC (2010). Cyclosporine A administered during reperfusion fails to restore cardioprotection in prediabetic Zucker obese rats in vivo. Nutr Metab Cardiovasc Dis 20:706-712.

Huhn R, Heinen A, Weber NC, Hollmann MW, Schlack W, Preckel B (2008). Hyperglycaemia blocks sevoflurane-induced postconditioning in the rat heart in vivo: cardioprotection can be restored by blocking the mitochondrial permeability transition pore. Br J Anaesth 100:465-471.

Huhn R, Heinen A, Weber NC, Kerindongo RP, Oei GT, Hollmann MW, *et al.* (2009). Helium-induced early preconditioning and postconditioning are abolished in obese Zucker rats in vivo. Pharmacol Exp Ther 329:600-607.

Hui Peng, Want LL, Aroda VR, *et al.* (2016). Safety and Tolerability of Glucagon-Like Peptide-1 Receptor Agonists Utilizing Data from the Exenatide Clinical Trial Development Program. Cur Diab Rep 16:44.

Huisamen B, Genis A, Marais E, Lochner A (2004). Pre-treatment with a DPP-4 inhibitor is infarct sparing in hearts from obese, pre-diabetic rats. Cardiovasc Drugs Ther 18:247-255.

Ichiki T, Tokunou T, Fukuyama K, Iino N, Masuda S, Takeshita A (2004). 15-Deoxy-delta12, 14-prostaglandin J2 and thiazolidinediones transactivate epidermal growth factor and plateletderived growth factor receptors in vascular smooth muscle cells. Biochem Biophys Res Commun 323:402–408.

Ichinomiya T, Cho S, Higashijima U, Matsumoto S, Maekawa T, Sumikawa K. (2012). High-dose fasudil preserves postconditioning against myocardial infarction under hyperglycemia in rats: role of mitochondrial KATP channels. Cardiovasc Diabetol 11:28.

Ingalls AM, Dickie MM, Snell GD (1950). Obese, a new mutation in the house mouse. J Hered 41:317-318.

Inserte J, Garcia-Dorado D, Ruiz-Meana M, Agullo L, Pina P, Soler-Soler J (2004). Ischemic preconditioning attenuates calpain-mediated degradation of structural proteins through a protein kinase A-dependent mechanism. Cardiovasc Res 64:105–114.

Inserte J, Ruiz-Meana M, Rodríguez-Sinovas A, Barba I, Garcia-Dorado D (2011). Contribution of delayed intracellular pH recovery to ischemic postconditioning protection. Antioxid Redox Signal 14:923-939.

For Peer Review

Ishibashi S, Goldstein JL, Brown MS, Herz J, Burns DK (1994). Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J Clin Invest 93: 1885-1893.

Ishihara M, Inoue I, Kawagoe T, Shimatani Y, Kurisu S, Nishioka K, *et al.* (2001). Diabetes mellitus prevents ischemic preconditioning in patients with a first acute anterior wall myocardial infarction. J Am Coll Cardiol 38:1007-1011.

Ishihara M, Sato H, Tateishi H, Kawagoe T, Shimatani Y, Kurisu S, *et al.* (1997). Implications of prodromal angina pectoris in anterior wall acute myocardial infarction: acute angiographic findings and long-term prognosis. J Am Coll Cardiol 30:970-975.

Istenes I, Körei AE, Putz Z, Németh N, Martos T, Keresztes K, *et al.* (2014). Heart rate variability is severely impaired among type 2 diabetic patients with hypertension. Diabetes Metab Res Rev 30:305-312.

Jamwal S, Kumar K, Reddy BV (2016). Beneficial effect of zinc chloride and zinc ionophore pyrithione on attenuated cardioprotective potential of preconditioning phenomenon in STZ-induced diabetic rat heart. Perfusion 31:334-342.

Janahmadi Z, Nekooeian AA, Mozafari M (2015). Hydroalcoholic extract of Allium eriophyllum leaves attenuates cardiac impairment in rats with simultaneous type 2 diabetes and renal hypertension. Res Pharm Sci 10:125-33.

Janssen SW, Martens GJ, Sweep CG, Ross HA, Hermus AR (1999). In Zucker diabetic fatty rats plasma leptin levels are correlated with plasma insulin levels rather than with body weight. Horm Metab Res 31: 610-615.

Jensen RV, Støttrup NB, Kristiansen SB, Bøtker HE (2012). Release of a humoral circulating cardioprotective factor by remote ischemic preconditioning is dependent on preserved neural pathways in diabetic patients. Basic Res Cardiol 107:285.

Ji L, Zhang X, Liu W, Huang Q, Yang W, Fu F, *et al.* (2013). AMPK-regulated and Akt-dependent enhancement of glucose uptake is essential in ischemic preconditioning-alleviated reperfusion injury. PLoS One 8:69910.

Jonassen AK, Sack MN, Mjøs OD, Yellon DM (2001). Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. Circ Res 89:1191-1198.

Jones SP, Tang XL, Guo Y, Steenbergen C, Lefer DJ, Kukreja RC, *et al.* (2015). The NHLBI-sponsored Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR): a new paradigm for rigorous, accurate, and reproducible evaluation of putative infarct-sparing interventions in mice, rabbits, and pigs. Circ Res 116:572-586.

Jordan JE, Simandle SA, Tulbert CD, Busija DW, Miller AW (2003). Fructose-fed rats are protected against ischemia/reperfusion injury. J Pharmacol Exp Ther 307:1007-1011.

Joyeux M, Faure P, Godin-Ribuot D, Halimi S, Patel A, Yellon DM, *et al.* (1999). Demenge P, Ribuot C. Heat stress fails to protect myocardium of streptozotocin-induced diabetic rats against infarction. Cardiovasc Res 43:939-946.

Jun JH, Jun NH, Shim JK, Shin EJ, Kwak YL (2014). Erythropoietin protects myocardium against ischemia-reperfusion injury under moderate hyperglycemia. Eur J Pharmacol 745:1-9.

For Peer Review

Katakam PV, Jordan JE, Snipes JA, Tulbert CD, Miller AW, Busija DW (2007). Myocardial preconditioning against ischemia-reperfusion injury is abolished in Zucker obese rats with insulin resistance. Am J Physiol Regul Integr Comp Physiol 292:920-926.

Kavianipour M, Ehlers MR, Malmberg K, Ronquist G, Ryden L, Wikstrom G, *et al.* (2003). Glucagon-like peptide-1 (7-36) amide prevents the accumulation of pyruvate and lactate in the ischemic and non-ischemic porcine myocardium. Peptides 24:569–578.

Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T (1992). Spontaneous Long-Term Hyperglycemic Rat with Diabetic Complications - Otsuka Long-Evans Tokushima Fatty (Oletf) Strain. Diabetes 41:1422-1428.

Kazumi T, Odaka H, Hozumi T, Ishida Y, Amano N, Yoshino G (1997). Effects of dietary fructose or glucose on triglyceride production and lipogenic enzyme activities in the liver of Wistar fatty rats, an animal model of NIDDM. Endocr J 44:239-245.

Kehl F, Krolikowski JG, Mraovic B, Pagel PS, Warltier DC, Kersten JR (2002). Hyperglycemia prevents isoflurane-induced preconditioning against myocardial infarction. Anesthesiology 96:183-188.

Kersten JR, Montgomery MW, Ghassemi T, Gross ER, Toller WG, Pagel PS, *et al.* (2001). Warltier DC. Diabetes and hyperglycemia impair activation of mitochondrial K(ATP) channels. Am J Physiol Heart Circ Physiol 280:1744-1750.

Kersten JR, Schmeling TJ, Orth KG, Pagel PS, Warltier DC (1998). Acute hyperglycemia abolishes ischemic preconditioning in vivo. Am J Physiol 275:721-725.

Kersten JR, Toller WG, Gross ER, Pagel PS, Warltier DC (2000). Diabetes abolishes ischemic preconditioning: role of glucose, insulin, and osmolality. Am J Physiol Heart Circ Physiol 278:1218-1224.

Kiss A, Tratsiakovich Y, Gonon AT, Fedotovskaya O, Lanner JT, Andersson DC, *et al.* (2014). The role of arginase and rho kinase in cardioprotection from remote ischemic perconditioning in non-diabetic and diabetic rat in vivo. PLoS One 9:104731.

Kleinbongard P, Bøtker HE, Ovize M, Hausenloy DJ, Heusch G (2019). Co-morbidities and co-medications as confounders of cardioprotection - does it matter in the clinical setting? Br J Pharmacol. DOI: 10.1111/bph.14839.

Kloner RA, Shook T, Antman EM, Cannon CP, Przyklenk K, Yoo K, *et al.* (1998). Prospective temporal analysis of the onset of preinfarction angina versus outcome: an ancillary study in TIMI-9B. Circulation 97:1042-1045.

Koka S, Das A, Salloum FN, Kukreja RC (2013). Phosphodiesterase-5 inhibitor tadalafil attenuates oxidative stress and protects against myocardial ischemia/reperfusion injury in type 2 diabetic mice. Free Radic Biol Med 60:80-88.

Korkmaz-Icöz S, Lehner A, Li S, Vater A, Radovits T, Hegedűs P, *et al.* (2015). Mild Type 2 Diabetes Mellitus Reduces the Susceptibility of the Heart to Ischemia/Reperfusion Injury: Identification of Underlying Gene Expression Changes. J Diabetes Res 396414.

Kosiborod M, Rathore SS, Inzucchi SE, Masoudi FA, Wang Y, Havranek EP, *et al.* (2005). Admission glucose and mortality in elderly patients hospitalized with acute myocardial infarction: implications for patients with and without recognized diabetes. Circulation 111:3078-86.

For Peer Review

Kottenberg E, Thielmann M, Kleinbongard P, Frey UH, Heine T, Jakob H, *et al.* (2014). Myocardial protection by remote ischaemic pre-conditioning is abolished in sulphonylurea-treated diabetics undergoing coronary revascularisation. Acta Anaesthesiol Scand 58:453-462.

Kristensen J, Mortensen UM, Schmidt M, Nielsen PH, Nielsen TT, Maeng M (2009). Lack of cardioprotection from subcutaneously and preischemic administered liraglutide in a closed chest porcine ischemia reperfusion model. BMC Cardiovasc Disord 9:31.

Kristiansen SB, Løfgren B, Støttrup NB, Khatir D, Nielsen-Kudsk JE, Nielsen TT, *et al.* (2004). A Ischaemic preconditioning does not protect the heart in obese and lean animal models of type 2 diabetes Diabetologia 47:1716-1721.

La Bonte LR, Davis-Gorman G, Stahl GL, McDonagh PF (2008). Complement inhibition reduces injury in the type 2 diabetic heart following ischemia and reperfusion. Am J Physiol Heart Circ Physiol 294:1282-1290.

Lacerda L, Opie LH, Lecour S (2012). Influence of tumour necrosis factor alpha on the outcome of ischaemic postconditioning in the presence of obesity and diabetes. Exp Diabetes Res 502654.

Lacerda L, Somers S, Opie LH, Lecour S (2009). Ischaemic postconditioning protects against reperfusion injury via the SAFE pathway. Cardiovasc Res 84:201-208.

Lambert EA, Thomas CJ, Hemmes R, Eikelis N, Pathak A, Schlaich MP, *et al.* (2016). Sympathetic nervous response to ischemia-reperfusion injury in humans is altered with remote ischemic preconditioning. Am J Physiol Heart Circ Physiol 311:364-370.

Lee TM, Chou TF (2003). Impairment of myocardial protection in type 2 diabetic patients. J Clin Endocrinol Metab 88:531-537

Lemoine S, Durand C, Zhu L, Ivasceau C, Lepage O, Babatasi G, *et al.* (2010). Desflurane-induced postconditioning of diabetic human right atrial myocardium in vitro. Diabetes Metab 36:21-28.

Lemoine S, Zhu L, Buléon C, Massetti M, Gérard JL, Galera P, *et al.* (2011). Mechanisms involved in the desflurane-induced post-conditioning of isolated human right atria from patients with type 2 diabetes. Br J Anaesth 107:510-518.

Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. Am J Physiol 273:725-730.

Li H, Bian Y, Zhang N, Guo J, Wang C, Lau WB, *et al.* (2013a). Intermedin protects against myocardial ischemia-reperfusion injury in diabetic rats. Cardiovasc Diabetol 12:91.

Li H, Liu Z, Wang J, Wong GT, Cheung CW, Zhang L, *et al.* (2013b). Susceptibility to myocardial ischemia reperfusion injury at early stage of type 1 diabetes in rats. Cardiovasc Diabetol 12:133.

Li J, Ren Y, Shi E, Tan Z, Xiong J, Yan L, *et al.* (2016). Inhibition of the Let-7 Family MicroRNAs Induces Cardioprotection Against Ischemia-Reperfusion Injury in Diabetic Rats. Ann Thorac Surg 102:829-835.

Li TP, Guo Z, Liu CJ, Sun T, Chen L, Zhao X. (2017). Association of down-regulation of calcitonin gene-related peptide and substance P with increase of myocardial vulnerability in diabetic neuropathic rats. Peptides 96:1-7.

For Peer Review

Lim VG, Bell RM, Arjun S, Kolatsi-Joannou M, Long DA, Yellon DM (2019). SGLT2 Inhibitor, Canagliflozin, attenuates myocardial infarction in the diabetic and nondiabetic heart. JACC Basic Transl Sci 4:15-26.

Lin J, Wang T, Li Y, Wang M, Li H, Irwin MG, Xia Z (2016). N-Acetylcysteine Restores Sevoflurane Postconditioning Cardioprotection against Myocardial Ischemia-Reperfusion Injury in Diabetic Rats. J Diabetes Res 2016:9213034.

Lincoff AM, Wolski K, Nicholls SJ, Nissen SE (2007). Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. JAMA 2007 298:1180-1188.

Liu B, Wang J, Li M, Yuan Q, Xue M, Xu F, *et al.* (2017a). Inhibition of ALDH2 by O-GlcNAcylation contributes to the hyperglycemic exacerbation of myocardial ischemia/reperfusion injury. Oncotarget 8:19413-19426.

Liu M, Zhou B, Xia ZY, Zhao B, Lei SQ, Yang QJ, Xue R, *et al.* (2013). Hyperglycemia-induced inhibition of DJ-1 expression compromised the effectiveness of ischemic postconditioning cardioprotection in rats. Oxid Med Cell Longev 2013:564902.

Liu X, Wei J, Peng DH, Layne MD, Yet SF (2005). Absence of heme oxygenase-1 exacerbates myocardial ischemia/reperfusion injury in diabetic mice. Diabetes 54:778-784.

Liu Y, Qu Y, Wang R, Ma Y, Xia C, Gao C, *et al.* (2012). The alternative crosstalk between RAGE and nitrative thioredoxin inactivation during diabetic myocardial ischemia-reperfusion injury. Am J Physiol Endocrinol Metab 303:E841-852.

Liu Y, Xia C, Wang R, Zhang J, Yin T, Ma Y, *et al.* (2017b). The opposite effects of nitric oxide donor, S-nitrosoglutathione, on myocardial ischaemia/reperfusion injury in diabetic and non-diabetic mice. Clin Exp Pharmacol Physiol 44:854-861.

Lønborg J, Engstrøm T, Ahtarovski KA, Nepper-Christensen L, Helqvist S, Vejlstrup N, *et al.* (2017). Myocardial Damage in Patients With Deferred Stenting After STEMI: A DANAMI-3-DEFER Substudy. J Am Coll Cardiol 69:2794-2804.

Loukogeorgakis Williams R, Panagiotidou AT, Kolvekar SK, Donald A, Cole TJ, Yellon DM, *et al.* (2007). Transient limb ischemia induces remote preconditioning and remote postconditioning in humans by a K(ATP)-channel dependent mechanism. Circulation 116:1386-1395.

Lovshin JA and Drucker DJ (2009). Incretin-based therapies for type 2 diabetes mellitus. Nat Rev Endocrinol 5:262-269.

Ma G, Al-Shabrawey M, Johnson JA, Datar R, Tawfik HE, Guo D, *et al.* (2006). Protection against myocardial ischemia/reperfusion injury by short-term diabetes: enhancement of VEGF formation, capillary density, and activation of cell survival signaling. Naunyn Schmiedebergs Arch Pharmacol 373:415-427.

Maddock HL, Siedlecka SM, Yellon DM (2004). Myocardial protection from either ischaemic preconditioning or nicorandil is not blocked by gliclazide. Cardiovasc Drugs Ther 18:113–119.

Maioli TU, Goncalves JL, Miranda MC, Martins VD, Horta LS, Moreira TG, *et al.* (2016). High sugar and butter (HSB) diet induces obesity and metabolic syndrome with decrease in regulatory T cells in adipose tissue of mice. Inflamm Res 65: 169-178.

Majewski C, Bakris GL (2015). Blood pressure reduction: an added benefit of sodium-glucose cotransporter 2 inhibitors in patients with type 2 diabetes. Diabetes Care 38:429-430.

For Peer Review

Malmberg K, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D, *et al.* (2000). Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry. Circulation 102:1014-1019.

Mapanga RF, Joseph D, Symington B, Garson KL, Kimar C, Kelly-Laubscher R, *et al.* (2014). Detrimental effects of acute hyperglycaemia on the rat heart. Acta Physiol (Oxf) 210:546-564.

Marfella R, D'Amico M, Di Filippo C, Piegari E, Nappo F, Esposito K, *et al.* (2002). Myocardial infarction in diabetic rats: role of hyperglycaemia on infarct size and early expression of hypoxia-inducible factor 1. Diabetologia 45:1172-1181.

Marfella R, Esposito K, Nappo F, Siniscalchi M, Sasso FC, Portoghese M, *et al.* (2004). Expression of angiogenic factors during acute coronary syndromes in human type 2 diabetes. Diabetes 53:2383-2391.

Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, *et al.* (2016a). Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med 375:1834-1844.

Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen *et al.* (2016b). Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 375:311-322.

Mathew V, Gersh BJ, Williams BA, Laskey WK, Willerson JT, Tilbury RT, et. al. (2004). Outcomes in patients with diabetes mellitus undergoing percutaneous coronary intervention in the current era: a report from the Prevention of REStenosis with Tranilast and its Outcomes (PRESTO) trial. Circulation 109:476-480.

Matsubara M, Kanemoto S, Leshnower BG, Albone EF, Hinmon R, Plappert T, *et al.* (2009). Single dose GLP-1-Tf ameliorates myocardial ischemia/reperfusion injury. J Surg Res 165:38–45.

Matsumoto S, Cho S, Tosaka S, Higashijima U, Maekawa T, Hara T, *et al.* (2012). Hyperglycemia raises the threshold of levosimendan- but not milrinone-induced postconditioning in rat hearts. Cardiovasc Diabetol 11:4.

Matsumoto S, Cho S, Tosaka S, Ureshino H, Maekawa T, Hara T, *et al.* (2009). Pharmacological preconditioning in type 2 diabetic rat hearts: the roles of mitochondrial ATP-sensitive potassium channels and the phosphatidylinositol 3-kinase-Akt pathway. Cardiovasc Drugs Ther 23:263-270.

McKenney-Drake ML, Rodenbeck SD, Owen MK, Schultz KA, Alloosh M, Tune JD, *et al.* (2016). Repeat cross-sectional data on the progression of the metabolic syndrome in Ossabaw miniature swine. Data in Brief 7: 1393–1395.

Mering JV MO (1889). Diabetes mellitus nach Pankreasextirpation. Centralblatt für klinische Medicin, Leipzig: 10:383.

Miki T, Itoh T, Sunaga D, Miura T (2012). Effects of diabetes on myocardial infarct size and cardioprotection by preconditioning and postconditioning. Cardiovasc Diabetol 11:67.

Miki T, Miura T, Hotta H, Tanno M, Yano T, Sato T, *et al.* (2009). Endoplasmic reticulum stress in diabetic hearts abolishes erythropoietin-induced myocardial protection by impairment of phospho-glycogen synthase kinase-3beta-mediated suppression of mitochondrial permeability transition. Diabetes 58:2863-2872.

For Peer Review

Mizuno M, Kuno A, Yano T, Miki T, Oshima H, Sato T, *et al.* (2018). Empagliflozin normalizes the size and number of mitochondria and prevents reduction in mitochondrial size after myocardial infarction in diabetic hearts. Physiol Rep 2018 6:e13741.

Mocanu MM, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM (2001). Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. Circulation 103:3111-3116.

Mocanu MM, Yellon DM (2007). PTEN, the Achilles' heel of myocardial ischaemia/reperfusion injury? Br J Pharmacol 150:833-838.

Mohsin AA, Chen Q, Quan N, Rousselle T, Maceyka MW, Samidurai A, *et al.* (2019). Mitochondrial Complex I Inhibition by Metformin Limits Reperfusion Injury. J Pharmacol Exp Ther 369:282-290.

Muravyeva M, Baotic I, Bienengraeber M, Lazar J, Bosnjak ZJ, Sedlic F, *et al.* (2014). Cardioprotection during diabetes: the role of mitochondrial DNA. Anesthesiology 120:870-879.

Murry CE, Jennings RB, Reimer KA (1986). Preconditioning with ischemia**:** a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124-1136.

Najafi M, Farajnia S, Mohammadi M, Badalzadeh R, Ahmadi Asl N, Baradaran B, *et al.* (2014). Inhibition of mitochondrial permeability transition pore restores the cardioprotection by postconditioning in diabetic hearts. J Diabetes Metab Disord 13:106.

Nakadate Y, Sato H, Oguchi T, Sato T, Kawakami A, Ishiyama T, *et al.* (2017). Glycemia and the cardioprotective effects of insulin pre-conditioning in the isolated rat heart. Cardiovasc Diabetol 16:43.

Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, *et al.* (2017). Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 377:644-657.

Neeb ZP, Edwards JM, Alloosh M, Long X, Mokelke EA, Sturek M (2010). Metabolic syndrome and coronary artery disease in Ossabaw compared with Yucatan swine. Comparative Medicine 60:300–315.

Ng KM, Lau YM, Dhandhania V, Cai ZJ, Lee YK, Lai WH, *et al.* (2018). Empagliflozin Ammeliorates High Glucose Induced-Cardiac Dysfuntion in Human iPSC-Derived Cardiomyocytes. Sci Rep 8:14872.

Nieszner E, Posa I, Kocsis E, Pogátsa G, Préda I, Koltai MZ (2002). Influence of diabetic state and that of different sulfonylureas on the size of myocardial infarction with and without ischemic preconditioning in rabbits. Exp Clin Endocrinol Diabetes 110:212-218.

Nissen SE, Nicholls SJ, Wolski K, Nesto R, Kupfer S, Perez A, *et al.* (2008). Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. JAMA 299:1561-1573.

Okazaki T, Otani H, Shimazu T, Yoshioka K, Fujita M, Katano T, *et al.* (2011). Reversal of inducible nitric oxide synthase uncoupling unmasks tolerance to ischemia/reperfu sion injury in the diabetic rat heart. J Mol Cell Cardiol 50:534-544.

Oosterlinck W, Dresselaers T, Geldhof V, Nevelsteen I, Janssens S, Himmelreich U, *et al.* (2013). Diabetes mellitus and the metabolic syndrome do not abolish, but might reduce, the cardioprotective effect of ischemic postconditioning. J Thorac Cardiovasc Surg 145:1595-1602.

For Peer Review

Oshima H, Miki T, Kuno A, Mizuno M, Sato T, Tanno M, *et al.* (2019). Empagliflozin, an SGLT2 Inhibitor, reduced the mortality rate after acute myocardial infarction with modification of cardiac metabolomes and antioxidants in diabetic rats. J Pharmacol Exp Ther 368:524-534.

Ovünç K. (2000). Effects of glibenclamide, a K(ATP) channel blocker, on warm-up phenomenon in type II diabetic patients with chronic stable angina pectoris. Clin Cardiol 23:535-539.

Pælestik KB, Jespersen NR, Jensen RV, Johnsen J, Bøtker HE, Kristiansen SB (2017). Effects of hypoglycemia on myocardial susceptibility to ischemia-reperfusion injury and preconditioning in hearts from rats with and without type 2 diabetes. Cardiovasc Diabetol 16:148.

Pagliaro P, Moro F, Tullio F, Perrelli MG, Penna C (2011). Cardioprotective pathways during reperfusion: focus on redox signaling and other modalities of cell signaling. Antioxid Redox Signal 14:833-850.

Pagliaro P, Penna C (2015). Redox signalling and cardioprotection: translatability and mechanism. Br J Pharmacol 172:1974-1995.

Pauly RP, Rosche F, Wermann M, McIntosh CH, Pederson RA, Demuth HU (1996). Investigation of glucose-dependent insulinotropic polypeptide-(1-42) and glucagon-like peptide-1-(7-36) degradation in vitro by dipeptidyl peptidase IV using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. A novel kinetic approach. J Biol Chem 271: 23222–23229.

Peake BF, Nicholson CK, Lambert JP, Hood RL, Amin H, Amin S, *et al.* (2013). Hydrogen sulfide preconditions the db/db diabetic mouse heart against ischemia-reperfusion injury by activating Nrf2 signaling in an Erk-dependent manner. Am J Physiol Heart Circ Physiol 304:H1215-1224.

Pei H, Qu Y, Lu X, Yu Q, Lian K, Liu P, *et al.* (2013). Cardiac-derived adiponectin induced by long-term insulin treatment ameliorates myocardial ischemia/reperfusion injury in type 1 diabetic mice via AMPK signaling. Basic Res Cardiol 108:322.

Penna C, Granata R, Tocchetti CG, Gallo MP, Alloatti G, Pagliaro P (2015). Endogenous Cardioprotective Agents**:** Role in Pre and Postconditioning. Curr Drug Targets 16:843-867.

Penna C, Mancardi D, Raimondo S, Geuna S, Pagliaro P (2008). The paradigm of postconditioning to protect the heart. J Cell Mol Med 12:435-458.

Penna C, Rastaldo R, Mancardi D, Raimondo S, Cappello S, Gattullo D, *et al.* (2006). Post-conditioning induced cardioprotection requires signalling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K+ channel and protein kinase C activation. Basic Res Cardiol 101: 180-189.

Pfeffer MA, Claggett B, Diaz R, Dickstein K, Gerstein HC, Køber LV, *et al.*; ELIXA Investigators. (2015). Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. N Engl J Med 373:2247-2257.

Pickard JM, Bøtker HE, Crimi G, Davidson B, Davidson SM, Dutka D, *et al.* (2015). Remote ischemic conditioning: from experimental observation to clinical application: report from the 8th Biennial Hatter Cardiovascular Institute Workshop. Basic Res Cardiol 110:453.

Poncelas M, Inserte J, Vilardosa Ú, Rodriguez-Sinovas A, Bañeras J, *et al.* (2015). Obesity induced by high fat diet attenuates postinfarct myocardial remodeling and dysfunction in adult B6D2F1 mice. J Mol Cell Cardiol 84:154-161.

For Peer Review

Pons S, Martin V, Portal L, Zini R, Morin D, Berdeaux A, Ghaleb B (2013). Regular treadmill exercise restores cardioprotective signaling pathways in obese mice independently from improvement in associated co-morbidities. J Mol Cell Cardiol 54:82-89.

Potier L, Waeckel L, Vincent MP, Chollet C, Gobeil F Jr, Marre M, *et al.* (2013). Selective kinin receptor agonists as cardioprotective agents in myocardial ischemia and diabetes. J Pharmacol Exp Ther 346:23-30.

Pourkhalili K, Hajizadeh S, Akbari Z, Dehaj ME, Akbarzadeh S, Alizadeh A (2012). Hyperoxic preconditioning fails to confer additional protection against ischemia-reperfusion injury in acute diabetic rat heart. EXCLI J 11:263-273.

Przyklenk K, Maynard M, Greiner DL, Whittaker P (2011). Cardioprotection with postconditioning: loss of efficacy in murine models of type-2 and type-1 diabetes. Antioxid Redox Signal 14:781-790.

Qi K, Li X, Geng Y, Cui H, Jin C, Wang P, *et al.* (2018). Tongxinluo attenuates reperfusion injury in diabetic hearts by angiopoietin-like 4-mediated protection of endothelial barrier integrity via PPAR-α pathway. PLoS One 13:e0198403.

Qian J, Chen H, Birnbaum Y, Nanhwan MK, Bajaj M, Ye Y (2016). Aleglitazar, a Balanced Dual PPARα and -γ Agonist, Protects the Heart Against Ischemia-Reperfusion Injury. Cardiovasc Drugs Ther 30:129-141.

Qiu Y, Wu Y, Meng M, Luo M, Zhao H, Sun H, *et al.* (2018). GYY4137 protects against myocardial ischemia/reperfusion injury via activation of the PHLPP-1/Akt/Nrf2 signaling pathway in diabetic mice. J Surg Res 225:29-39.

Qiu Z, Lei S, Zhao B, Wu Y, Su W, Liu M, *et al.* (2017). NLRP3 Inflammasome Activation-Mediated Pyroptosis Aggravates Myocardial Ischemia/Reperfusion Injury in Diabetic Rats. Oxid Med Cell Longev 2017:9743280.

Rakieten N, Rakieten ML, Nadkarni MV (1963). Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother Rep 29: 91-98.

Ramezani-Aliakbari F, Badavi M, Dianat M, Mard SA, Ahangarpour A (2017). Effects of gallic acid on hemodynamic parameters and infarct size after ischemia-reperfusion in isolated rat hearts with alloxan-induced diabetes. Biomed Pharmacother 96:612-618.

Rana A, Sharma S (2016). Mechanism of sphingosine-1-phosphate induced cardioprotection against I/R injury in diabetic rat heart: Possible involvement of glycogen synthase kinase 3β and mitochondrial permeability transition pore. Clin Exp Pharmacol Physiol 43:166-173.

Ranjbar K, Zarrinkalam E, Salehi I, Komaki A, Fayazi B (2018). Cardioprotective effect of resistance training and Crataegus oxyacantha extract on ischemia reperfusion-induced oxidative stress in diabetic rats. Biomed Pharmacother 100:455-460.

Raphael J, Gozal Y, Navot N, Zuo Z (2010). Hyperglycemia inhibits anesthetic-induced postconditioning in the rabbit heart via modulation of phosphatidylinositol-3-kinase/Akt and endothelial nitric oxide synthase signaling. J Cardiovasc Pharmacol 55:348-357.

Raphael J, Gozal Y, Navot N, Zuo Z (2015). Activation of Adenosine Triphosphate-regulated Potassium Channels during Reperfusion Restores Isoflurane Postconditioning-induced Cardiac Protection in Acutely Hyperglycemic Rabbits. Anesthesiology 122:1299-1311.

Ravingerová T, Neckár J, Kolár F (2003). Ischemic tolerance of rat hearts in acute and chronic phases of experimental diabetes. Mol Cell Biochem 249:167-174.

For Peer Review

Reinstadler SJ, Stiermaier T, Eitel C, Metzler B, de Waha S, Fuernau G, *et al.* (2017). Relationship between diabetes and ischaemic injury among patients with revascularized ST-elevation myocardial infarction. Diabetes Obes Metab 19:1706-1713.

Ren JY, Song JX, Lu MY, Chen H (2011). Cardioprotection by ischemic postconditioning is lost in isolated perfused heart from diabetic rats: Involvement of transient receptor potential vanilloid 1, calcitonin gene-related peptide and substance P. Regul Pept 169:49-57.

Rerup C, Tarding F (1969). Streptozotocin- and alloxan-diabetes in mice. Eur J Pharmacol 7: 89-96.

Rerup CC (1970). Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22: 485-518.

Roberto S, Milia R, Doneddu A, Pinna V, Palazzolo G, Serra S, *et al.* (1985). Hemodynamic abnormalities during muscle metaboreflex activation in patients with type 2 diabetes mellitus. J Appl Physiol 126:444-453.

Russell WMS, Burch RL (1992). The principles of humane experimental technique (Universities Federation for Animal Welfare) Herts, England 238.

Saito S, Thuc LC, Teshima Y, Nakada C, Nishio S, Kondo H, *et al.* (2016). Glucose Fluctuations Aggravate Cardiac Susceptibility to Ischemia/Reperfusion Injury by Modulating MicroRNAs Expression. Circ J 80:186-195.

Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, Ishikawa K, Watanabe S, Picatoste B, *et al.* (2019). Empagliflozin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. J Am Coll Cardiol 73:1931-1944.

Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, *et al.*; SAVOR-TIMI 53 Steering Committee and Investigators. (2013). Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med 369:1317-1326.

Sharma NK, Mahadevan N, Balakumar P (2013). Adenosine transport blockade restores attenuated cardioprotective effects of adenosine preconditioning in the isolated diabetic rat heart: potential crosstalk with opioid receptors. Cardiovasc Toxicol 13:22-32.

Shi-Ting W, Mang-Hua X, Wen-Ting C, Feng-Hou G, ZhuYing G (2011). Study on tolerance to ischemia-reperfusion injury and protection of ischemic preconditioning of type 1 diabetes rat heart. Biomed Aging Pathol 1:56-60.

Sivaraman V, Hausenloy DJ, Wynne AM, Yellon DM (2010). Preconditioning the diabetic human myocardium. J Cell Mol Med 14:1740-1746.

Sivaraman V, Pickard JM, Hausenloy DJ (2015). Remote ischaemic conditioning: cardiac protection from afar. Anaesthesia 70:732–748.

Skyschally A, Kleinbongard P, Lieder H, Gedik N, Stoian L, Amanakis G, *et al.* (2018). Humoral transfer and intramyocardial signal transduction of protection by remote ischemic perconditioning in pigs, rats, and mice. Am J Physiol Heart Circ Physiol315:H159-H172.

Skyschally A, van Caster P, Iliodromitis EK, Schulz R, Kremastinos DT, Heusch G (2009). Ischemic postconditioning: experimental models and protocol algorithms. Basic Res Cardiol 104:469-483.

Soh S, Jun JH, Song JW, Shin EJ, Kwak YL, Shim JK. (2018). Ethyl pyruvate attenuates myocardial ischemia-reperfusion injury exacerbated by hyperglycemia via retained inhibitory effect on HMGB1. Int J Cardiol 252:156-162.

For Peer Review

Sonne DP, Engstrom T, Treiman M (2008). Protective effects of GLP-1 analogues exendin-4 and GLP-1(9-36) amide against ischemia–reperfusion injury in rat heart. Regul Pept 146:243–249.

Sörensson P, Saleh N, Bouvier F, Böhm F, Settergren M, Caidahl K, *et al.* (2010). Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction. Heart 96:1710-1715.

Spurlock ME, Gabler NK (2008). The development of porcine models of obesity and the metabolic syndrome. J Nutr138:397-402.

Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, *et al.* (2005). Postconditioning the human heart. Circulation 112:2143-2148.

Stanley WC, Dabkowski ER, Ribeiro RF, Jr., O'Connell KA (2012). Dietary fat and heart failure: moving from lipotoxicity to lipoprotection. Circ Res 110:764-776.

Su H, Sun X, Ma H, Zhang HF, Yu QJ, Huang C, *et al.* (2007). Acute hyperglycemia exacerbates myocardial ischemia/reperfusion injury and blunts cardioprotective effect of GIK. Am J Physiol Endocrinol Metab 293:E629-635.

Sun D, Narsinh K, Wang H, Li C, Li W, Zhang Z, *et al.* (2013). Effect of autologous bone marrow mononuclear cells transplantation in diabetic patients with ST-segment elevation myocardial infarction. Int J Cardiol 167:537-547.

Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, *et al.* (1995). Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 44: 645-651.

Tai W, Shi E, Yan L, Jiang X, Ma H, Ai C (2012). Diabetes abolishes the cardioprotection induced by sevoflurane postconditioning in the rat heart in vivo: roles of glycogen synthase kinase-3β and its upstream pathways. J Surg Res 178:96-104.

Tanajak P, Sa-Nguanmoo P, Sivasinprasasn S, Thummasorn S, Siri-Angkul N, Chattipakorn SC, *et al.* (2018). Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury. J Endocrinol 236:69-84.

Theiss, HD, Gross, L, Vallaster, M (2013). Antidiabetic gliptins in combination with G-CSF enhances myocardial function and survival after acute myocardial infarction. Int J Cardiol 168:3359–3369.

Thibault H, Piot C, Ovize M (2007). Postconditioning in man. Heart Fail Rev 12:245-248.

Thirunavukkarasu M, Penumathsa SV, Koneru S, Juhasz B, Zhan L, Otani H, *et al.* (2007). Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. Free Radic Biol Med 43:720-729.

Thomaz Neto FJ, Koike MK, Abrahão Mde S, Carillo Neto F, Pereira RK, Machado JL, *et al.* (2013). Ischemic preconditioning attenuates remote pulmonary inflammatory infiltration of diabetic rats with an intestinal and hepatic ischemia-reperfusion injury. Acta Cir Bras 28:174-178.

Thourani VH, Weintraub WS, Stein B, Gebhart SS, Craver JM, Jones EL, *et al.* (1999). Influence of diabetes mellitus on early and late outcome after coronary artery bypass grafting. Ann Thorac Surg 67:1045-1052.

Tian Y, French BA, Kron IL, Yang Z (2015). Splenic leukocytes mediate the hyperglycemic exacerbation of myocardial infarct size in mice. Basic Res Cardiol 110:39.

For Peer Review

Timmers L Henriques JP, de Kleijn DP, Devries JH, Kemperman H, Steendijk P, *et al.* (2009). Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. J Am Coll Cardiol 53:501–510.

Trask AJ, Katz PS, Kelly AP, Galantowicz ML, Cismowski MJ, West TA, *et al.* (2012). Dynamic micro- and macrovascular remodeling in coronary circulation of obese Ossabaw pigs with metabolic syndrome. Journal of Applied Physiology, 113, 1128–1140.

Tratsiakovich Y, Kiss A, Gonon AT, Yang J, Sjöquist PO, Pernow J (2017). Inhibition of Rho kinase protects from ischaemia-reperfusion injury via regulation of arginase activity and nitric oxide synthase in type 1 diabetes. Diab Vasc Dis Res 14:236-245.

Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM (2005). Preconditioning the diabetic heart: the importance of Akt phosphorylation. Diabetes 54:2360-2364.

Tyagi S, Singh N, Virdi JK, Jaggi AS (2019). Diabetes abolish cardioprotective effects of remote ischemic conditioning: evidences and possible mechanisms. J Physiol Biochem 75:19-28.

Uthman L, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JWT, Koeman A, *et al.* (2018). Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na(+)/H(+) exchanger, lowering of cytosolic Na(+) and vasodilation. Diabetologia 61:722-726.

Uthman L, Nederlof R, Eerbeek O, Baartscheer A, Schumacher C, Buchholtz N, *et al.* (2019). Delayed ischemic contracture onset by Empagliflozin associates with NHE-1 inhibition and is dependent on insulin in isolated mouse hearts. Cardiovasc Res 115:1533-1545.

Van den Bergh A, Flameng W, Herijgers P (2006). Type II diabetic mice exhibit contractile dysfunction but maintain cardiac output by favourable loading conditions. Eur J Heart Fail 8: 777-783.

Van der Mieren G, Nevelsteen I, Vanderper A, Oosterlinck W, Flameng W, Herijgers P (2013). Angiotensin-converting enzyme inhibition and food restriction restore delayed preconditioning in diabetic mice. Cardiovasc Diabetol 12:36.

Van der Mieren G, Nevelsteen I, Vanderper A, Oosterlinck W, Flameng W, Herijgers P (2012). Angiotensin-converting enzyme inhibition and food restriction in diabetic mice do not correct the increased sensitivity for ischemia-reperfusion injury. Cardiovasc Diabetol 11:89.

Varjabedian L, Bourji M, Pourafkari L, Nader ND (2018). Cardioprotection by Metformin: Beneficial Effects Beyond Glucose Reduction. Am J Cardiovasc Drugs 18:181-193.

Verma S, Mazer CD, Yan AT, Mason T, Garg V, Teoh H, *et al.* ; EMPA-HEART CardioLink-6 Investigators. (2019). Effect of Empagliflozin on Left Ventricular Mass in Patients with Type 2 Diabetes and Coronary Artery Disease: The EMPA-HEART CardioLink-6 Randomized Clinical Trial. Circulation DOI: 10.1161/CIRCULATIONAHA.119.042375.

Vinokur V, Weksler-Zangen S, Berenshtein E, Eliashar R, Chevion M (2016). The Loss of Myocardial Benefit following Ischemic Preconditioning Is Associated with Dysregulation of Iron Homeostasis in Diet-Induced Diabetes. PLoS One 11:e0159908.

Vogel WM, Apstein CS (1988). Effects of alloxan-induced diabetes on ischemia-reperfusion injury in rabbit hearts Circ Res 1988 62:975-982.

Wang C, Li H, Wang S, Mao X, Yan D, Wong SS, *et al.* (2018a). Repeated Non-Invasive Limb Ischemic Preconditioning Confers Cardioprotection Through PKC-Ɛ/STAT3 Signaling in Diabetic Rats. Cell Physiol Biochem 45:2107-2121.

For Peer Review

Wang D, Hu X, Lee SH, Chen F, Jiang K, Tu Z, *et al.* (2018b). Diabetes Exacerbates Myocardial Ischemia/Reperfusion Injury by Down-Regulation of MicroRNA and Up-Regulation of O-GlcNAcylation. JACC Basic Transl Sci 3:350-362.

Wang TT, Shi MM, Liao X, Li YQ, Yuan HX, Li YQ, *et al.* (2019). Overexpression of inducible nitric oxide synthase in the diabetic heart compromises ischemic postconditioning. J Mol Cell Cardiol 129:144-153.

Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, *et al.* (2016). Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. N Engl J Med 375:323-334.

Wayman NS, Hattori Y, McDonald MC, Mota-Filipe H, Cuzzocrea S, Pisano B, *et al.* (2002). Ligands of the peroxisome proliferator-activated receptors (PPAR-gamma and PPAR-alpha) reduce myocardial infarct size. Faseb J 16:1027–1040.

Weber NC, Goletz C, Huhn R, Grueber Y, Preckel B, Schlack W, *et al.* (2008). Blockade of anaesthetic-induced preconditioning in the hyperglycaemic myocardium: the regulation of different mitogen-activated protein kinases. Eur J Pharmacol 592:48-54.

White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, *et al.* (2013). Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med 369:1327-1335.

Whittington HJ, Harding I, Stephenson CI, Bell R, Hausenloy DJ, Mocanu MM, *et al.* (2013). Cardioprotection in the aging, diabetic heart: the loss of protective Akt signalling. Cardiovasc Res 99:694-704.

Wider J, Przyklenk K. (2014). Ischemic conditioning**:**the challenge of protecting the diabetic heart. Cardiovasc Diagn Ther 4:383-396.

Wider J, Undyala VVR, Whittaker P, Woods J, Chen X, Przyklenk K. (2018). Remote ischemic preconditioning fails to reduce infarct size in the Zucker fatty rat model of type-2 diabetes: role of defective humoral communication. Basic Res Cardiol 113:16.

Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, *et al.* (2019). Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 380:347-357.

Wolf E, Braun-Reichhart C, Streckel E, Renner S (2014). Genetically engineered pig models for diabetes research. Transgenic Res 23:27-38.

Wong VW, Mardini M, Cheung NW, Mihailidou AS (2011). High-dose insulin in experimental myocardial infarction in rabbits: protection against effects of hyperglycaemia. J Diabetes Complications 25:122-128.

Wroge J, Williams NT (2016). Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists in Cardiac Disorders. Ann Pharmacother 50:1041-1050.

Wu Y, Xia ZY, Zhao B, Leng Y, Dou J, Meng QT, *et al.* (2017). (-)-Epigallocatechin-3-gallate attenuates myocardial injury induced by ischemia/reperfusion in diabetic rats and in H9c2 cells under hyperglycemic conditions. Int J Mol Med 40:389-399.

Wynne AM, Mocanu MM, Yellon DM (2005). Pioglitazone mimics preconditioning in the isolated perfused rat heart: a role for the prosurvival kinases PI3K and P42/44MAPK. J Cardiovasc Pharmacol 46:817–822.

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Xiao CY, Chen M, Zsengellér Z, Szabó C (2004). Poly(ADP-ribose) polymerase contributes to the development of myocardial infarction in diabetic rats and regulates the nuclear translocation of apoptosis-inducing factor. J Pharmacol Exp Ther 310:498-504.

Xie P, Yang L, Talaiti A, Wu JJ, Yu J, Yu T, *et al.* (2017). Deferoxamine-activated hypoxia-inducible factor-1 restores cardioprotective effects of sevoflurane postconditioning in diabetic rats. Acta Physiol (Oxf) 221:98-114.

Xu G, Takashi E, Kudo M, Ishiwata T, Naito Z (2004). Contradictory effects of short- and long-term hyperglycemias on ischemic injury of myocardium via intracellular signaling pathway. Exp Mol Pathol 76:57-65.

Xue F, Yang X, Zhang B, Zhao C, Song J, Jiang T, *et al.* (2010). Postconditioning the human heart in percutaneous coronary intervention. Clin Cardiol 33:439-444.

Xue M, Li T, Wang Y, Chang Y, Cheng Y, Lu Y, Liu X, *et al.* (2019). Empagliflozin prevents cardiomyopathy via sGC-cGMP-PKG pathway in type 2 diabetes mice. Clin Sci (Lond) 133:1705-1720.

Yadav HN, Singh M, Sharma PL (2010). Involvement of GSK-3β in attenuation of the cardioprotective effect of ischemic preconditioning in diabetic rat heart. Mol Cell Biochem 343:75-81.

Yang Z, Tian Y, Liu Y, Hennessy S, Kron IL, French BA (2013). Acute hyperglycemia abolishes ischemic preconditioning by inhibiting Akt phosphorylation: normalizing blood glucose before ischemia restores ischemic preconditioning. Oxid Med Cell Longev 2013:329183.

Yao X, Li Y, Tao M, Wang S, Zhang L, Lin J, *et al.* (2015). Effects of Glucose Concentration on Propofol Cardioprotection against Myocardial Ischemia Reperfusion Injury in Isolated Rat Hearts. J Diabetes Res 2015:592028.

Ye Y, Hu Z, Lin Y, Zhang C, Perez-Polo JR (2010a). Downregulation of microRNA-29 by antisense inhibitors and a PPAR-gamma agonist protects against myocardial ischaemia–reperfusion injury. Cardiovasc Res 87:535–544.

Ye Y, Keyes KT, Zhang C, Perez-Polo JR, Lin Y, Birnbaum Y (2010b). The myocardial infarct size-limiting effect of sitagliptin is PKA-dependent, whereas the protective effect of pioglitazone is partially dependent on PKA. Am J Physiol Heart Circ Physiol 298:H1454–H1465.

Ye Y, Lin Y, Manickavasagam S, Perez-Polo JR, Tieu BC, Birnbaum Y (2008). Pioglitazone protects the myocardium against ischemia–reperfusion injury in eNOS and iNOS knockout mice. Am J Physiol Heart Circ Physiol 295:H2436–H2446.

Yoon AH, Ye Y, Birnbaum Y (2014). Dipeptidyl peptidase IV inhibitors and ischemic myocardial injury. J Cardiovasc Pharmacol Ther 19:417-425.

Yu J, Maimaitili Y, Xie P, Wu JJ, Wang J, Yang YN, *et al.* (2017). High glucose concentration abrogates sevoflurane post-conditioning cardioprotection by advancing mitochondrial fission but dynamin-related protein 1 inhibitor restores these effects. Acta Physiol (Oxf) 220:83-98.

Yu L, Fan C, Li Z, Zhang J, Xue , Xu Y, *et al.* (2017). Melatonin rescues cardiac thioredoxin system during ischemia-reperfusion injury in acute hyperglycemic state by restoring Notch1/Hes1/Akt signaling in a membrane receptor-dependent manner. J Pineal Res 62.

Yu Q, Zhou N, Nan Y, Zhang L, Li Y, Hao X, *et al.* (2014). Effective glycaemic control critically determines insulin cardioprotection against ischaemia/reperfusion injury in anaesthetized dogs. Cardiovasc Res 103:238–247.

For Peer Review

Yue TL, Bao W, Gu JL, Cui J, Tao L, Ma XL, *et al.* (2005). Rosiglitazone treatment in Zucker diabetic Fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. Diabetes 54:554-562.

Yumei Ye, Perez-Polo JR, Aguilar D, Birnbaum Y (2011). The potential effects of anti-diabetic medications on myocardial ischemia-reperfusion injury. Basic Res Cardiol 106:925–952.

Yurista SR, Silljé HHW, Oberdorf-Maass SU, Schouten EM, Pavez Giani MG, Hillebrands JL, et al (2019). Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. Eur J Heart Fail 21:862-873.

Zálešák M, BlaŽíček P, Pancza D, Gablovský I, Štrbák V, Ravingerová T (2016). Hyperosmotic environment blunts effectivity of ischemic preconditioning against ischemia-reperfusion injury and improves ischemic tolerance in non-preconditioned isolated rat hearts. Physiol Res 65:1045-1051.

Zálešák M, Blažíček P, Pancza D, Ledvényiová V, Barteková M, Nemčeková M, *et al.* (2014). Severity of lethal ischemia/reperfusion injury in rat hearts subjected to ischemic preconditioning is increased under conditions of simulated hyperglycemia. Physiol Res 63:577-585.

Zhang XJ, Xiong ZB, Tang AL, Ma H, Ma YD, Wu JG, *et al.* (2010). Rosiglitazone-induced myocardial protection against ischaemia–reperfusion injury is mediated via a phosphatidylinositol 3-kinase/Akt-dependent pathway. Clin Exp Pharmacol Pysiol 37:156-161.

Zhang Y, Zhang L, Gu E, Zhu B, Zhao X, Chen J (2016). Long-term insulin treatment restores cardioprotection induced by sufentanil postconditioning in diabetic rat heart. Exp Biol Med (Maywood) 241:650-657.

Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, *et al.* (2004). Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 285(2):H579-588. Erratum in: Am J Physiol Heart Circ Physiol 286:H477.

Zhou B, Leng Y, Lei SQ, Xia ZY (2017). AMPK activation restores ischemic post-conditioning cardioprotection in STZ-induced type 1 diabetic rats: Role of autophagy. Mol Med Rep 16:3648-3656.

Zhu HJ, Wang DG, Yan J, Xu J (2015). Up-regulation of microRNA-135a protects against myocardial ischemia/reperfusion injury by decreasing TXNIP expression in diabetic mice. Am J Transl Res 7:2661-2671.

Zhu SG, Xi L, Kukreja RC (2012). Type 2 diabetic obese db/db mice are refractory to myocardial ischaemic post-conditioning in vivo: potential role for Hsp20, F1-ATPase delta and Echs1. J Cell Mol Med 16:950–958.

Zuurbier CJ, Eerbeek O, Meijer AJ (2005). Ischemic preconditioning, insulin, and morphine all cause hexokinase redistribution. Am J Physiol Heart Circ Physiol 289:H496-499.

Zuurbier CJ, Heinen A, Koeman A, Stuifbergen R, Hakvoort TB, Weber NC, *et al.* (2014). Cardioprotective efficacy depends critically on pharmacological dose, duration of ischaemia, health status of animals and choice of anaesthetic regimen: a case study with folic acid. J Transl Med 12:325.

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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	Hyperglycaemi	Hyperlipidaemi	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 (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 insulinotropic drugs. • ZDF were selectively bred from Zucker fatty rat which are similar but without hyperglycaemia.	• Hydronephrosis. • Due to different genetic backgrounds, no comparisons can be made between ZDF and Zucker fatty rats. • Special diet requirements. • Some degree of infertility.

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		Pathophysiological changes									
Model (specie) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemi	Hyperlipidaemi	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.	• 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.

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									• Increased LV mass, fatty acid oxidation and lipid content.		
		Pathophysiological changes									
Model (specie) Ref.	Type	DM type	Obesity	HI & Ins Resist	Hyperglycaemi	Hyperlipidaemi	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.

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Table 2 – Ischaemic and Pharmacological Preconditioning in Diabetic and Hyperglycaemic Models (Selected studies from 2013)

Model	Ischaemia/ Reperfusion protocol	Preconditioning protocol	Results	Refs
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	(Yang *et al.,* 2013)
Acute hyperglycaemia **Rats**: Male Wistar - (22 mmol/l)	**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	(Zálešák *et al.,* 2014, 2016)
T1DM **Rats:** STZ, 50 mg/kg i.p	**Ex vivo**, 30 min ischaemia/2 hr reperfusion	2 cycles of 5 min ischaemia/5 min reperfusion	IPC was attenuated but was restored by zinc chloride and zinc ionophore pyrithione	(Jamwal *et al.,* 2016)
T1DM **Rats**: Male Sprague-Dawley; STZ, 70 mg/kg	**In vivo**, 30 min ischaemia/3 hr reperfusion	2 cycles of 5 min ischaemia/5 min reperfusion	T1DM did not affect IRI but IPC was abrogated Exogenous insulin supplementation restored IPC cardioprotection	(Ji *et al.,* 2013)
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	(Hjortbak *et al.,* 2018)
TD2M **Rats**: Cohen diabetes-resistant (CDr) rats (Panel 1B) and 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.	(Vinokur *et al.,* 2016)
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	(Whittington *et al.,* 2013)
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.	(Pælestik *et al.,* 2017)
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.	(Hausenloy *et al.,* 2013)
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	(Nakadate *et al.,* 2017)
T1DM	**In vivo** 30 min ischaemia/4 hr reperfusion	Geniposide, intragastric administration	Geniposide PPC reduced IRI in T1DM	(Wang *et al.,* 2019)

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Rats: Sprague-Dawley, Diabetes injected with 1% streptozotocin (55 mg/kg)		(100 mg/kg) before, once a day for 7 days.		
T1DM **Rat**: Wistar either sex, a single dose of alloxan monohydrate (120 mg/kg)	**Ex vivo**, 30-min ischaemia/2 hr reperfusion	Atrial natriuretic peptide (ANP) 0.1 μM/L	ANP PPC reduced IRI in T1DM	(Charan *et al.,* 2016)
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	NaHS (20 μM) for 15 min prior to I/R	H_2S PPC reduced IRI in both models	(Ansari *et al.,* 2019)
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	(Rana & Sharma 2016)
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	(Peake *et al.,* 2013)

Krebs-Henseleit, KH; Streptozotocin, STZ.

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Table 3 –Ischaemic and Pharmacological Postconditioning in DM and Hyperglycaemic Models

Model	Ischaemia/ Reperfusion protocol	Postconditioning protocol	Results	References
Ischaemic Postconditioning (IPost)				
Hyperglycaemia H9c2 cardiomyoblasts incubated with 25 mM glucose for 48 h	**In vitro**, 5 hr hypoxia (95% N_2 and 5% CO_2)/4 hr reoxygenation	One cycle of 5-min hypoxia after 5-min reoxygenation at the onset Remifentanil PPost (1-µM) at the onset of reoxygenation	Hypoxic postconditioning and Remifentanil PPC attenuated by high glucose concentration.	(Chen *et al.,* 2016)
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	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	(Lacerda *et al.,* 2012)
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	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	(Ren *et al.,* 2011)
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	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	(Przyklenk *et al.,* 2011)
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	(Zhu *et al.,* 2012)
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	(Oosterlinck *et al.,* 2013)
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	(Raphael *et al.,* 2010; 2015)
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	(Huhn *et al.,* 2008)

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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	(Yu *et al.,* 2017)
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.	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	(Drenger *et al.,* 2011)
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	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	(Grievink *et al.,* 2019)
T1DM **Rats**: male Sprague-Dawley, DM induced by a single injection of STZ (55 mg/kg)	**In vivo**, 30 min ischaemia/2 hr reperfusion	Sufentanil PPost (1 µg/kg, i.v.) 5 min before the onset of reperfusion	Sufentanil PPC restored by long-term insulin treatment.	(Zhang *et al.,* 2016)
T1DM **Rats**: Sprague-Dawley male, DM induced by high-fat diet after 4 weeks, STZ (45 mg/kg)	**In vivo**, 40 min ischaemia/ 3 hr reperfusion	Atorvastatin	Atorvastatin PPC effective	(Chen *et al.,* 2017)
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	Sevoflurane 2% for 15 min in the second half of ischaemia	N-Acetylcysteine co-administered with STZ restored sevoflurane PPost cardioprotection	(Lin *et al.,* 2016)
T1DM **Rats**: Wistar, Hyperglycemia 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; 10-100 µg/kg) given 5 min before reperfusion	Normal dose of milrinone and high dose of levosimendan, were protective in hyperglycaemic conditions.	(Matsumoto *et al.,* 2012)
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	Sevoflurane (1-MAC) first 5 min after the onset of reperfusion.	DM blocked sevoflurane PPost	(Tai *et al.,* 2012)
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	(Lemoine *et al.,* 2010; 2011)
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.	(Gao *et al.,* 2016)
T2DM **Rats**: Sprague-Dawley, DM induced by high-fat and high-sugar diet for 6 weeks and a single injection of SPZ (40 mg/kg, i.p.)	**In vivo**, 40 min ischaemia/2 hr reperfusion	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	(Xie *et al.,* 2017)
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	(Huhn *et al.,* 2010) PMID: 19819119

Diabetes Mellitus, DM; Minimal Alveolar Concentration, 1-MAC; Streptozotocin, STZ.

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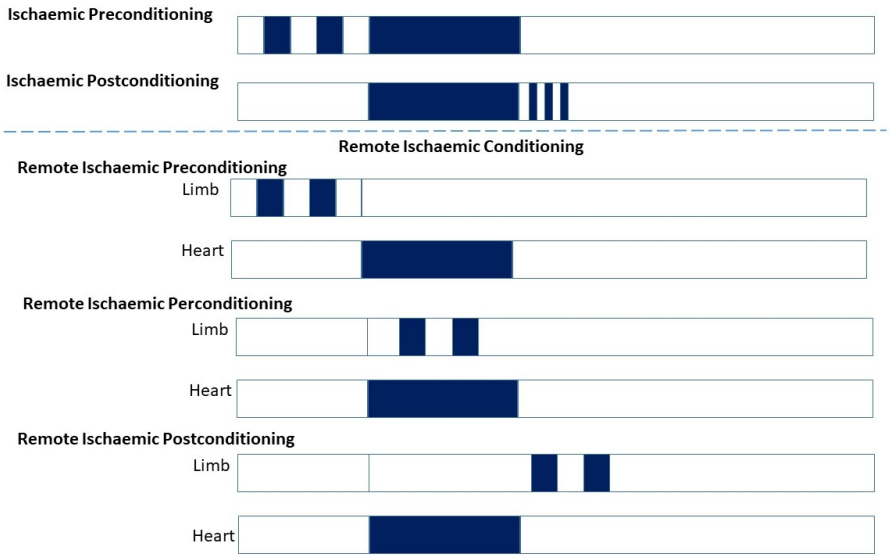

Figure 1: Schematic representation of the various protocols of ischaemic conditioning.

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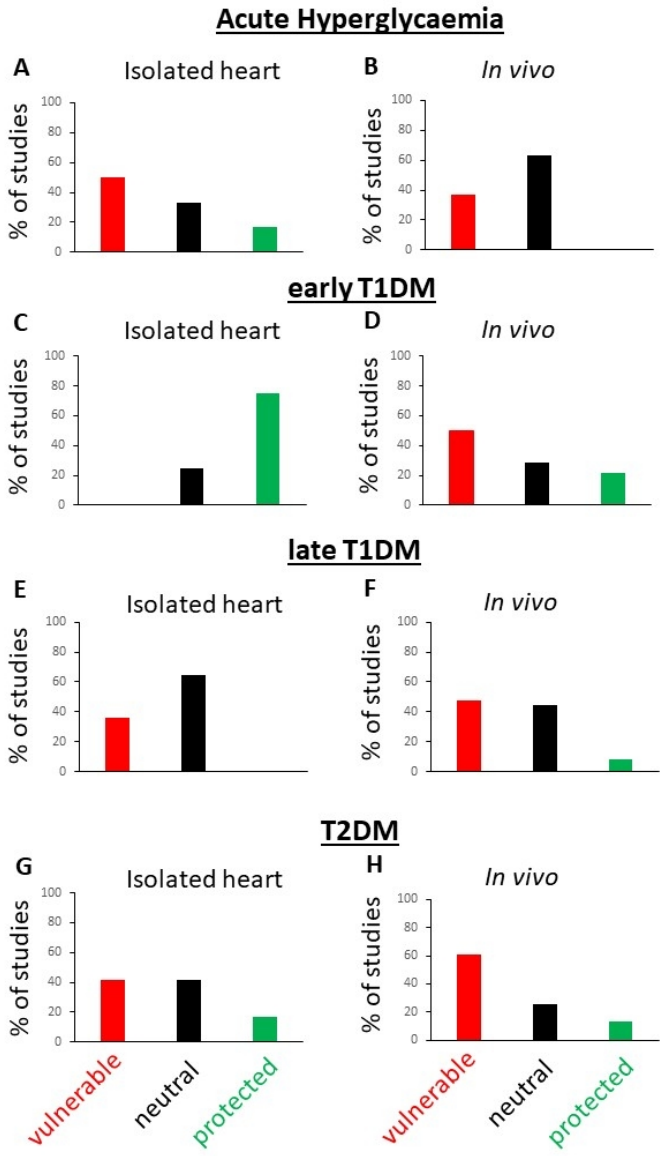

Figure 2: Schematic summary of 130 studies reporting effects on cardiac sensitivity to IRI of acute hyperglycaemia (A, B), early type 1 Diabetes Mellitus (C, D), late type 1 Diabetes Mellitus (E, F) and Type 2 Diabetes Mellitus (G, H) for the isolated heart (A, C, E and G) or the in vivo condition (B, D, F and H), respectively. Within each of the 8 categories the number of studies reporting increased vulnerability, neutral or protective effects is divided by the total number of studies in that category.

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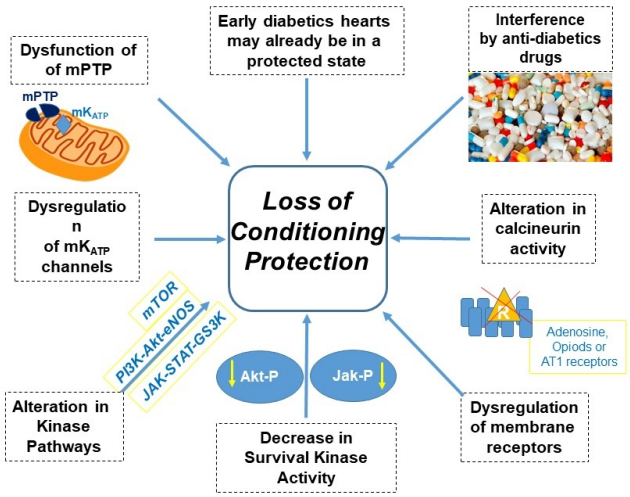

Figure 3: Mechanisms of loss of conditioning protection in diabetic hearts. In the early stages of diabetes, the heart is in a paradoxical state of protection. Subsequently, diabetic hearts may have an increased threshold for conditioning protection, the reasons for which are multifactorial. These include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

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