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# Preconditioning the diabetic human myocardium

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## Abstract

Our objective was to determine whether human diabetic myocardium is amenable to the cardioprotective actions of ischaemic preconditioning. Human right atrial appendages were harvested from diabetic and non-diabetic patients undergoing elective coronary artery bypass graft surgery. The atrial trabeculae were isolated and subjected to 90 min. of hypoxia followed by 120 min. of reoxygenation, following which the percentage recovery of baseline contractile function was determined. The atrial trabeculae were randomized to: (*i*) controls (groups 1 and 3); (*ii*) standard hypoxic preconditioning (HPC) protocol consisting of 4 min. of hypoxia/16 min. of reoxygenation before the 90 min. index hypoxic period (groups 2 and 4); (*iii*) Prolonged HPC protocol consisting of: 7 min. of hypoxia /16 min. of reoxygenation before the index hypoxic period (group 5). In addition, basal levels of Akt phosphorylation were determined in right atrial appendages harvested from non-diabetic patients and diabetic patients to determine whether PI3K-Akt signalling is down-regulated in the diabetic heart. Standard HPC improved baseline contractile function in human atrial trabeculae harvested from non-diabetic patients (52.4 ± 3.8% with HPC *versus* 30.0 ± 3.2% in control: $P = 0.001$; $N = 6$/group), but not in atrial trabeculae isolated from diabetic patients (22.6 ± 3.3% with HPC *versus* 28.5 ± 1.9% in control: $P > 0.05$; $N = 6$/group). However, the prolonged HPC protocol did improve baseline contractile function in atrial trabeculae harvested from diabetic patients (42.0 ± 2.4% with HPC *versus* 28.5 ± 1.9% in control: $P = 0.001$; $N \geq 6$/group). Western blot analysis demonstrated lower levels of phosphorylated Akt in diabetic myocardium compared to non-diabetic myocardium (0.13 ± 0.03 arbitrary units *versus* 0.39 ± 0.11 arbitrary units: $P = 0.047$; $N \geq 4$/group). From the data obtained it appears that the threshold for preconditioning the diabetic myocardium is elevated which may be related to the down-regulation of the PI3K-Akt pathway.

**Keywords:** preconditioning • human • myocardium • diabetes mellitus • myocardial infarction

## Introduction

Coronary heart disease (CHD) is a major cause of morbidity and mortality in patients with type 2 diabetes, with the incidence of cardiovascular disease in diabetic patients being twice that of non-diabetic men and three times that of non-diabetic women [1]. More recent estimates suggest that women in the age group of 35–54 years are five times more likely to have a myocardial infarction than women without diabetes [2]. Interventions such as a coronary artery bypass graft (CABG) surgery, are also associated with a significantly lower 5-year survival in diabetic patients than in non-diabetic with increased post-operative and long-term morbidity [3–5]. Furthermore, diabetic patients who survive an MI are more likely to develop heart failure than non-diabetic patients [6]. Therefore, new cardioprotective strategies which are capable of protecting the diabetic myocardium are required in order to improve clinical outcomes in diabetic patients with CHD.

In this respect, the endogenous cardioprotective phenomenon of ischaemic preconditioning (IPC) represents a powerful interventional strategy for protecting against myocardial injury arising from ischaemia-reperfusion injury. IPC is elicited by brief non-lethal episodes of myocardial ischaemia and reperfusion applied before the lethal ischaemic insult [7]. However, experimental animal studies suggest that the diabetic heart may be resistant to the cardioprotective effects of IPC [8], and this may be the case in diabetic patients [9, 10]. An experimental study from our laboratory suggests that a more robust IPC stimulus is used, the diabetic heart is still amenable to the infarct-limiting effects of IPC [11]. The apparent resistance of the diabetic heart to a standard IPC protocol may be attributed to down-regulation of the PI3K-Akt pathway [11], a major component of the signal transduction pathway which underlies IPC [12, 13], as demonstrated in previous experimental animal studies [14]. In the current study we investigate whether myocardial tissue harvested from diabetic patients undergoing CABG is amenable to the cardioprotection elicited by IPC.

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**Table 1** Profile of diabetic patients included in the study. (WB – White British, WO – White other)

| No. | Age/sex | Race | Procedure | Lab exp | $HbA_1C$ | Oral hypoglycaemic |
|---|---|---|---|---|---|---|
| 1. | 52/M | Asian | CABG | Trabeculae | 6.5 | Glipizide |
| 2. | 58/M | WB | CABG | Trabeculae | NA | Metformin |
| 3. | 58/M | WB | AVR | Trabeculae | 5.3 | Metformin |
| 4. | 65/M | Asian | CABG | Trabeculae | 9.1 | Repaglinide |
| 5. | 61/M | WB | CABG | Trabeculae | 9.3 | Glimepride |
| 6. | 70/M | WB | AVR | Trabeculae | NA | Metformin |
| 7. | 69/F | Mixed | AVR/CABG | Trabeculae | 6.8 | Metformin |
| 8. | 76/M | WO | CABG | Trabeculae | 8.5 | Metformin/Glipizide |
| 9. | 79/F | WB | CABG | Westerns | NA | Metformin/Gliclazide |
| 10. | 71/M | WB | CABG | Westerns | 6.5 | Metformin |
| 11. | 66/M | WB | CABG | Westerns | 7.8 | Metformin/Gliclazide |
| 12. | 47/F | Asian | AVR | Westerns | NA | Gliclazide/Rosiglitazone |
| 13. | 65/M | WO | AVR | Westerns | NA | Gliclazide/Metformin |

# Materials and methods

## Study patients

Ethical approval for the study was obtained from the Research and Ethics Committee (REC), at the University College London/University College London Hospitals. Elective patients admitted for coronary artery bypass surgery or valve surgery, were consented. Patients over the age of 80, with a troponin positive event in the last 6 weeks, unstable angina, congestive cardiac failure ($EF < 50\%$), renal failure, arrhythmias or on anti-arrhythmic medication were excluded. Patients diagnosed with type 2 diabetes on oral hypoglycaemic agents were consented for the study. The average duration of diabetes in these patients was 4.7 years. Type 2 diabetic patients who were either on insulin or glibenclamide were excluded from the study as insulin is a known cardioprotective agent and glibenclamide is known to block the protective effect of preconditioning [15, 16].

During the surgery, and immediately prior to the insertion of the venous cannula of the bypass machine, a piece of the right atrial appendage was harvested. The appendage was either transported to the laboratory in modified Tyrode's buffer (comprising mmol/l 118.5 NaCl, 4.8 KCl, 24.8 $NaHCO_3$, 1.2 $KH_2PO_4$, 1.44 $MgSO_4 \cdot 7H_2O$, 1.8 $CaCl_2 \cdot 2H_2O$, 10.0 glucose and 10.0 pyruvic acid, oxygenated with a 95% $O_2$–5% $CO_2$ gas mixture, to maintain pH between 7.35 and 7.45, and maintain a $pO_2 > 55$ kPa and a $pCO_2$ between 4.0 and 6.0 kPa) at less than 4°C in order to dissect atrial trabeculae, or snap frozen in liquid nitrogen within 5 sec. of harvesting from the patient, in order to conduct Western blot analysis to determine the levels of phosphorylated and total Akt. Of the non-diabetic patients, 10 were consented for the study of which six appendages were used for trabecula experiments and four were used for Western blot analysis. There were seven males and three females with ages ranging from 57 to 80 years. The average age was 71 ± 2.3 years. A profile of diabetic patients consented along with the $HbA_1C$ levels and their oral hypoglycemic therapy is shown in Table 1.

## The human atrial trabecula model of simulated ischaemia-reperfusion injury

This experimental model was first established in our research laboratory and is both a robust and reproducible technique for assessing cardioprotective treatment strategies using human atrial trabeculae subjected to simulated ischaemia-reperfusion injury [17, 18]. In the laboratory, the human atrial trabeculae are dissected from the inner surface of the appendage and immersed in an organ bath containing modified Tyrode's buffer and placed between two pacing electrodes. The temperature of the bath is kept at 37°C with a heat exchanger. A minimum of two trabeculae were dissected from each appendage as one of the trabeculae served as a control. The trabeculae were paced at 1 Hz for a period of 75 min. to allow it to stabilize. Ischaemia was simulated by replacing modified Tyrode's buffer with a hypoxic glucose free buffer (containing in mmol/l 118.5NaCl, 4.8 KCl, 24.8 $NaHCO_3$, 1.2 $KH_2PO_4$, 1.44 $MgSO_4 \cdot 7H_2O$, 1.8 $CaCl_2 \cdot 2H_2O$, 7.0 choline chloride bubbled with 95%$N_2$–5%$CO_2$ to keep $pO_2$ between 6 and 8 kPa, $pCO_2$ between 4 and 6 kPa and pH between 7.30 and 7.35) and the pacing increased to 3 Hz. Reperfusion was simulated by replacing the organ bath with modified Tyrode's buffer and reducing the pacing frequency to 1 Hz. The force of contraction of the trabeculae was calculated *via* a pacing transducer and software (Chart 5 for Windows). Trabecula diameter greater than 1.2 mm, damaged trabeculae determined visually, irregularly contracting trabeculae either at baseline or at the end of reperfusion, or trabeculae with an amplitude of contraction less than 0.5 g were excluded. These exclusion criteria have been developed by our group following 12 years of experience with this model [17, 18].

The atrial trabeculae harvested from patients were randomized to the following groups (Fig. 1): (1) Non-diabetic control ($n = 6$): Atrial trabeculae harvested from non-diabetic patients were subjected to 75 min. of stabilization, 90 min. of index hypoxia and 120 min. reoxygenation; (2) Non-diabetic standard hypoxic preconditioning (HPC) ($n = 6$): Atrial trabeculae harvested from non-diabetic patients were subjected to a PC stimulus comprising 4 min. of hypoxia and 16 min. of reoxygenation

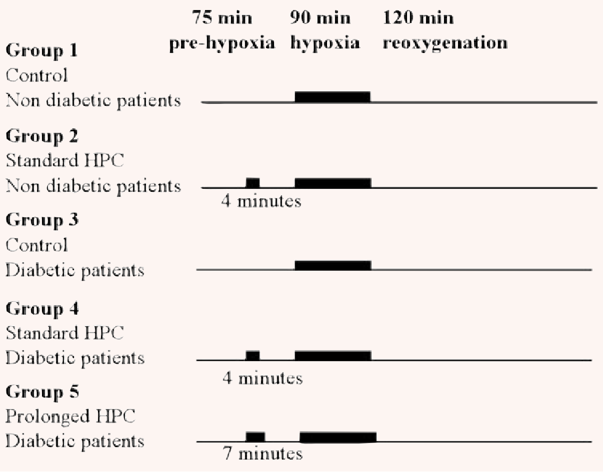

**Fig. 1** Experimental protocol used in the trabecula experiments.

prior to the 90 min. index hypoxia; (3) Diabetic control ($n = 6$): Atrial trabeculae harvested from diabetic patients were subjected to 75 min. of stabilization, 90 min. of hypoxia and 120 min. of reoxygenation; (4) Diabetic standard HPC ($n = 6$): Atrial trabeculae harvested from diabetic patients were subjected to a preconditioning stimulus comprising 4 min. hypoxia and 16 min. reoxygenation prior to the 90 min. index hypoxia; (5) Diabetic prolonged HPC ($n = 7$): Atrial trabeculae harvested from diabetic patients were subjected to a more robust preconditioning stimulus comprising 7 min. hypoxia and 16 min. reoxygenation prior to the 90 min. index hypoxia.

The recovery of baseline contractile function was calculated by dividing the force of contraction at the end of the protocol by the baseline, which was recorded at the end of the period of stabilization. This was expressed as a percentage of the baseline and is used as a surrogate marker of tissue injury.

## Western blot analysis

Right atrial appendages from non diabetic ($n = 4$) and diabetic ($n = 5$) patients, were immediately frozen after being excised from the heart at the time of CABG surgery. They were stored in a –80°C freezer and were subsequently analysed for phosphorylation of Akt (serine 473) and normalized to the total Akt level. Equal loading was confirmed by probing for β-actin levels. SDS-PAGE immunoelectrophoresis was used to determine this along with primary and secondary antibodies obtained from New England Bio Labs (Ipswich, MA, USA) as previously described [12].

## Statistical analysis

Data are presented as mean ± S.E.M. A factorial one-way ANOVA was used for comparison between more than two groups in the functional recovery experiments. Where a significant $F$-value was obtained, Fisher's protected least significant difference *post hoc* to test for significance has been applied. A $P$-value of $<0.05$ has been taken to indicate significance. Protein band quantifications are expressed as mean value ± S.E.M. and differences between groups have been tested using a t-test. All data was analysed using Statview 4.5 (Abacus Concepts Inc., Berkley, CA, USA). Proteins were detected using chemiluminescence and bands visualized by exposure to photographic film and relative densitometry assessed using NIH Image-1.63 software.

**Table 2** Baseline functional data for the human atrial trabecula experiments

| Group | *N* | Average contraction at baseline | *P*-value |
|---|---|---|---|
| Non diabetic control | 6 | 0.93 ± 0.2 | NA |
| Non diabetic standard HPC | 6 | 0.96 ± 0.1 | 0.9 |
| Diabetic control | 6 | 0.83 ± 0.1 | 0.6 |
| Diabetic standard HPC | 6 | 0.80 ± 0.1 | 0.5 |
| Diabetic prolonged HPC | 7 | 0.88 ± 0.2 | 0.8 |

# Results

## Diabetic myocardium requires a stronger preconditioning stimulus

There were no significant differences in the contractile functions of the atrial trabeculae at baseline (Table 2). Standard HPC of atrial trabeculae harvested from non-diabetic patients resulted in a significant improvement in recovery of baseline contractile function (52.4 ± 3.8% with HPC *versus* 30.0 ± 3.2% in control: $P = 0.0011$). This was not the case in atrial trabeculae isolated from diabetic patients where the recovery of baseline function was 22.6 ± 3.3% in the standard HPC group *versus* 28.5 ± 1.9% in the diabetic control group ($P > 0.05$). Interestingly, the more robust HPC protocol did manage to improve baseline contractile function in atrial trabeculae isolated from diabetic patients (42.0 ± 2.4% with prolonged HPC *versus* 28.5 ± 1.9% in diabetic control: $P = 0.0014$) (Fig. 2).

## Reduced levels of baseline Akt phosphorylation in human diabetic myocardium

Compared to non-diabetic myocardium, the extent of phosphorylation of Akt in diabetic myocardium was significantly lower (0.39 ± 0.11 arbitrary units in non-diabetic *versus* 0.13 ± 0.03 arbitrary units in diabetic myocardium: ($P = 0.047$). In both non-diabetic and diabetic patients, there was no significant difference in the total Akt level when normalized against β-actin (1.37 ± 0.29 arbitrary units *versus* 0.84 ± 0.12 arbitrary units in non diabetic and diabetic myocardium, respectively) (Fig. 3).

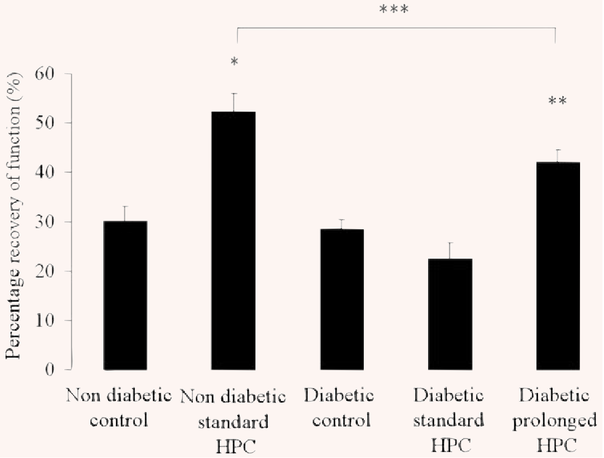

**Fig. 2** Standard hypoxic preconditioning (HPC – 4 min. hypoxia and 16 min. reoxygenation) results in better recovery of function compared to the control group in non-diabetic trabeculae ($*P < 0.005$; $n = 6$/group). The standard HPC protocol fails to protect diabetic trabeculae ($n = 6$) but a prolonged HPC protocol (7 min. hypoxia and 16 min. reoxygenation; $n = 7$) results in significantly better recovery of function compared to the control group ($n = 8$) ($**P < 0.005$). However, there is also a significant difference between the standard HPC in the non-diabetic trabeculae and the prolonged HPC in the diabetic trabeculae, suggesting that there may be room for further protection ($***P < 0.05$).

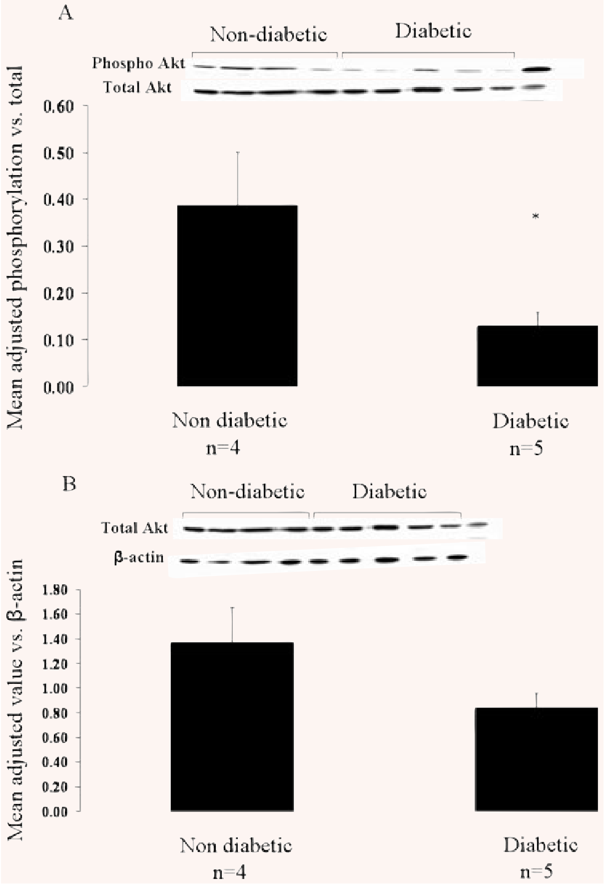

**Fig. 3** In diabetic myocardium, phospho-Akt is significantly lower when compared to non-diabetic myocardium ($*P < 0.05$). Note that the tenth blot shown in the Western blotting strip at the top of the figure is a positive control in an insulin stimulated mouse heart harvested a few minutes into reperfusion (**A**). There is no significant difference in the total Akt, normalized to β-actin, between diabetic and non diabetic myocardium ($*P = 0.11$). The β-actin blots in the strip at the top of the figure suggest equal loading in all wells. Once again, the tenth blot in the total Akt strip is a positive control in an insulin stimulated mouse heart harvested a few minutes into reperfusion. The β-actin signal in the insulin stimulated mouse heart was too weak to be picked up at the exposure required for human myocardium (**B**). Values on the *y*-axis are in arbitrary units.

# Discussion

The major findings in this study are as follows: (*i*) Human atrial trabeculae harvested from diabetic patients undergoing CABG surgery are resistant to a standard HPC protocol; (*ii*) A more robust HPC stimulus is required in order to demonstrate a preconditioning effect in human atrial muscle isolated from diabetic patients; (*iii*) Baseline levels of Akt phosphorylation were significantly lower in right atrial appendages harvested from diabetic patients when compared to non-diabetic control patients. Therefore, we have demonstrated that the human diabetic myocardium is amenable to IPC provided that a sufficient stimulus is used. This apparent resistance to the cardioprotective effects of IPC were associated with lower levels of Akt phosphorylation at baseline in human diabetic hearts.

Our findings in the human heart are in keeping with our previous animal studies using type 2 diabetic rat hearts. Although a standard IPC protocol comprising one cycle of 5 min. ischaemia and reperfusion did not reduce myocardial infarct size in diabetic rat hearts, three cycles of IPC did result in a significantly smaller myocardial infarct size [11]. Interestingly, the resistance of the diabetic rat heart following the standard IPC protocol of one cycle was attributed to insufficient activation of the PI3K-Akt pathway, whereas the three cycles of IPC succeeded in limiting myocardial infarct size, as the PI3K-Akt pathway was sufficiently activated [11], suggesting a threshold level of kinase activity which needs to be achieved to confer cardioprotection in the setting of IPC. In contrast, Ghosh and colleagues [9] failed to precondition human right atrial appendages obtained from type 2 diabetic patients despite applying repeated cycles of hypoxia and reoxygenation. This discordant result may be attributed to that fact that the experimental model used in their study differs considerably from the one used by our laboratory. In their study the entire right atrial appendage sample is cut into thin slices and the whole specimen is immersed in hypoxic buffer and subjected to simulated ischaemia-reperfusion injury, at the end of which the amount of

CK released is used to assess cardioprotection [9] as opposed to our study which examines the percentage recovery of function. It is possible that an aggressive HPC protocol resulting in a total hypoxic period of 15 min. during preconditioning may have initiated necrosis in the atrial appendages. We have tried to avoid such an aggressive hypoxic insult by using a slightly prolonged protocol of HPC instead and using a different end-point of injury.

Early experimental animal studies examining IPC in diabetic animal hearts produced contradictory data. Many groups used streptozotocin (STZ) or alloxan to induce a diabetic state. Although some reported additional protection, others noted no protection against end-points such as arrhythmias and myocardial stunning with the amount of protection achieved varying according to the age of the animal [8]. This has led to criticism of models that use chemicals to induce diabetes as the effect of the chemical could be widespread and non specific. Both these substances are highly toxic and induce diabetes by destroying islet cells in the pancreas and their effect on the cardiovascular system is unpredictable. Moreover, most STZ-induced mice models are male, as female mice appear to be less sensitive to the effects of STZ [19]. In other more reliable models of type 2 diabetes such as the lean Goto–Kakizaki (GK) and the Zucker diabetic fatty rats, IPC did not offer any protection [20], just as in diabetic animal models of the dog, the rabbit and the sheep. If anything, outcome seemed to worsen in the larger animal models [8].

In the clinical setting, IPC is known to be more difficult in diabetic patients than in non-diabetic patients. Ishihara *et al.* studied 611 patients presenting with their first AMI, of whom 121 had diabetes. They noted that the onset of prodromal angina 24 hrs prior to the AMI was associated with a lower CK level after procedure, higher left ventricular ejection fractions and lower in hospital mortality in non-diabetic patients. Diabetic patients, on the other hand, did not benefit from the onset of angina, with no change in the three mentioned parameters [10]. The authors suggested that this may be due to a number of reasons: diabetic hearts may have an altered $K_{ATP}$ channel [21]; acute hyperglycaemia has been shown to abolish IPC [22] and oral hypoglycaemic agents like glibenclamide which block $K_{ATP}$ channel opening can prevent IPC [16]. In addition, it is also known that the initial steps of insulin signalling and glucose transport are defective in the type 2 diabetic heart [23], steps which may be inherent to insulin mediated IPC [24]. Furthermore, basal protein kinase B levels and insulin stimulated Akt, ERK and PI3-K have been shown to be defective in diabetic animals [14, 25]. Mitochondrial dysfunction has also been suggested as a potential reason for failure to precondition the human diabetic myocardium [26]. Hassouna *et al.* harvested appendages from non-diabetic patients and diabetic patients who were insulin dependent, or were being treated with metformin and glibenclamide. They noted that although diazoxide depolarized mitochondrial membrane potential in isolates from non-diabetic patients, it failed to do the same in diabetic mitochondrial isolates. They also reported that although ischemia, phenylephrine, adenosine and diazoxide failed to precondition diabetic appendages, protein kinase C and p38MAPK activators were able to precondition them. This group, as discussed earlier, used CK release as an end-point as compared to the recovery of function used by us [26].

In addition to the above, we have shown that the levels of phosphorylated Akt at baseline are lower in atrial appendages harvested from diabetic patients, when compared to non-diabetic patients, which, to our knowledge, has never been reported. This may offer a mechanistic explanation as to why a prolonged or more robust HPC stimulus is needed to cause protection in diabetic trabeculae. However, it is also likely that the defect could be at any level in the signalling pathway and a firm conclusion cannot be drawn from this data. Animal studies suggest that levels of phosphorylated Akt are lower in diabetic hearts whether or not an intervention is applied [27, 28]. In addition, studies suggest that standard stimuli such as insulin and isoproterenol, that cause phosphorylation of Akt in control animal hearts, fail to cause the same level of phosphorylation in diabetic models. Yet, stimuli were additive, a finding that is in keeping with human hearts, where an additional HPC stimulus resulted in a better recovery of function [14]. We speculate that the additional HPC stimulus crossed a threshold activating the signalling pathway resulting in cardioprotection however additional experiments are necessary to clarify his point.

Metformin had been prescribed to 9 of the 13 diabetic patients recruited into the study. In the United Kingdom Prospective Diabetes Study (UKPDS), there was a significant reduction in the incidence of myocardial infarction in type 2 diabetic patients who were treated with metformin, which additionally, has been shown to be superior to sulphonylurea therapy in reducing both all cause mortality and cardiovascular mortality [29, 30]. It has been suggested that the cardioprotective effects of metformin could be attributable to actions other than glucose lowering, specifically, through the activation of AMP-activated protein kinase (AMPK), an enzyme regulating lipid and glucose metabolism [31]. In fact, the acute administration of metformin in low doses has been shown to activate AMPK-eNOS in diabetic and non-diabetic murine hearts, while causing infarct size reduction following ischaemia-reperfusion injury [32]. However, the effect of chronic metformin therapy on the activity of AMPK has not been demonstrated.

Of the 13 diabetic patients consented for our study, 6 patients were on sulphonylureas, which are thought to inhibit mitochondrial $K_{ATP}$ channels that may inhibit the cardioprotection elicited by preconditioning. However, the role of the mitochondrial $K_{ATP}$ channel in IPC has not been established without doubt [33, 34]. Furthermore, some sulphonylureas such as gliclazide have been shown to be more selective for pancreatic mitochondrial $K_{ATP}$ channels as opposed to myocardial channels [35]. Indeed it has been shown that newer sulphonlyureas, such as gliclazide and glimepiride, do not abolish IPC in rats whereas glibenclamide does [16, 36, 37]. Four patients were prescribed gliclazide which, incidentally, has been shown to inhibit preconditioning using supramaximal doses in human atrial muscle [38]. Two patients were on glipizide, which does not inhibit myocardial preconditioning in rabbits *in vivo* [39]. Additionally, one patient each was prescribed repaglinide and rosiglitazone. Although repaglinide has been shown to have no selectivity for pancreatic or myocardial $K_{ATP}$

channels, the results were included because of the debatable role of $K_{ATP}$ channel in preconditioning. Pioglitazone, a thiazolidinedione and PPAR-γ receptor agonist similar to rosiglitazone, has been shown to activate PI3k-Akt and induce cardioprotection when administered acutely. However, it is likely that chronic administration of any PI3K-Akt activators would result in negative inhibition *via* activation of phosphatase and tensin homolog [40], as has shown to be the case with chronic atorvastatin administration by our group [41, 42].

A major limitation of our study is that it does not provide direct evidence that the prolonged HPC stimulus would result in greater phosphorylation of Akt. The evidence is not causal but only an association. To prove a causal relationship, it would be necessary to precondition trabeculae with the standard and prolonged HPC stimulus and compare the levels of Akt phosphorylation between the two groups. However, in our experience trabeculae do not provide sufficient tissue to be able to blot proteins by current methods. It may have been possible to subject an entire appendage to the standard and prolonged HPC protocol but difficulty in recruiting, collecting and handling human material have limited our options for additional measurements. Another limitation of our study was the unfortunate lack of $HbA_1C$ levels in some patients to co-relate with our findings. In the trabeculae experimental group, eight diabetic patients were included of which six had $HbA_1C$ levels. The mean $HbA_1C$ in the trabeculae group was 7.6% (7.0–8.2). Despite this, it was possible to precondition this group with a prolonged HPC stimulus. Thus, it is likely that diabetes associated with higher $HbA_1C$ levels may result in glycation of many cellular signalling proteins but it is still possible to activate these enzymes by using a stronger than normal preconditioning stimulus. A further drawback of our study is the use of human atrial tissue as opposed to ventricular tissue. This is based on the easier availability of atrial tissue.

In the UKPDS, intensive treatment with a target $HbA_1C$ of 7.0% was associated with no reduction in macrovascular complications as compared to the conventional treatment group [43]. Additionally, it has been suggested that despite intensive treatment in diabetic patients, mortality following AMI reduces only in the short term and in the long term the trend is maintained only in the non-diabetic patients [44, 45]. It may be possible, however, to improve both mortality and morbidity in both diabetic and non-diabetic patients if we could pharmacologically or mechanically activate the protective signalling pathway associated with pre- or post-conditioning and reduce infarct size following an AMI. Clinical studies are needed to evaluate whether this is possible and a better understanding of the pathology of the diabetic myocardium will perhaps improve long-term morbidity and mortality rates in this large subset of patients.

In conclusion, the present study demonstrates for the first time that myocardium from diabetic patients can be preconditioned *ex vivo*; however, the threshold for protection is raised. The findings from the current study suggest that diabetic patients are still amenable to the benefits of IPC but that they may require a stronger preconditioning stimulus compared to non-diabetic patients.

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