Evaluation of the relationship between hyperinsulinaemia and myocardial ischaemia/reperfusion injury in a rat model of depression

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ABSTRACT

Major depression is associated with medical co-morbidity, such as ischaemic heart disease and diabetes, but the underlying pathophysiological mechanisms remain unclear. The FSL (Flinders Sensitive Line) rat is a genetic animal model of depression exhibiting features similar to those of depressed individuals. The aim of the present study was to compare the myocardial responsiveness to I/R (ischaemia/reperfusion) injury and the effects of IPC (ischaemic preconditioning) in hearts from FSL rats using SD (Sprague–Dawley) rats as controls and to characterize differences in glucose metabolism and insulin sensitivity between FSL and SD rats. Hearts were perfused in a Langendorff model and were subjected or not to IPC before 40 min of global ischaemia, followed by 120 min of reperfusion. Myocardial infarct size was found to be significantly larger in the FSL rats than in the SD rats following I/R injury (62.4 ± 4.2 compared with 46.9 ± 2.9 %; \( P < 0.05 \)). IPC reduced the infarct size (\( P < 0.01 \)) and improved haemodynamic function (\( P < 0.01 \)) in both FSL and SD rats. No significant difference was found in blood glucose levels between the two groups measured after 12 h of fasting, but fasting plasma insulin (70.1 ± 8.9 compared with 40.9 ± 4.7 pmol/l; \( P < 0.05 \)) and the HOMA (homoeostatic model assessment) index (\( P < 0.01 \)) were significantly higher in FSL rats compared with SD rats. In conclusion, FSL rats had larger infarct sizes following I/R injury and were found to be hyperinsulinaemic compared with SD rats, but appeared to have a maintained cardioprotective mechanism against I/R injury, as IPC reduced infarct size in these rats. This animal model may be useful in future studies when examining the mechanisms that contribute to the cardiovascular complications associated with depression.

Key words: cardiovascular disease, co-morbidity, depression, diabetes, infarct size, ischaemia/reperfusion injury, ischaemic preconditioning.

Abbreviations: AAR, area at risk; BRS, baroreflex sensitivity; CORT, corticosterone; CVD, cardiovascular disease; FSL, Flinders Sensitive Line; FST, forced swim test; HbA1c, glycated haemoglobin; HOMA, homoeostatic model assessment; HPA, hypothalamic–pituitary–adrenal; HRV, heart rate variability; I/R, ischaemia/reperfusion; IL–6, interleukin–6; IPC, ischaemic preconditioning; LV, left ventricular; LVDP, LV developed pressure; MI, myocardial infarction; AMI, acute MI; OGTT, oral glucose tolerance test; RPP, rate pressure product; SD, Sprague–Dawley; TNF–α, tumour necrosis factor–α.

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INTRODUCTION

Depression is a mental disorder often associated with medical co-morbidities such as heart disease and diabetes [1]. Depression is a well-known risk factor for the development of ischaemic heart disease and is associated with increased cardiovascular morbidity and mortality [2–5]. Major depression doubles the risk of adverse cardiovascular events within 12 months in patients with newly diagnosed coronary heart disease [6] and increases the risk of mortality after AMI [acute MI (myocardial infarction)] [7]. The presence of diabetes has been found to double the risk of co-morbid depression [8], and a meta-analysis has shown that depression increases the risk of developing Type 2 diabetes in adults 37% [9]. Some of the suggested biological mechanisms that link depression with CVD (cardiovascular disease) include increased HPA (hypothalamic–pituitary–adrenal) axis function [10–12], decreased HRV (heart rate variability) [13], dysregulation of inflammatory and immune functioning [14], and increased platelet/endothelial aggregation [15]. Other explanations why depression increases the risk of, as well as the mortality from, CVD could be behavioural mediators such as smoking, obesity and a sedentary lifestyle [16–18].

Although there appears to be an obvious co-occurrence of depression with heart disease and diabetes [1], the shared pathophysiology between these co-morbid disorders is not fully clarified. To obtain further insight into these potential mechanisms, the use of an animal model of depression that might also elicit cardiovascular and metabolic dysfunctions could enhance the understanding of common mechanisms that lead to the increased risk of co-morbidities in depression.

The FSL (Flinders Sensitive Line) rat is a well-validated genetic animal model of depression, bred from the SD (Sprague–Dawley) rat, which has behavioural, neurochemical and pharmacological features similar to depressed individuals [19]. The FSL rat has depressive-like symptoms on several behavioural tests, for example increased immobility in the FST (forced swim test), which can be neutralized by chronic antidepressant treatment [20,21]. Reduced food intake, body weight and irregular sleeping patterns have been observed in FSL rats and they may have serotonergic and dopaminergic abnormalities, as well as HPA axis dysfunctions compared with control strains [21–23].

It has been demonstrated that FSL rats have reduced HRV and BRS (baroreflex sensitivity) compared with control strains [24,25], both predictive markers of prognosis and cardiac mortality in clinical settings. Similar regulatory abnormalities are evident in patients with depression. Hence the FSL rat may serve as a useful model for investigating the susceptibility to myocardial ischaemia and the response to IPC (ischaemic preconditioning), an intervention that offers cardioprotective benefits against I/R (ischaemia/reperfusion) injury. Whether the FSL rat has pathophysiological or clinical resemblances to those observed in patients with the metabolic syndrome or Type 2 diabetes is still not clarified.

The aim of the present study was to compare the responsiveness to I/R injury between FSL and SD rats, and to investigate effects of IPC. Furthermore, we wanted to determine whether there were any pre-diabetic changes in fasting plasma blood glucose and insulin levels between the rats. The present study may help to determine whether the FSL rat could serve as a suitable animal model for future studies looking at mechanisms and associations between depression, diabetes and ischaemic heart disease.

Part of the present study was presented at the 43rd EASD Annual Meeting, held in Amsterdam on 17–21 September 2007, and at the 49th Meeting of the SCNP, held in Juan Le Pines on 2–4 April 2008, and were subsequently published in abstract form [25a,25b].

MATERIALS AND METHODS

Animals

Male FSL rats were supplied from the Center of Psychiatric Research, Risskov Hospital, Risskov, Denmark. Male SD rats were from M&B Taconic. The rats were handled according to national guidelines in Denmark for animal research (permission ID: 2007/531-1378). Animals were housed in a temperature-controlled (22–23 °C) and light-controlled (12/12-h light/dark cycle) room, and were given free access to water and a standard rat diet.

Study design

The experimental protocol is illustrated in Figure 1. The present study consisted of two series of experiments, involving aged-matched rats (FSL, n = 27, 9–10-weeks old and 300–350 g; and SD, n = 30, 9–10-weeks old, and 300–350 g). In experiment 1, the rats were tested in a FST and the hearts were Langendorff-perfused. Prior to perfusion, the rats were divided into four groups: SD without IPC (n = 9), SD with IPC (n = 9), FSL without IPC (n = 8) and FSL with IPC (n = 9). The perfusion of the hearts in the Langendorff model was performed 4 weeks after the FST to avoid any possible influence of the swimming procedure on the experiments. In experiment 2, the rats were initially exposed to an OGTT (oral glucose tolerance test), and 4 weeks later the rats were tested in a FST. Blood samples were collected on day 33. Weekly body weight and food consumption were assessed.

Isolated perfused hearts

Rats were anaesthetized with a mixture of Dormicum® (0.5 mg of midazolam/kg of body weight; Matrix Pharmaceuticals) and Hypnorm® (0.158 mg of fentanyl citrate/kg of body weight and 5 mg of fluanisone/kg of body weight; VetaPharma Ltd), each diluted with an equal volume of sterile water prior to mixing and
were administered as a single subcutaneous injection. When an adequate depth of anaesthesia was confirmed, a tracheotomy was performed and the rat was connected to a rodent ventillator (Zoovent). Respiration was maintained by mechanical ventilation with 50% room air and 50% O2. After laparotomy and thoracotomy, the heart was disected free from surrounding structures. A heparin bolus (1000 international units/kg of body weight; Leo Pharma) was given through the femoral vein. The aorta was cannulated and retrograde perfusion of the heart was started in situ. The heart was rapidly excised under continuous perfusion and mounted in an isolated perfused heart system. Hearts were perfused at constant pressure of 80 mmHg with a modified Krebs–Henseleit buffer (118.5 mmol/l NaCl, 4.7 mmol/l KCl, 25.0 mmol/l NaHCO3, 1.1 mmol/l glucose monohydrate, 1.2 mmol/l MgSO4·7H2O, 2.4 mmol/l CaCl2 and 1.2 mmol/l KH2PO4, pH 7.4). The perfusion buffer was oxygenated with a mixture of 95% O2 and 5% CO2 and kept at 37°C. The hearts were allowed to stabilize for 40 min and then subjected to global ischaemia for 40 min, followed by 120 min of reperfusion. Global ischaemia was induced by discontinuing the retrograde perfusion. Animals subjected to IPC were exposed to two cycles of 5 min global ischaemia and 5 min reperfusion during the last 20 min of the stabilization period (Figure 1).

**Evaluation of LV (left ventricular) function and coronary flow**

A latex balloon (Hugo Sachs Electronics) was inserted in the left ventricle through an incision in the left atrium and was kept in place by the mitral valve. A pressure transducer (Baxter Cardiovascular Group) was connected to the latex balloon allowing recording of LV function. Coronary flow was measured continuously using an inline flow probe (Transonic). All data were digitally converted (DT9804; Data Translation) and were analysed using a data acquisition system (Notocord Hem Software).

**Assessment of MI**

To assess infarct size, hearts were sliced (approx. 1.5 mm) and immersed in 0.1 mol/l phosphate buffer (Bie & Berntsen) at 37°C and pH 7.4 with 1% (w/v) 2,3,5-triphenyl tetrazolium chloride (Sigma) for 3 min to delineate areas of infarction. Following fixation in 4% formaldehyde buffer (Lillies solution; VWR International), the slices were weighed, scanned (HP ScanJet 4300C; Hewlett Packard) and digitally saved as JPEG files. The viable myocardium stained deep red, whereas necrotic tissue was pale. The entire AAR (area at risk; area of left ventricle–cavities) and infarct size (area of infarction of the left ventricle) were quantified using image analysis software (UTHSCA ImageTool version 3.0). The infarct size/left ventricle ratio was then calculated and corrected for the weight of each individual slice. All measurements were done in a blinded fashion.

**FST**

Increased immobility during forced swimming is an accepted index of depressive-like behaviour in rodent depressive models and can be determined in the FST. The FST was performed as described previously [26,27] with minor modifications. Briefly, rats were immersed individually in a cylinder of acrylic plastic (54 cm in height and 24 cm in diameter) containing 38 cm of water (25°C) for 15 min. At 24 h later, a 5 min re-test was conducted during which the total immobility time was recorded (in s) on videotape. The animals were judged immobile when they made no further attempts to escape.
Clinical and biochemical measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SD rats</th>
<th>FSL rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>415 ± 3</td>
<td>386 ± 9†</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.31 ± 0.02</td>
<td>1.31 ± 0.03</td>
</tr>
<tr>
<td>Heart weight/body weight ratio (g/kg)</td>
<td>3.16 ± 0.05</td>
<td>3.40 ± 0.09†</td>
</tr>
<tr>
<td>Fructosamine (μmol/l)</td>
<td>162.6 ± 10.5</td>
<td>157.1 ± 10.4</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>2.28 ± 0.07</td>
<td>4.07 ± 0.18†</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.25 ± 0.10</td>
<td>1.42 ± 0.08</td>
</tr>
</tbody>
</table>

OGTT

The OGTT was performed in the morning after the rats had been starved for 12 h. Blood glucose and insulin were measured from samples obtained by tail bleeding before administration of 2.5 g of glucose/kg of body weight by gavage, as well as 15, 60 and 120 min after glucose loading. Blood glucose levels were determined from all samples using a OneTouch Ultra Blood Glucose Monitoring System (LifeScan). Plasma insulin levels were determined using an ultrasensitive rat insulin ELISA kit from DRG Diagnostics. HOMA (homeostatic model assessment) is a method used to quantify whole-body insulin resistance and β-cell function. The HOMA index was calculated as follows: HOMA index = fasting glucose (mmol/l) × fasting insulin (pmol/l)/155 [28].

Blood samples

On the last day of experiment 2, the animals were killed by decapitation and blood was collected. The blood was centrifuged at 1200 g for 10 min and plasma was collected and frozen at −80 °C. All rats had been starved for 12 h. Hearts were removed and weighed for evaluation of the heart weight (in g)/body weight (in kg) ratio. Plasma levels of cholesterol and triacylglycerols (triglycerides) were determined on a Cobas Integra Analyser (Roche Diagnostics). Plasma fructosamine, which can be used as a surrogate for HbA1c (glycated haemoglobin) and reflects an average blood glucose level over a period of 2–3 weeks, was measured on a Cobas Mira Plus Chemistry System (Roche Diagnostics). Plasma IL-6 (interleukin-6) and TNF-α (tumour necrosis factor-α) concentrations were measured in duplicate using rat ELISA kits (R&D Systems).

Statistics and calculations

All values are expressed as means ± S.E.M. LVDP (LV developed pressure) was calculated as \( P_{LV, systolic} - P_{LV, diastolic} \) (systolic and diastolic LV pressure respectively), and the RPP (rate pressure product) as LVDP × HR (heart rate). Haemodynamic data were compared using two-way ANOVA with repeated measures. The infarct size/AAR ratio was evaluated with one-way ANOVA supplemented with Bonferroni’s multiple comparison test.

The statistical evaluation of the data concerning the clinical and biochemical measurements was compared using an unpaired Student’s \( t \) test. SPSS 10 was used for statistical calculations, and \( P < 0.05 \) was considered statistically significant.

RESULTS

Clinical and biochemical parameters

Clinical and biochemical parameters, recorded at the end of experiment 2, are shown in Table 1. There was no significant difference in body weight between the groups before the start of the study. At the end of the study, body weight was 8% higher (\( P < 0.01 \)) in the SD rats compared with the FSL rats. The decrease in body weight might be caused by a reduced food intake in the FSL rats which (on average) was 24 g/day compared with 27 g/day in the SD rats. Although no difference was found in heart weight between the FSL and SD rats, the heart weight/body weight ratio, which can be used as an objective measurement of cardiac hypertrophy in animal experiments, was significantly increased in the FSL rats (\( P = 0.05 \)) compared with the SD rats. The FSL rats were also characterized as having higher levels of total cholesterol (78% increase; \( P < 0.01 \)) and triacylglycerols (14% increase; \( P = 0.18 \)) compared with the SD rats. Fructosamine, a surrogate for HbA1c, was not different between the FSL and SD rats. Plasma IL-6 and TNF-α concentrations were at very low levels in both the FSL and SD rats (around the lower detection limit for the assay used) and no difference was found between the two strains (results not shown). All measurements were done in starved rats.

OGTT

The FSL rats were characterized by significantly increased fasting plasma insulin (71% increase; \( P < 0.05 \)) and increased 2-h insulin values (66% increase; \( P < 0.01 \)) compared with the SD rats (Table 2). There was a significant increase in the HOMA index in the FSL rats (82% increase; \( P < 0.01 \)) compared with the SD rats. No significant difference was found in fasting plasma glucose and the 2-h glucose value between the two groups (Table 2).

FST

The first trial (day 1) lasted 15 min and the second trial, which was performed 24 h later (day 2), lasted 5 min.
### Table 2  Data related to the OGTT

Values are means ± S.E.M. *P < 0.05 and †P < 0.01 compared with SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SD</th>
<th>FSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.85 ± 0.16</td>
<td>5.16 ± 0.12</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>40.9 ± 4.7</td>
<td>70.1 ± 8.9</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.28 ± 0.14</td>
<td>2.33 ± 0.28</td>
</tr>
<tr>
<td>Insulin at 2h (pmol/l)</td>
<td>83.3 ± 7.5</td>
<td>138.5 ± 16.4</td>
</tr>
<tr>
<td>Glucose at 2 h (mmol/l)</td>
<td>6.62 ± 0.26</td>
<td>7.11 ± 0.25</td>
</tr>
</tbody>
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**Figure 2**  Infarct size, expressed as a percentage of the AAR, in hearts stabilized for 40 min and subjected to 40 min of global ischaemia, followed by 120 min of reperfusion.

The depressed FSL rats had significantly larger infarct sizes than the SD control rats. IPC significantly reduced the infarct size in both the FSL (FSL + IPC) and SD (SD + IPC) rats. No significant difference in infarct size was found between the SD + IPC and FSL + IPC groups. Values are means ± S.E.M. *P < 0.05 and ‡P < 0.01 compared with SD rats; †P < 0.01 compared with FSL rats.

In experiment 1, the immobility time recorded during the 5 min on day 2 was significantly higher in the FSL rats compared with the SD rats (254.3 ± 4.9 compared with 213.2 ± 7.7 s; P < 0.01). In experiment 2, the immobility time recorded during the 5 min on day 2 was significantly higher in the FSL rats compared with the SD rats (211.4 ± 8.8 compared with 154.0 ± 7.0 s; P < 0.01), indicating a depressive-like behaviour in the FSL rats.

### Myocardial infarct size and AAR

Infarct size, expressed as a percentage of the AAR (infarct size/AAR), following I/R was significantly larger in the FSL rats compared with the SD rats (62.4 ± 4.2 and 46.9 ± 2.9 % respectively; P < 0.01) (Figure 2). IPC significantly reduced infarct size/AAR in both the FSL (62.4 ± 4.2 % for I/R alone to 30.0 ± 4.4 %; P < 0.01) and the SD rats (46.9 ± 2.9 % for I/R alone 24.0 ± 4.2 %; P < 0.01) compared with I/R alone (Figure 2). No significant difference in infarct size/AAR was observed between the FSL + IPC and SD + IPC rats (30.0 ± 4.4 and 24.0 ± 4.2 % respectively; Figure 2). There were no significant differences in AAR in the Langendorff-perfused hearts between the FSL and SD rats (results not shown). The weight of the Langendorff-perfused hearts did not differ significantly between the FSL and SD rats (1.25 ± 0.39 compared with 1.18 ± 0.44 g) or the FSL + IPC and the SD + IPC rats (1.25 ± 0.34 compared with 1.20 ± 0.35 g).

### LV function and coronary flow

There were no significant differences in LVDP, RPP and dP/dt\text{max/min} between the FSL and SD rats during stabilization (Figures 3 and 4). IPC significantly improved LVDP, RPP, dP/dt\text{max} and dP/dt\text{max/min} during reperfusion in both the FSL and SD rats (P < 0.01). There were no significant differences in LVDP, RPP and dP/dt\text{max/min} during reperfusion between the FSL and SD rats.

No significant differences were found in the mean coronary flow before or after ischaemia between the FSL and SD rats. IPC increased the mean coronary flow during reperfusion in the FSL (14 % increase) and SD rats (25 % increase), although this increase was not significant (results not shown).
DISCUSSION

The results of the present study demonstrate that infarct size after global ischaemia is significantly larger in FSL rats than SD control rats. In contrast, IPC reduced infarct size and improved haemodynamic recovery in both the FSL and SD rats. Fasting plasma insulin levels and the HOMA index, both markers of insulin resistance, were significantly increased in the FSL rats. Furthermore, after an OGTT, the FSL rats had a significant increase in the 2-h insulin value, but no significant change in the 2-h glucose value. Taken together, this indicates an impaired glucometabolic function in the FSL rats.

Although patients with depression, as well as patients with diabetes, have an increased risk of developing ischaemic heart disease and an increased risk of adverse cardiovascular events following an AMI, understanding the common mechanisms between these co-morbid disorders remains unclear. In the present study, we used the FSL rat, a genetic animal model of depression, which had a clear depressive-like behaviour measured by increased immobility during forced swimming compared with the SD rats. We have demonstrated that hearts from depressed FSL rats developed significantly larger infarct sizes than the SD control rats, indicating that these rats are less extensively protected against myocardial ischaemia. Although no statistically significant differences were found in post-ischaemic LV functions between the FSL and SD rats, these findings suggest that infarct size might play a role in the prognosis of depression following an AMI, as infarct size has been indicated to be an important determinant of mortality and heart failure after MI [29,30].

Previous studies have demonstrated that hearts from animals with obesity and features of the metabolic syndrome [31] and animals with Type 2 diabetes [32] had either unchanged or smaller infarct sizes, demonstrating a reduced susceptibility to I/R injury in these animals. In contrast with this, other studies have demonstrated greater myocardial damage in insulin-resistant and obese rats compared with control rats, indicating increased susceptibility to I/R injury in these rats [33–35].

Whether the FSL rats have any metabolic disturbances that could resemble those observed in an insulin-resistant state has not been clarified, but it has been demonstrated previously [23] that FSL rats have higher basal levels of CORT (corticosterone) compared with SD rats. Despite lower levels of CORT after social isolation compared with basal levels, it was suggested that FSL rats might have chronic HPA axis up-regulation. One of the proposed mechanisms responsible for the increased cardiovascular risk in depression includes HPA axis dysfunction [10–12], which can lead to increased levels of cortisol in humans caused by an increased activity of the HPA axis. Hypercortisolaemia can lead to risk factors observed in the metabolic syndrome, such as hypercholesterolaemia, dyslipidaemia and reduced glucose tolerance. Therefore measurements of several components observed in the metabolic syndrome were performed in the present study.

There were no differences in fasting plasma glucose (Table 2) and, in accordance with this, no difference was found in fructosamine levels (Table 1), as this reflects an average blood glucose level over a period of 2–3 weeks, which demonstrates that the FSL rats were not diabetic. Interestingly fasting plasma insulin levels were significantly higher in the FSL rats compared with the SD rats (Table 2) and, when calculating the HOMA index, a marker of whole-body insulin resistance and β-cell function, the FSL rats had a significant 82% increase compared with the SD rats, indicating an insulin-resistant state in these rats. Additionally, after an OGTT, the FSL rats had a 66% significant increase in the 2-h insulin value (Table 2). Dyslipidaemia is an important component of the metabolic syndrome and is characterized by hypertriglycerolaemia and low serum levels of HDL (high-density lipoprotein)-cholesterol. Elevation of LDL (low-density lipoprotein)-cholesterol or total cholesterol
is frequently present, but is not a criterion of the metabolic syndrome. In the present study, we found significantly increased levels of total cholesterol and a trend towards higher triacylglycerols in the FSL rats, which indicate that these rats, to some extent, may have a dyslipidaemic status. Taken together, these findings suggest that FSL rats have some metabolic alterations, reflecting those observed in the metabolic syndrome. Further studies examining the possible role of HPA axis dysregulation and the relationship to the behavioural and metabolic deficits in FSL rats are required and could help to clarify the understanding and exact role of HPA axis dysfunction in depression, diabetes and ischaemic heart disease.

IPC is a cardioprotective mechanism, first described by Murry et al. [36], which can be used as a protective intervention to attenuate myocardial I/R injury in animals and may have beneficial clinical effects [37,38]. Interestingly, animal studies have shown that, in the presence of pathological conditions such as insulin resistance or diabetes, the effect of IPC in the myocardium may be abolished [32,39,40], whereas other studies have shown that the diabetic myocardium is amenable to cardioprotection elicited by IPC [41,42]. The results of the present study demonstrate that IPC, which consisted of two cycles of 5 min of global ischaemia and 5 min of reperfusion before prolonged ischaemia, significantly reduced infarct size (Figure 2) and improved post-ischaemic LV function in both the FSL and SD rats compared with the non-IPC groups (Figures 3 and 4). Although the FSL rats had larger infarct sizes, these findings indicate that these rats have a maintained cardioprotective mechanism against I/R injury. Previous studies have demonstrated that hyperglycaemia may have adverse effects on the ischaemic myocardium and prevent reductions in myocardial infarct size produced by IPC [43,44]. Although the FSL rats used in the present study had hyperinsulinaemia, they were not hyperglycaemic, which could explain in part the maintained effects of IPC in these animals. This may also explain some of the differences in the findings compared with those observed in overt diabetic rats, as the effect of IPC was abolished in these rats [32,39]. On the other hand, the lack of protection afforded by IPC in insulin-resistant animals may not solely be related to hyperglycaemia as it was demonstrated by Katakan et al. [40] that IPC was abolished in euglycaemic obese Zucker rats. Further studies are required to clarify the underlying mechanisms responsible for the cardioprotective effect of IPC and the lack of protection afforded by IPC in insulin-resistant animals.

The mechanisms responsible for the increased cardiovascular risk in depression are still not clarified, but several possible mechanisms have been suggested [1]. For example, in patients with depression changes in autonomic regulation have been associated with decreased HRV and eventually an increased risk of CVD [13]. It has been demonstrated previously that FSL rats have reduced HRV and BRS compared with control strains [24], which could be due to impaired serotonergic control of cardiac reflex function [25]. Similar changes have been observed in other animal models of depression [45,46]. In relation to this, we found an increase in the heart weight/body weight ratio in the FSL rats compared with the SD rats (Table 1), indicating cardiac hypertrophy in these rats, since this ratio may be used as an objective measurement of cardiac hypertrophy in animal experiments. Cardiac hypertrophy has been shown to be independently associated with reduced HRV [47] and, furthermore, suggested as a contributing mechanism to increased infarct size [48].

As blood pressure has been demonstrated to be similar in the FSL and SD rats in previous studies [24,25] and therefore most likely does not influence on the size of the myocardium, the apparent 'hypertrophy' of the myocardium in the FSL rats is therefore presumably body-weight-related due to the decreased body weight observed in the FSL rats. This results in an increase in the heart weight/body weight ratio that may represent a plausible explanation for our findings in the FSL rats.

In conclusion, isolated perfused hearts from FSL rats subjected to global ischaemia developed significantly larger infarct sizes, indicating that these rats are not protected against myocardial ischaemic damage compared with SD control rats. IPC reduced infarct size and improved haemodynamic recovery to almost the same proportion in SD and FSL rats, indicating that FSL rats have a maintained cardioprotective mechanism against I/R injury that is not affected by the hyperinsulinaemic state observed in this animal model.

The findings reported in the present study indicate that the FSL rat may serve as a suitable model to provide insight into the shared pathophysiological mechanisms that link depression, diabetes and ischaemic heart disease, and might contribute to the development of more suitable treatment strategies to reduce the co-morbidities of these diseases.

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