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Evaluation of the Relation between Hyperinsulinaemia and Myocardial Ischaemia-Reperfusion Injury in a rat model of Depression

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Abstract

Major depression is associated with medical comorbidity such as ischaemic heart disease and diabetes but the underlying pathophysiological mechanisms remain unclear. The Flinders Sensitive Line (FSL) 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 ischaemia-reperfusion injury and effects of ischaemic preconditioning (IPC) in hearts from FSL rats using Sprague-Dawley (SD) rats as controls and to characterize differences in glucose metabolism and insulin sensitivity between the FSL and SD rats. Hearts were perfused in a Langendorff model and subjected or not to IPC before 40 minutes of global ischaemia followed by 120 minutes of reperfusion. Myocardial infarct size was found to be significantly larger in the FSL rats (I/R: 62.4±4.2 vs. 46.9±2.9%, $P<0.05$) than in the SD rats. IPC reduced the infarct size ($P<0.01$) and improved haemodynamic function ($P<0.01$) in both the FSL and the SD rats. No significant difference was found in blood glucose levels between the two groups measured after 12 hours of fasting but fasting plasma insulin (70.1±8.9 vs. 40.9±4.7 pmol/l, $P<0.05$) and HOMA (homeostatic model assessment) index ($P<0.01$) were significantly higher in the FSL rats compared to the SD rats. In conclusion, FSL rats had larger infarct sizes and were found to be hyperinsulinaemic compared to SD rats but seemed to have a maintained cardioprotective mechanism against ischaemia-reperfusion 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.

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Introduction

Depression is a mental disorder often associated with medical comorbidities 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 acute myocardial infarction [7]. The presence of diabetes has been found to double the risk of comorbid depression [8] and a recent meta-analysis showed that depression increases the risk of developing type 2 diabetes in adults by 37% [9]. Some of the suggested biological mechanisms that link depression with cardiovascular disease (CVD) include increased hypothalamic-pituitary-adrenal (HPA) function [10-12], decreased 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 behavioral mediators such as smoking, obesity and sedentary lifestyle [16-18].

Although there seems to be an obvious co-occurrence of depression with heart disease and diabetes [1], the shared pathophysiology between these comorbid disorders is not fully clarified. To obtain further insight into these potential mechanisms implementation 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 comorbidities in depression.

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

Recently it has been demonstrated that FSL rats compared with control strains exhibit reduced heart rate variability (HRV) and baroreflex sensitivity (BRS) [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 investigations of susceptibility to myocardial ischaemia and response to ischaemic preconditioning (IPC), an intervention that offers cardioprotective benefits against ischaemia and reperfusion injury. Whether the FSL rat exhibits 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 study was to compare the responsiveness to ischaemia and reperfusion injury between FSL and the 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. This study may help to determine whether the Flinders Sensitive Line rat could serve as a suitable animal model for future studies looking at mechanisms and associations between depression, diabetes and ischaemic heart disease.

Materials and Methods

Animals

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

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Study design

The experimental protocol is illustrated in Fig 1. The present study consisted of two series of experiments, involving aged matched rats, FSL (n = 27, 9 - 10 weeks old, 300-350 gram) and SD (n = 30, 9 - 10 weeks old, 300-350 gram) rats. In *experiment 1* the rats were tested in a forced swim test (FST) and Langendorff-perfused. Prior to perfusion the rats were divided into 4 groups. SD without IPC (n=9); SD with IPC (n=9); FSL without IPC (n=8); FSL with IPC (n=9). The perfusion of the hearts in the Langendorff model was performed 4 weeks after the FST to avoid possible influence of the swimming procedure on the experiments. In *experiment 2* the rats were initially exposed to an oral glucose tolerance test. 4 weeks later the rats were forced swim tested. 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® (midazolam, 0.5 mg/kg bwt; Matrix Pharmaceuticals, Herlev, Denmark) and Hypnorm® (fentanyl citrate 0.158 mg/kg bwt and fluanisone 5 mg/kg bwt; VetaPharma Ltd, Leeds UK) each diluted with an equal volume of sterile water prior to mixing and administered as a single subcutaneous injection. When adequate depth of anaesthesia was confirmed a tracheotomy was made and the rat was connected to a rodent ventilator (Zoovent, Newport Pagnell, UK). The respiration was maintained by mechanical ventilation with 50% room air and 50% O_2. After laparotomy and thoracotomy the heart was dissected free from surrounding structures. A bolus of 1000 IU/kg Heparin (Leo Pharma, Copenhagen, Denmark) was given through the femoral vein. 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 (NaCl 118.5 mmol/l, KCl 4.7 mmol/l, $NaHCO_3$ 25.0 mmol/l, glucosemonohydrate 11.1 mmol/l, $MgSO_4 \cdot 7h_2O$ 1.2 mmol/l, $CaCl_2$ 2.4 mmol/l and KH_2PO_4 1.2 mmol/l). The perfusion buffer was oxygenated with a mixture of 95 % O_2 and 5 % CO_2 and kept at 37 ^oC. The hearts were allowed to stabilize for 40 minutes and then subjected to global ischaemia for 40 minutes and 120 minutes reperfusion. Global ischaemia was induced by discontinuing the retrograde perfusion. Animals subjected to ischaemic preconditioning (IPC) were exposed to 2 cycles of 5 minutes global ischaemia and 5 minutes reperfusion during the last 20 minutes of the stabilisation period (Fig. 1).

Evaluation of left ventricular function and coronary flow

A latex balloon (Hugo Sachs Electronics, March-Hugstetten, Germany) was inserted in the left ventricle (LV) through an incision in the left atrium and kept in place by the mitral valve. A pressure transducer (Baxter Cardiovascular Group, Irvine, CA, US) was connected to the latex balloon allowing recording of left ventricular function. Coronary flow was continuously measured using an in-line flow probe (Transonic, Maastricht, The Netherlands). All data were digitally converted (DT9804; Data Translation, Marlboro, MA, USA) and analyzed using a data acquisition system (Notocord Hem Software, Croissy sur Seine, France).

Assessment of myocardial infarction

To asses infarct size hearts were sliced (~1,5 mm) and immersed in phosphate buffer at 37°C and pH 7,4 with 1% 2,3,5-triphenyl tetrazolium chloride (Sigma, St.Louis, MO, USA) for 3 minutes to delineate areas of infarction. Following fixation in 4% formaldehyde buffer (Lillies Solution, VWR International, Albertslund, Denmark) the slices were weighted, scanned (HP ScanJet 4300C, Hewlett Packard, Palo Alto, CA, USA) and digitally saved as JPEG-files. The viable myocardium stained deep red and necrotic tissue pale. The entire area at risk (AAR) (= area of left ventricle minus cavities) and area of infarction (IS) of the left ventricle were quantified using image analysis

software (UTHSCA ImageTool, Ver. 3.0). The IS:LV ratio was then calculated and corrected for the weight of each individual slice. All measurements were done in a blinded fashion.

Forced swim test

Increased immobility during forced swimming is an accepted index of depressive-like behavior in rodent depressive models and can be determined in the forced swim test (FST). The FST was performed with minor modifications relative to the previous descriptions [26;27]. Briefly, the rats were immersed individually in a cylinder of acrylic plastic (54 cm in height, 24 cm in diameter) containing 38 cm of water (25°C) for 15 minutes. Twenty-four hours later, a 5 minutes retest was conducted during which the total immobility time was recorded (in seconds) on videotape. The animals were judged immobile when they made no further attempts to escape and remained floating, making only movements necessary to keep their heads above water (the animals were unable to touch the bottom of the cylinder). The water was changed for each animal. At the end of the sessions, the rats were removed from the cylinder, carefully dried in towels and returned to their home cages. The experiments were performed between 0900 and 1700 hours. The video tapes were evaluated blinded.

Oral glucose tolerance test (OGTT)

The OGTT was performed in the morning after a 12-hour-fast. Blood glucose and insulin were measured from samples obtained by tail bleeding before administration of 2.5 g glucose/kg body weight loaded by gavage as well as 15, 60 and 120 minutes after glucose load. Blood glucose levels were determined from all samples using a OneTouch Ultra Blood Glucose Monitoring System (LifeScan Inc., Milpitas, CA, USA). Plasma insulin levels were determined using an ultrasensitive Rat Insulin ELISA (enzyme-linked immunosorbent assay) Kit from DRG Diagnostics (Marburg, Germany). The homeostatic model assessment (HOMA) is a method used to quantify whole body insulin resistance and beta-cell function. The HOMA index was calculated as follows: HOMA index = fasting glucose (mmol/L) x 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 3000 rpm for 10 minutes and plasma was collected and frozen at -80°C. All rats were fasted for 12 hours. Hearts were removed and weighed for evaluation of body-weight (gram) / heart-weight (kg) ratio. Plasma levels of cholesterol and triglycerides were determined on a Cobas Integra Analyzer (Roche Diagnostics, Basel, Switzerland). Plasma fructosamine, which can be used as a surrogate for HbA(1c), and reflects an average blood glucose level over a period of two to three weeks, was measured on a Cobas Mira Plus Chemistry System (Roche Diagnostics, Basel, Switzerland). Plasma IL-6 and TNF-α concentrations were measured in duplicate using a Rat ELISA Kit (R&D Systems, Minneapolis, MN, USA).

Statistics and calculations

All values are expressed as mean±SEM. Left ventricular developed pressure (LVDP) was calculated as $P_{LV,\ systolic} - P_{LV,\ diastolic}$ and rate pressure product (RPP) as LVDP x heart rate (HR). Haemodynamic data were compared using two-way ANOVA with repeated measures. IS/AAR 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 by using an unpaired t-test. SPSS 10 (SPSS Inc, USA) was used for statistical calculations and $P<0.05$ was considered statistically significant.

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Results

Clinical and biochemical parameters

Clinical and biochemical parameters, recorded at the end of the experiment 2, are presented in Table 1. There was no significant difference in body weight between the groups before initiation of the study. At the end of the study, body weight was 8% higher (P<0.01) in the SD rats compared to the FSL rats. The reduced body weight might be caused by a reduced food intake in the FSL rats which (in average) was 24 gram pr. day compared to 27 gram in the SD rats. Although no difference was found in heart weight between the FSL and SD rats, 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 to the SD rats. The FSL rats were also characterized by higher levels of total cholesterol (78% increase, P<0.01) and triglycerides (14% increase, P=0.18) compared to the SD rats. Fructosamine, a surrogate for HbA(1c), showed no differences between the FSL and SD rats. Plasma IL-6 and TNF-α concentrations were measured 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 (data not shown). All measurements were done in fasted rats.

Oral glucose tolerance test

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

Forced swim test (FST)

The first trial (day 1) lasted 15 minutes and the second trial, which was performed 24 hours later (day 2) lasted 5 minutes. In experiment 1 immobility time recorded during the 5 minutes on day 2 was significantly higher in the FSL rats (254.3±4.9 vs. 213.2±7.7 seconds, P<0.01) compared to the SD rats. In experiment 2 immobility time recorded during the 5 minutes on day 2 was significantly higher in the FSL rats (211.4±8.8 vs. 154.0±7.0 seconds, P<0.01) compared to the SD rats indicating a depressive-like behavior in the FSL rats (Fig. 2).

Myocardial infarct size and area at risk

The infarct size (IS) expressed as a percentage of the area at risk (IS : AAR) was significantly larger in the FSL rats (I/R: 62.4±4.2% vs. 46.9±2.9%, P < 0.01) compared to the SD rats (Fig. 3). IPC significantly reduced IS : AAR in both the FSL (I/R: 62.4±4.2% vs. 30.0±4.4%, P < 0.01) and the SD rats (I/R: 46.9±2.9% vs. 24.0±4.2%, P < 0.01) (Fig. 3). No significant difference in IS : AAR was observed between the FSL+IPC (I/R: 30.0±4.4% vs. 24.0±4.2%) and the SD+IPC rats (Fig. 3). There were no significant differences in AAR in the Langendorff-perfused hearts between the FSL rats compared to the SD rats (data not shown). The weight of the Langendorff-perfused hearts did not differ significantly between the FSL rats (1.25±0.39 vs. 1.18±0.44 gram) and the SD rats or the FSL+IPC (1.25±0.34 vs. 1.20±0.35 gram) and the SD+IPC rats (data not shown).

Left ventricular function and coronary flow

There were no significant differences in LVDP, RPP and $dP/dt_{max/min}$ between the FSL and the SD rats during stabilization (Figs. 4 - 5). IPC significantly improved LVDP, RPP and $dP/dt_{max/min}$ during reperfusion in both the FSL and the SD group (P < 0.01). There were no significant differences in LVDP, RPP and $dP/dt_{max/min}$ during reperfusion between the FSL and the SD rats.

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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 (data not shown).

Discussion

The present study demonstrates that infarct size after global ischaemia is significantly larger in the Flinders Sensitive Line (FSL) rats than the Sprague-Dawley (SD) control rat. In contrast IPC reduced infarct size and improved haemodynamic recovery in both the FSL and SD rats. Fasting plasma insulin levels and the HOMA (homeostatic model assessment) index, both markers of insulin resistance, were significantly increased in the FSL rats. Furthermore, after an oral glucose tolerance test (OGTT) the FSL rats displayed a significant increase in the 2-hour-insulin value but no significant change in the 2-hour-glucose value. 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 for the development of ischaemic heart disease and an increased risk of adverse cardiovascular events following an acute myocardial infarction, understanding of the common mechanisms between these comorbid disorders remains unclear. In this study we used the FSL rat, a genetic animal model of depression, which displayed a clear depressive-like behavior measured by increased immobility during forced swimming compared to the SD rats (Fig. 2). We demonstrate that hearts from depressed FSL rats developed significantly larger infarct sizes than the SD control rats indicating that these rats are less significant extensively protected against myocardial ischaemia. Although no statistically significant differences were found in post-ischaemic left ventricular functions between the FSL and the SD rats, these findings suggest that the 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 myocardial infarction [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] exhibited either unchanged or smaller infarct sizes demonstrating reduced susceptibility to ischaemia-reperfusion injury in these animals. In contrast to this other studies have demonstrated greater myocardial damage in insulin-resistant and obese rats compared to control rats indicating increased susceptibility to ischaemia-reperfusion injury in these rats [33-35].

Whether the FSL rats display any metabolic disturbances that could resemble those observed in an insulin resistant state has not been clarified, but recently it has been demonstrated [23] that the FSL rats showed higher basal levels of corticosterone (CORT) compared to SD rats. Despite lower levels of CORT after social isolation compared to basal levels it was suggested that the FSL rats might exhibit a chronic HPA axis up-regulation. One of the proposed mechanisms responsible for the increased cardiovascular risk in depression include HPA-axis dysfunction [10-12], which can lead to increased levels of cortisol in humans caused by an increased activity of the HPA axis. Hypercortisolemia can lead to risk factors seen in the metabolic syndrome (MS) such as hypercholesterolemia, dyslipidemia and reduced glucose tolerance. Therefore, measurements of several components observed in the MS were performed. There were no differences in fasting plasma glucose (FPG) (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 two to three weeks, which demonstrates that the FSL rats were not diabetic. Interestingly fasting plasma insulin levels were significantly higher in the FSL compared to the SD rats (table 2) and when calculating the HOMA (homeostatic model assessment) index, a marker of whole body insulin resistance and beta-cell function, the FSL rats showed an 82% significant increase compared to the SD rats, indicating an insulin resistant state in these rats. Additionally, after an OGTT the FSL rats displayed a 66% significant increase in the 2-hour-insulin value (table 2). Dyslipidemia is an important component of the MS and is characterized by hypertriglyceridemia and low serum levels

of high density lipoprotein cholesterol (HDL-C). Elevation of low density lipoprotein cholesterol (LDL-C) or total cholesterol is frequently present, but is not a criterion of the metabolic syndrome. In this study we found significantly increased levels of total cholesterol and a trend towards higher triglycerides in the FSL rats which indicates that the FSL rats to some extent may have a dyslipidemic status. Together these data suggest that the FSL rats display some metabolic alterations, reflecting those observed in the MS. Further studies examining the possible role of HPA axis dysregulation and the relation to the behavioral and metabolic deficits in the FSL rats are required and could help to clarify the understanding and exact role of HPA dysfunctions in depression, diabetes and ischaemic heart disease.

Ischaemic preconditioning (IPC) is a cardioprotective mechanism, first described by Murry et al. [36], which can be used as a protective intervention to attenuate the myocardial ischaemia-reperfusion 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], while other studies find that the diabetic myocardium is amenable to cardioprotection elicited by IPC [41;42]. The current study demonstrates that IPC, which consisted of two cycles of 5 minutes of global ischaemia and 5 minutes reperfusion before prolonged ischaemia, significantly reduced the infarct size (Fig. 3) and improved post ischaemic left ventricular function in both the FSL and SD rats compared to the non-IPC groups (Fig. 4-5). Although the FSL rats exhibited larger infarct sizes these findings indicate that the FSL have a maintained cardioprotective mechanism against ischaemia-reperfusion injury. Previous studies have demonstrated that hyperglycaemia may have adverse effects on the ischaemic myocardium and prevent reductions of myocardial infarct size produced by IPC [43;44]. Although the FSL rats used in the present study exhibited hyperinsulinaemia they were not hyperglycaemic which could in part explain the maintained effects of IPC in these animals. This may also explain some of the differences in the findings compared to 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 Katakam et al. [40] that IPC was abolished in euglycaemic, obese Zucker rats. Further studies are required to clarify the underlying mechanisms responsible for 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 the autonomic regulation have been associated with decreased heart rate variability (HRV) and eventually increased risk of CVD [13]. Recently it has been demonstrated that FSL rats compared with control strains exhibit reduced HRV and baroreflex sensitivity (BRS) [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 increased heart-weight/body-weight ratio in the FSL rats compared to the SD rats (table 1) indicating cardiac hypertrophy in these rats since heart-weight/body-weight 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]. Since blood pressure in previous studies has been demonstrated to be similar in the FSL and SD rats [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 increased heart-weight/body-weight ratio that may represent a plausible explanation to 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 to SD control rats. IPC reduced infarct size and improved

haemodynamic recovery almost to the same proportion in SD and FSL rats indicating that FSL rats have a maintained cardioprotective mechanism against ischaemia-reperfusion injury not affected by the hyperinsulinemic state observed in this animal model.
The data presented in this study indicate that the FSL rat may serve as a suitable model to improve 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 comorbidities of these diseases.

Legends to figures

Figure 1. Schematic diagram illustrating experimental protocol.

Figure 2. Immobility time in seconds spent in the forced swim test. The Flinders Sensitive Line (FSL) rats were significantly more immobile during forced swimming compared to the Sprague-Dawley (SD) control rats indicating a depressive-like behavior. Figure 2a, experiment 1. Figure 2b, experiment 2. Data are shown as means ± SEM. *$P < 0.01$ compared to SD rats.

Figure 3. Infarct size (IS) expressed as percentage infarction of the area at risk (AAR) in hearts stabilized for 40 minutes and subjected to 40 minutes of global ischaemia followed by 120 minutes of reperfusion. The depressed Flinders Sensitive Line (FSL) rats had significantly larger infarct sizes than the Sprague-Dawley (SD) control rats. Ischaemic preconditioning (IPC) significantly reduced the infarct size in both the FSL (FSL+IPC) and the SD (SD+IPC) rats. No significant difference in infarct size was found between the SD+IPC and FSL+IPC groups. Data are shown as means ± SEM. * $P<0.05$ compared to SD rats, ** $P<0.01$ compared to SD rats, † $P<0.01$ compared to FSL rats.

Figure 4. Left ventricular developed pressure (LVDP) (4a) and Rate-pressure product (RPP) (4b) during stabilization (stab.), ischaemia and reperfusion. No significant difference in LVDP and RPP during reperfusion was found between the Sprague-Dawley (SD) control and Flinders Sensitive Line (FSL) rats. Ischaemic preconditioning (IPC) significantly improved LVDP and RPP during reperfusion in both the SD (SD+IPC) and FSL (FSL+IPC) rats. Data are shown as means ± SEM. ** $P<0.01$ compared to SD rats, * $P<0.01$ compared to FSL rats.

Figure 5. Left ventricular dp/dt_{max} (5a) and dp/dt_{min} (5b) during stabilization (stab.), ischaemia and reperfusion. No significant difference in dp/dt_{max} and dp/dt_{min} during reperfusion was found between the Sprague-Dawley (SD) control and Flinders Sensitive Line (FSL) rats. Ischaemic preconditioning (IPC) significantly improved dp/dt_{max} and dp/dt_{min} during reperfusion in both the SD (SD+IPC) and the FSL (FSL+IPC) rats. Data are shown as means ± SEM. ** $P<0.01$ compared to SD rats, * $P<0.01$ compared to FSL rats.

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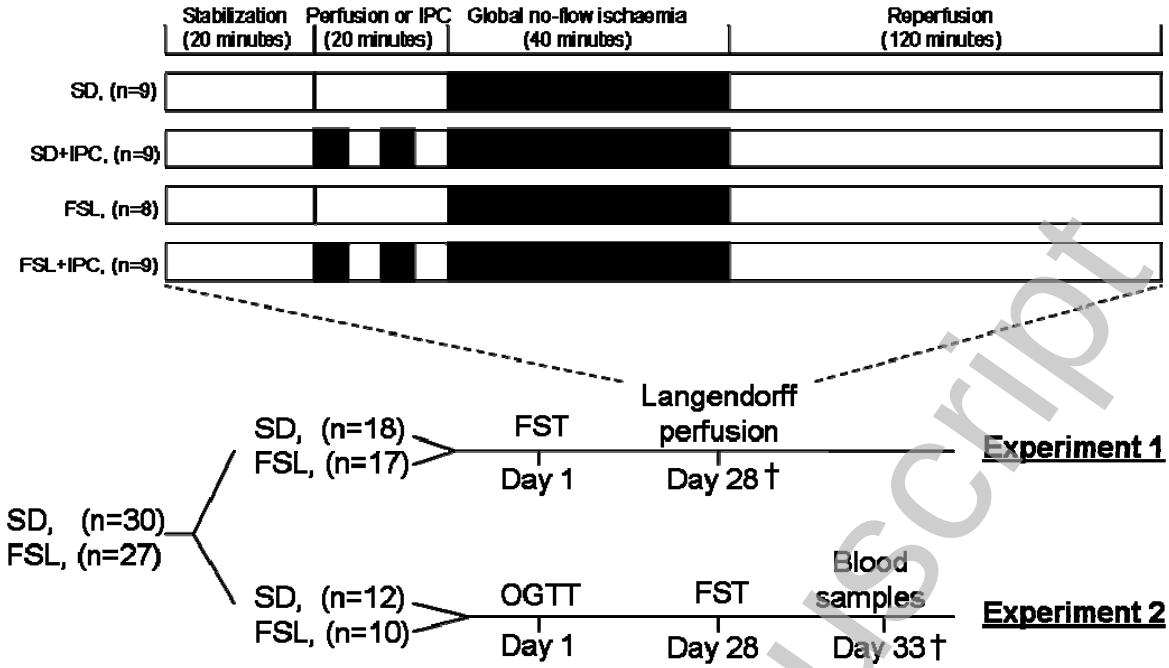

Figure 1

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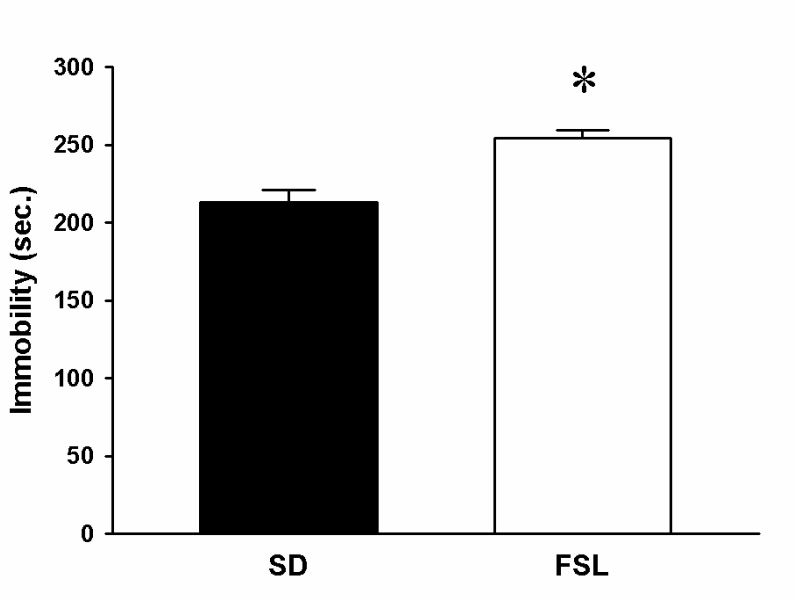

Figure 2a

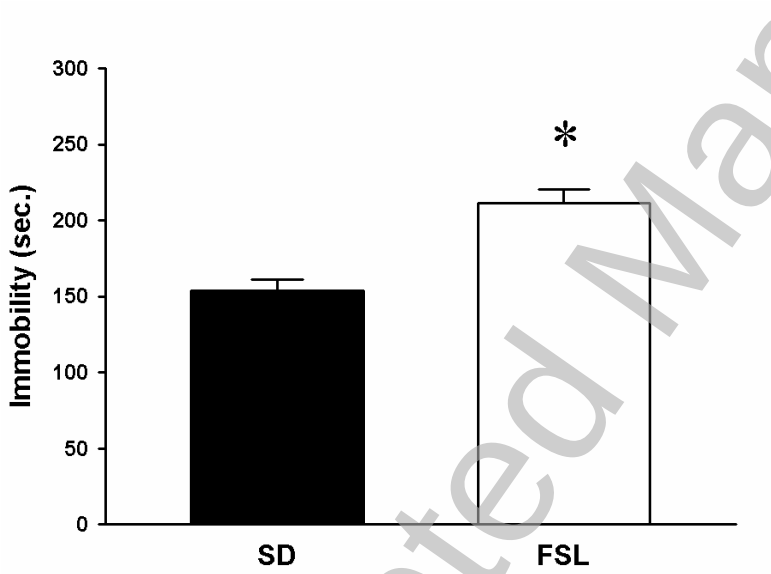

Figure 2b

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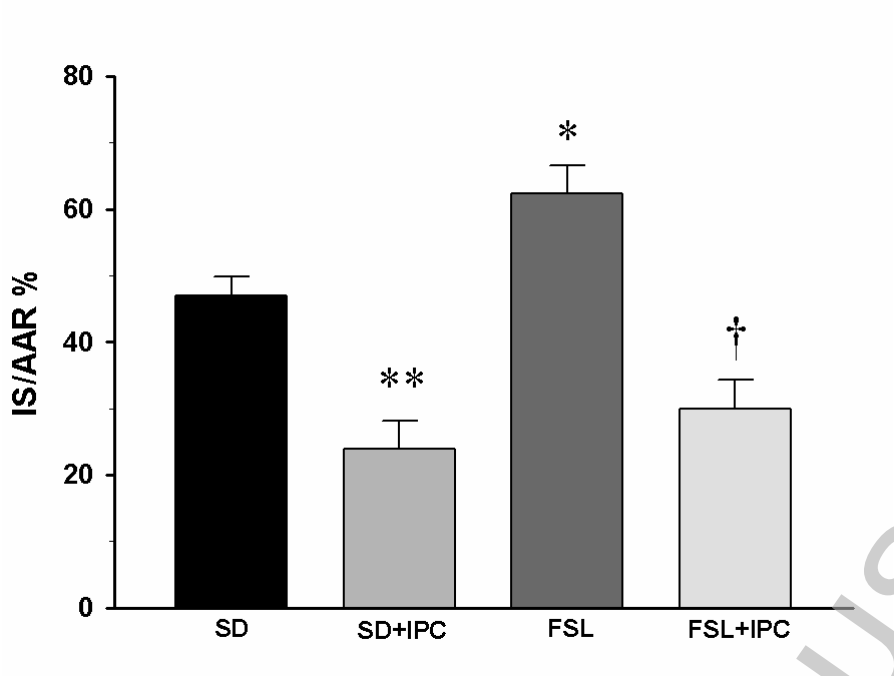

Figure 3

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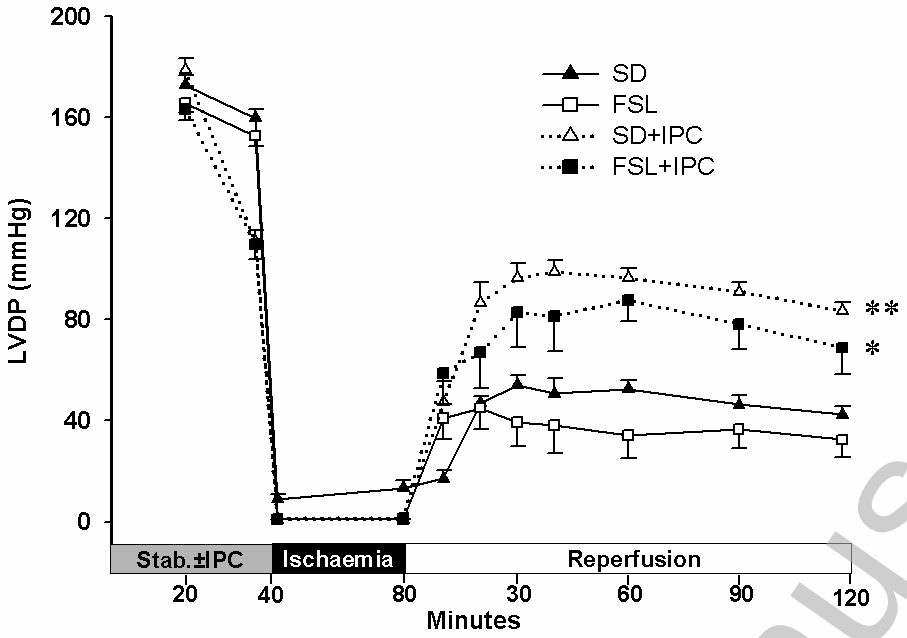

Figure 4a

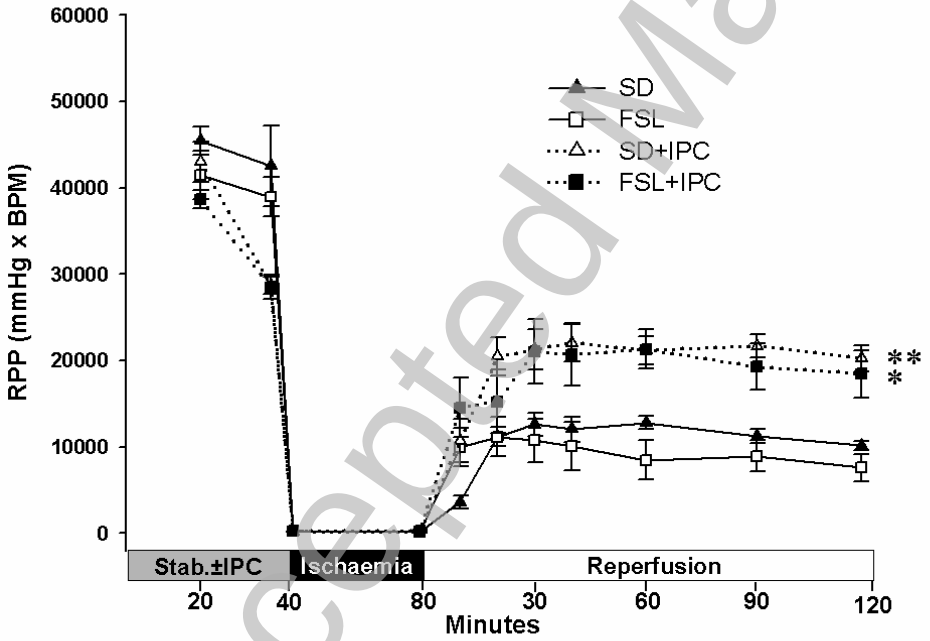

Figure 4b

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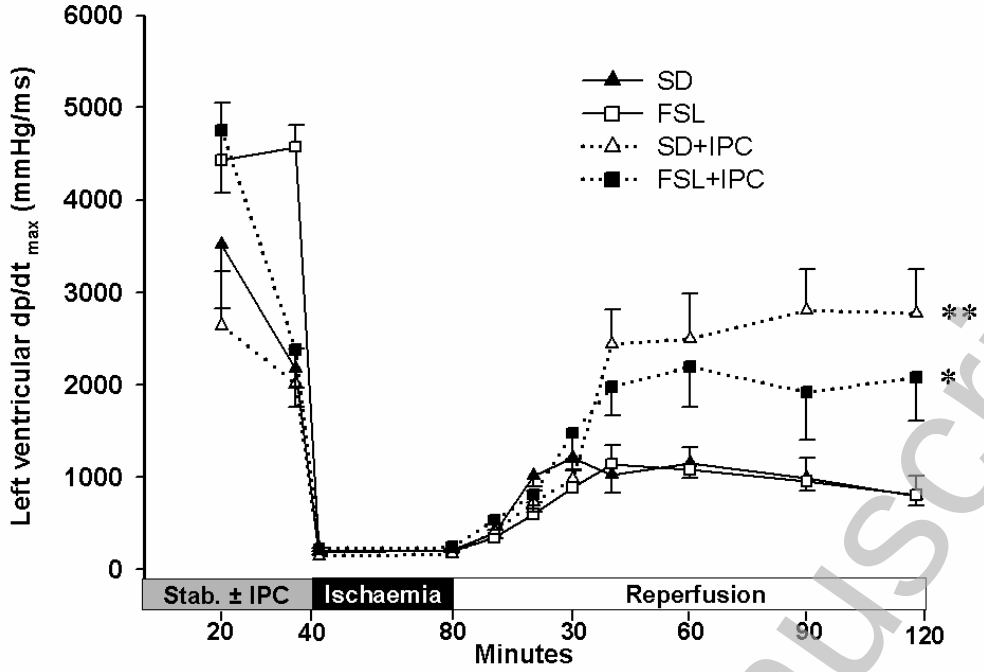

Figure 5a

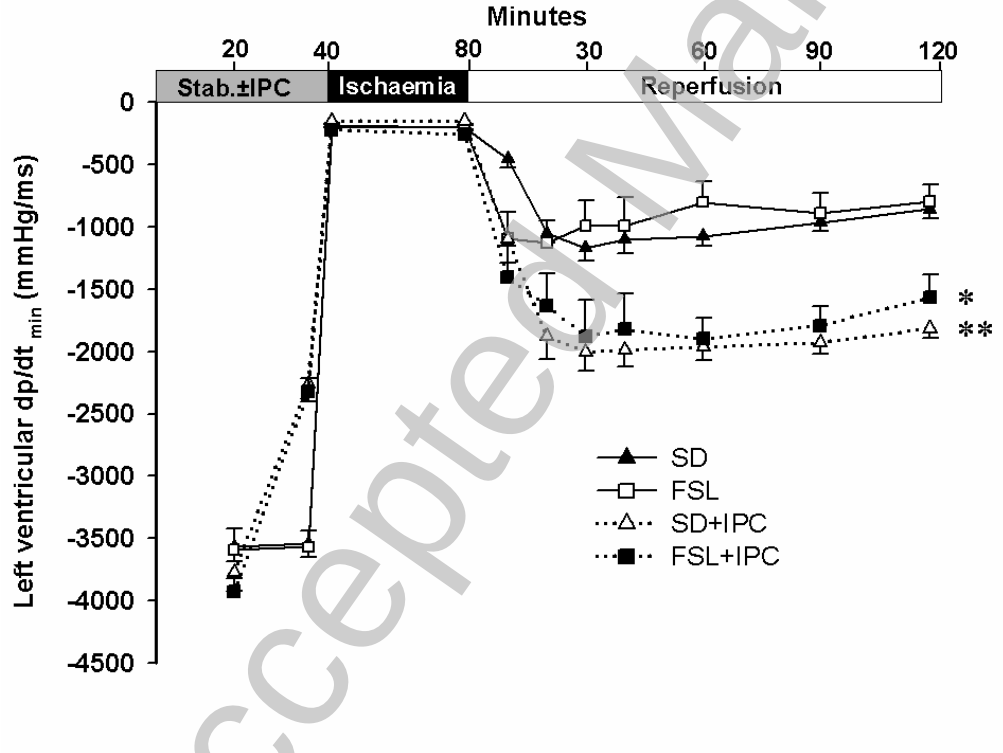

Figure 5b

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Table 1

Clinical and biochemical measurements

Values are means ± S.E.M. *P<0.05 and †P<0.01 compared to SD
SD = Sprague-Dawley rats and FSL = Flinders Sensitive Line rats

Parameter	SD	FSL
Body weight (gram)	415±3	386±9†
Heart weight (gram)	1.31±0.02	1.31±0.03
Heart weight/body weight ratio (g/kg)	3.16±0.05	3.40±0.09*
Fructosamine (µmol/l)	162.6±10.5	157.1±10.4
Total cholesterol (mmol/l)	2.28±0.07	4.07±0.18†
Triglycerides (mmol/l)	1.25±0.10	1.42±0.08

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Table 2

Data related to the oral glucose tolerance test (OGTT)

Values are means ± S.E.M. *P<0.05 and †P<0.01 compared to SD
SD = Sprague-Dawley rats and FSL = Flinders Sensitive Line rats

Parameter	SD	FSL
Fasting plasma glucose (mmol/l)	4.85±0.16	5.16±0.12
Fasting plasma insulin (pmol/l)	40.9±4.7	70.1±8.9*
HOMA-index	1.28±0.14	2.33±0.28†
2-hour-insulin value (pmol/l)	83.3±7.5	138.5±16.4†
2-hour-glucose value (mmol/l)	6.62±0.26	7.11±0.25

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