Insulin resistance, abnormal energy metabolism and increased ischemic damage in the chronically infarcted rat heart

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Abstract

Objective: Many patients with heart failure have whole-body insulin resistance and reduced cardiac fluorodeoxyglucose uptake, but whether these metabolic changes have detrimental effects on the heart is unknown. Here, we tested whether there is a link between insulin resistance and ischemic damage in the chronically infarcted Wistar rat heart, postulating that the heart would have decreased insulin sensitivity, with lower GLUT4 glucose transporter protein levels due to high circulating free fatty acid (FFA) concentrations. A decreased capacity for glucose uptake would lower glycolytic adenosine triphosphate (ATP) production and thereby increase ischemic injury in the infarcted heart.

Methods and results: In vivo left ventricular ejection fractions, measured using echocardiography, were 40% lower in rats 10 weeks after coronary artery ligation than in sham-operated control rats. Insulin-stimulated d[2,3H]glucose uptake was 42% lower in isolated, perfused, infarcted hearts. Myocardial GLUT4 glucose transporter protein levels were 28% lower in the infarcted hearts and correlated negatively with ejection fractions and with fasting plasma FFA concentrations. Compared with controls, chronically infarcted hearts had 46% lower total glucose uptake and three-fold faster ATP hydrolysis rates, measured using phosphorus-31 nuclear magnetic resonance spectroscopy, during 32-min ischemia at 0.4 ml/min/gww. During reperfusion, recovery of left ventricular developed pressure in infarcted hearts was 42% lower than in control hearts.

Conclusions: Glucose uptake, in response to insulin or ischemia, was lower in the chronically infarcted rat heart and associated with increased circulating FFA concentrations and decreased GLUT4 levels. Thus, infarcted hearts had greater ATP depletion, and consequently incurred greater damage, during ischemia.

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Keywords: Infarction; Heart failure; Ischemia; Insulin resistance; Energy metabolism

1. Introduction

Heart failure is characterized by cardiac dysfunction, abnormalities of the peripheral circulation and neurohumoral activation. Despite significant benefit resulting from inhibition of the sympathetic and the renin angiotensin systems, the prognosis and quality of life for chronic heart failure patients remain poor, the 5-year mortality being around 50% [1]. Depletion of high energy phosphate metabolites may contribute to failure, and a decreased PCr/ATP ratio has been found in cardiac muscle of heart failure patients [2,3] and animal models of heart failure [4]. Defects in energy metabolism may be caused by abnormalities in substrate supply or utilisation and/or oxidative phosphorylation, and may play a pivotal role in the contractile dysfunction associated with heart failure [5].

Myocardial glycolytic ATP production is important for preserving cardiac viability during ischemia [6]. A major
determinant of glycolytic flux is glucose transport, glucose entering heart cells via the facilitative glucose transporters, GLUT1 and GLUT4 [7]. A large proportion of GLUT1, the least abundant transporter, resides in the plasma membrane and mediates basal glucose uptake. GLUT1 overexpression in heart normalizes the PCR/ATP ratio and is protective against the development of failure induced by pressure overload [8]. GLUT4 resides in intracellular vesicles under basal conditions and translocates to the plasma membrane in response to insulin, ischemia and exercise; hence, GLUT4 translocation represents the major mechanism by which glucose uptake to the cardiomyocyte can be increased [7]. Chronic heart failure patients have whole-body insulin resistance [9–11], decreased myocardial fluorodeoxyglucose uptake during insulin clamp [12,13] and decreased glucose transporter activity [14]. In mice, GLUT4 ablation induces cardiac hypertrophy [7]; hence, impaired myocardial glucose uptake may contribute to the progression to failure, although the mechanisms for this are not completely understood.

Here, we have determined whether the chronically infarcted rat heart has lower glucose uptake in response to insulin or low-flow ischemia, and whether low glucose uptake during ischemia decreases glycolytic ATP production and exacerbates injury. D[2-3H]glucose uptake was measured before and during insulin stimulation and during low-flow ischemia, and levels of the insulin-responsive glucose transporter, GLUT4, were measured in control hearts and the residual viable tissue in chronically infarcted rat hearts. In order to examine the energetic changes that culminate in ischemic injury, phosphorus-31 nuclear magnetic resonance (31P NMR) spectroscopy was used to follow changes in ATP, phosphocreatine (PCr), inorganic phosphate (Pi) and pH during ischemia and reperfusion. A preliminary report of this work has been presented in abstract form [15].

2. Materials and methods

2.1. Animals

Male Wistar rats (~200g) (n=43) were obtained from a commercial breeder (Harlan, UK) and kept under controlled conditions for temperature, humidity and light, with rat chow and water available ad libitum. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The University of Oxford Animal Ethics Review Committees and the Home Office (London, UK) approved all procedures.

2.2. Chronic myocardial infarction and echocardiography

Animals were anaesthetised with 1.5–2% isoflurane, intubated and ventilated at 90 breaths/min, stroke volume 2.5ml. A thoracotomy was performed in the fourth intercostal space and a 5/0 Proline suture was tied around the left anterior descending coronary artery a few millimetres from its origin (n=25) as described previously [16]. In sham-operated rats (n=18), used throughout this study as controls, the ligation suture was not placed in the heart. Intramuscular buprenorphine 0.2mg/kg was given for pain relief. In the first 4h after surgery, approximately 40% of infarcted animals died due to ventricular fibrillation.

Echocardiography was performed at 10weeks post-surgery to determine the severity of left ventricular dysfunction. Animals were lightly sedated with 3.3–6.6mg/kg fluanisone and 0.11–0.21mg/kg fentanyl citrate (Hypnorm, Jansen Pharmaceuticals). Short axis two-dimensional echocardiographic images were taken through the left parasternal window using an Agilent Sonos 5500 with 5–12MHz transducer. Ejection fractions (EF) were calculated using:

\[ EF (\%) = \frac{\text{end-diastolic CSA} - \text{end-systolic CSA}}{\text{end-diastolic CSA}} \times 100 \]

CSA being the cross-sectional area at the papillary muscle level. Echocardiography was also used to measure end-diastolic diameter, posterior wall thickness, long axis length and heart rate in all animals.

2.3. Heart perfusion

Between 3 and 7 days after echocardiographic examination, rats were fasted overnight prior to sacrifice and were anaesthetised with a 1-ml i.p. injection of 60mg/ml sodium pentobarbitone (Sagatal, Rhône Mérieux, Dublin, Ireland). Following cessation of peripheral nervous function, blood samples, taken from the femoral artery, were immediately centrifuged at 4°C and the supernatant frozen at −80°C for determination of glucose, triglycerides and insulin, or frozen with a final concentration of 30μg/ml lipoprotein lipase inhibitor (tetrahydrolipstatin, Xenical, Roche) for determination of free fatty acids (FFAs). Hearts were excised, quickly weighed and arrested in ice-cold heparin-containing Krebs–Henseleit buffer containing 119mmol/l NaCl, 5.4mmol/l KCl, 1.2mmol/l MgSO4, 1.75mmol/l CaCl2, 25mmol/l NaHCO3, 0.5mmol/l EDTA, 1.2mmol/l KH2PO4 and 11mmol/l glucose. Hearts were cannulated via the ascending aorta for retrograde Langendorff perfusion at 37°C using Krebs–Henseleit buffer. The flow was continually gassed with 95% O2:5% CO2 to give a pH of 7.40 and filtered using an in-line prefilter, followed by 0.8μm and 0.45μm filters. Thebesian vein flow was removed from the left ventricle via a drain inserted through the apex of the heart. A water-filled polyethylene balloon, attached via polyethylene tubing to a bridge amplifier (ADInstruments Ltd., Chalgrove, Oxfordshire), was inserted into the left ventricular cavity via the mitral valve and inflated sufficiently to produce an end-diastolic pressure of...
4 mmHg. Heart rate and left ventricular (LV) pressures were recorded continuously using a PowerLab data acquisition system and Chart v4.0 software (ADInstruments Ltd., Chalgrove, Oxfordshire). Left ventricular developed pressure (DP) was calculated as the systolic pressure (SP) minus the end diastolic pressure (EDP).

2.4. Glucose uptake in response to insulin

Glucose uptake was measured as the rate of cleavage of H\(^+\) from glucose as previously described [17]. Hearts were perfused with 250-ml recirculating Krebs–Henseleit buffer containing 11 mmol/l glucose and 3\(\text{[2-3H]}\)glucose, with an activity of 14.5 μCi/mmol (Amersham, Amersham, Bucks). To determine insulin response, control (n=5) and chronically infarcted (n=9) rat hearts were perfused as described above and insulin was added to the buffer reservoir after 30min, to give a final concentration of 3U/l to ensure maximal stimulation of glucose transport. Recirculating perfusion was continued for another 30min and buffer samples from the reservoir were taken every 4min throughout the protocol. The glucose used (μmol) was plotted against time and the rates of glucose uptake (μmol/min/gww, after correcting the heart weight to exclude the weight of the infarct scar, which was approximately 7% of the total heart weight), without and with insulin, were calculated. At the end of the perfusion protocol, the hearts were perfused with non-radioactive Krebs–Henseleit buffer containing 11 mmol/l glucose for 15min, before infarcts were rapidly dissected away and the viable myocardium frozen at the temperature of liquid nitrogen for immunoblot analysis of GLUT4 protein (see below).

2.5. Glucose transporter protein expression

Tissue extraction and immunoblots for the determination of GLUT4 were performed on frozen viable myocardium from control (n=4) and chronically infarcted (n=7) rats, as previously described [18].

2.6. Glucose uptake during ischemia

Control (n=4) and chronically infarcted (n=8) rat hearts were perfused at a constant pressure of 100 mmHg for 30 min prior to ischemia with Krebs–Henseleit buffer containing 11 mmol/l glucose, 4.5 mmol/l pyruvate and 0.5 mmol/l lactate. For 32-min ischemia, hearts were perfused at 0.4 ml/min/gww with Krebs–Henseleit buffer containing 11 mmol/l glucose and 3\(\text{[2-3H]}\)glucose, with an activity of 14.5 μCi/mmol, but no pyruvate or lactate. Hearts were subsequently reperfused for 30 min at 100 mmHg constant pressure with the original Krebs–Henseleit buffer containing 11 mmol/l glucose, 4.5 mmol/l pyruvate and 0.5 mmol/l lactate. Buffer samples were collected immediately prior to ischemia to establish baseline counts and total effluent from each heart was collected over consecutive 4-min intervals during the 32 min of low-flow ischemia for glucose uptake measurements (see above).

2.7. \(^{31}\text{P} \)NMR spectroscopy

Control (n=6) and chronically infarcted (n=5) rat hearts were perfused at a constant pressure of 100 mmHg with Krebs–Henseleit buffer containing 11 mmol/l glucose, 4.5 mmol/l pyruvate and 0.5 mmol/l lactate, but without phosphate, so as to decrease the signal from phosphate in the buffer surrounding the heart [6]. The perfused hearts were placed in a 20 mm diameter glass NMR tube, which was inserted into a 400 MHz, 9.4 T vertical wide bore superconducting magnet (Oxford Instruments Inc., Oxford, UK). The temperature of the heart was maintained at 37°C using the variable temperature unit attached to the spectrometer. Peak resolution was enhanced by shimming the proton signal to a linewidth between 20 and 35 Hz. Consecutive 4-min \(^{31}\text{P} \)NMR spectra were acquired using a Varian Inova Unity spectrometer (Varian, Palo Alto, CA) at a phosphorus resonance frequency of 161.92 MHz, as previously described [6]. Saturated spectra were acquired using a 60° pulse angle with an interpulse delay of 2.14s. A total of 112 summed transients gave an acquisition time of 4 min. After 30 min normal perfusion, 32 min of low-flow ischemia at 0.4 ml/min/gww with 11 mmol/l glucose as the sole substrate was followed by 20 min of reperfusion at constant pressure and using the original buffer. The signal-to-noise ratio was increased by multiplying the \(^{31}\text{P} \)NMR free induction decays by an exponential function, sufficient to generate a line broadening of 20 Hz, before Fourier transformation. The areas of the spectral peaks were fitted to sum of Lorentzian and Gaussian line shapes using a computer program (NMR1, Tripos, St. Louis, MO). After correcting for spectral saturation, absolute \(^{31}\text{P} \)metabolite concentrations were calculated by assigning ATP concentrations of 10.6 mmol/l to the initial β-ATP peak area for sham hearts [19] and expressing all other ATP and PCR peak areas relative to this area. Intracellular pH throughout ischemia was estimated from the chemical shift of the inorganic phosphate (Pi) peak (δ\(\text{Pi} \)) relative to that of the PCR peak using the following equation derived from titration solutions: [18]

\[
pH = 6.72 + \log(\delta_{\text{Pi}} - 3.17)/(5.72 - \delta_{\text{Pi}})
\]

Glycogen concentrations were determined [20] in other groups of control (n=3) and chronically infarcted (n=3) rat hearts that were perfused for 30 min at a constant pressure of 100 mmHg with Krebs–Henseleit buffer containing 11 mmol/l glucose, 4.5 mmol/l pyruvate and 0.5 mmol/l lactate, before being freeze clamped at the temperature of liquid nitrogen and stored at -80°C prior to extraction and assay.

2.8. Biochemical analyses

Plasma glucose, insulin, FFAs and triglycerides were measured using kits (Sigma, St. Louis, MO; Mercodia AB,
2.9. Statistical analysis

Data are presented as means±S.E.M. Statistical significance was assessed using a two-way ANOVA followed by Bonferroni’s post hoc test (Instat v3.05, GraphPad Software, San Diego, CA). Differences were considered significant at \( P<0.05 \).

3. Results

3.1. In vivo echocardiographic measurements, heart and body weights and fasting plasma metabolites

Ejection fractions were 82±2% for all control rat hearts \((n=18)\) and 49±3% for chronically infarcted hearts \((n=25)\) 10 weeks post-infarction \((P<0.0001)\) (Table 1). Compared with control hearts, end-diastolic and end-systolic areas were 60% \((P<0.0001)\) and 360% \((P<0.0001)\) higher, respectively, in chronically infarcted hearts. End-diastolic diameters were 20% higher in chronically infarcted hearts than in controls \((P<0.0001)\). Posterior wall thicknesses, long axis lengths and heart rates were the same in all hearts. Chronically infarcted rat hearts weighed 30% more \((P<0.0001)\) than control rat hearts. Body weights of all rats were the same; consequently, the heart/body weight ratios were 30% higher \((P<0.001)\) for chronically infarcted rats. Rats with chronically infarcted hearts had 58% higher plasma FFAs \((P<0.01)\) than control rats, whereas fasting plasma glucose, insulin and triglyceride concentrations were the same for the all rats.

3.2. Insulin-stimulated glucose uptake and glucose transporter protein levels

The basal glucose uptake rate was the same for all hearts at 0.37±0.03 \(\text{\mu mol glucose/min/gww}\) (Fig. 1). Upon insulin stimulation, the total myocardial glucose uptake increased to 1.29±0.04 \(\text{\mu mol glucose/min/gww}\) in controls and 0.90±0.03 \(\text{\mu mol glucose/min/gww}\) in the infarcted hearts. Thus, the insulin-stimulated (non-basal) glucose uptake rate was 0.92±0.08 \(\text{\mu mol glucose/min/gww}\) in control hearts, but was 42% lower at 0.54±0.04 \(\text{\mu mol glucose/min/gww}\) in chronically infarcted rat hearts \((P<0.01)\). Total GLUT4 protein content was reduced by 28% \((P<0.001)\) in all the infarcted rat hearts, compared with control rat hearts (Fig. 1). For all rats, levels of cardiac GLUT4 correlated positively with in vivo left ventricular ejection fractions, measured using echocardiography \((r=0.82, P<0.01)\) (Fig. 2) and correlated negatively with plasma FFA concentrations \((r = −0.76, P<0.01)\) (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Echocardiography, body and heart weights and plasma metabolite concentrations in control and chronically infarcted rats</th>
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<tbody>
<tr>
<td><strong>Control ((n=18))</strong></td>
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<tr>
<td><strong>Echocardiography</strong></td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
</tr>
<tr>
<td>End-diastolic area (cm(^2))</td>
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<tr>
<td>End-systolic area (cm(^2))</td>
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<tr>
<td>End-diastolic diameter (cm)</td>
</tr>
<tr>
<td>Posterior wall thickness (cm)</td>
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<tr>
<td>Long axis length (cm)</td>
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<tr>
<td>Heart rate (bpm)</td>
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<tr>
<td><strong>Weights</strong></td>
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<tr>
<td>Heart weight (g)</td>
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<tr>
<td>Body weight (g)</td>
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<tr>
<td>HW: BW × 1000</td>
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<tr>
<td>Infarct scar weight (g)</td>
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<tr>
<td><strong>Plasma metabolites</strong></td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
</tr>
<tr>
<td>Plasma insulin ((\mu g/l))</td>
</tr>
<tr>
<td>Plasma FFA (mM)</td>
</tr>
<tr>
<td>Plasma triglycerides (mM)</td>
</tr>
</tbody>
</table>

*\(P<0.01\), **\(P<0.001\) compared with corresponding control values.
3.3. Myocardial glucose uptake during ischemia

The total glucose uptake during ischemia was 46% lower in the infarcted rat hearts than in control rat hearts \((P<0.01)\) (Fig. 3).

3.4. Contractile function before, during and after ischemia

There was no difference in heart rate, end-diastolic pressure or coronary flow rates between control and chronically infarcted hearts throughout the ischemia protocol (Table 2). Prior to ischemia, left ventricular developed pressures (LVDP) and rate pressure products (RPP) were the same in all hearts. Control hearts, after ischemia, recovered to pre-ischemic levels. However, the LVDP and RPP in chronically infarcted hearts post-ischemia was only 46% of those in control hearts post-ischemia \((P<0.001)\) (Table 2).

3.5. \(^{31}P\) NMR spectroscopy

\(^{31}P\) NMR spectra of control \((n=6)\) and chronically infarcted \((n=5)\) hearts were acquired at 4-min intervals prior to, during and after 32 min of low-flow ischemia. During ischemia, ATP was hydrolysed by 12% in control rat hearts and by 40% in chronically infarcted rat hearts (Fig. 4). Thus, the rate of ATP loss throughout the ischemic period was almost three-fold greater, at 0.11 ± 0.04 mmol/l/min/gww, in chronically infarcted hearts, than in controls, at 0.04 ± 0.01 mmol/l/min/gww \((P<0.01)\) (Fig. 4). ATP did not recover throughout 30-min reperfusion in either heart group, probably due to adenosine washout during low-flow ischemia [6].

Pre-ischemic intracellular PCr concentrations were 35% lower \((P<0.001)\) in chronically infarcted hearts than control hearts (Fig. 4) and, during ischemia, fell from 20.3 ± 1.0 to 7.0 ± 1.0 mmol/l in control hearts and from 13.1 ± 1.0 to 2.5 ± 1.3 mmol/l in chronically infarcted hearts (Fig. 4). During reperfusion, PCr increased to 96% of pre-ischemic concentrations in control hearts and to 66% of pre-ischemic levels in chronically infarcted hearts. Therefore, at the end of reperfusion, PCr was 55% lower in chronically infarcted hearts than in controls \((P<0.05)\).

Pre-ischemic pH\(_i\) was the same for all hearts (Fig. 4), but was 0.4 pH units lower in chronically infarcted hearts than in control hearts. The pH\(_i\) of control hearts increased by 0.4 pH units during reperfusion, whereas in chronically infarcted hearts the pH\(_i\) continued to decrease by 0.2 pH units during reperfusion.

3.6. GLUT4 expression

Levels of cardiac GLUT4 correlated positively with in vivo left ventricular ejection fractions \((r=0.82, P<0.01)\) (upper panel) and correlated negatively with levels of plasma free fatty acids \((r=-0.76, P<0.01)\) (\(n=11\)) (middle panel). Left ventricular ejection fractions correlated negatively with plasma levels of free fatty acids \((r=-0.76, P<0.01)\) (lower panel).

### Table 2

Functional parameters measured in isolated, Langendorff-perfused control and chronically infarcted rat hearts, before and after 32 min low-flow ischemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control ((n=10))</th>
<th>Chronically infarcted ((n=13))</th>
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<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>268 ± 15</td>
<td>283 ± 5</td>
</tr>
<tr>
<td>Post-ischemia</td>
<td>265 ± 17</td>
<td>271 ± 10</td>
</tr>
<tr>
<td>End-diastolic pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>4 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Post-ischemia</td>
<td>3 ± 3</td>
<td>19 ± 10</td>
</tr>
<tr>
<td>LV developed pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>121 ± 10</td>
<td>91 ± 11</td>
</tr>
<tr>
<td>Post-ischemia</td>
<td>123 ± 10</td>
<td>57 ± 12**</td>
</tr>
<tr>
<td>Rate pressure product (mm Hg/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>32,074 ± 2298</td>
<td>25,709 ± 3095</td>
</tr>
<tr>
<td>Post-ischemia</td>
<td>31,575 ± 2202</td>
<td>14,285 ± 3079**</td>
</tr>
<tr>
<td>Coronary flow (ml/min/gww)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>16 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Post-ischemia</td>
<td>16 ± 1</td>
<td>15 ± 1</td>
</tr>
</tbody>
</table>

* \(P<0.05\) compared with corresponding pre-ischemic value.
** \(P<0.01\) compared with controls.
†† \(P<0.001\) compared with corresponding control value.
control hearts at the end of ischemia. During reperfusion, no Pi could be detected in any heart, as PCr had been formed in the creatine kinase reaction using intracellular Pi and phosphate had been omitted from the buffer for the MR experiments. Consequently, pH could not be measured during reperfusion.

3.6. Cardiac glycogen levels prior to ischemia

Cardiac glycogen content prior to ischemia was the same in control and chronically infarcted hearts, at 15 µmol glucosyl units/gww.

4. Discussion

We have found that high energy phosphate metabolism, as indicated by the PCr concentration, was abnormal in the chronically infarcted rat heart, as others have reported both in this model [19,21,22] and in the failing human heart [3,23]. The causes and consequences of dysfunctional energy metabolism in heart failure are poorly understood, although there is growing evidence to suggest that alterations in energy substrate metabolism contribute to cardiac hypertrophy, left ventricular remodelling and systolic dysfunction [24]. Chronic heart failure patients have decreased myocardial fluorodeoxyglucose uptake during insulin clamp [12,13], and here we found that chronically infarcted rat hearts have decreased glucose uptake in response to insulin or ischemia, which resulted in greater loss of ATP and increased injury during ischemia.

Impaired insulin sensitivity is an independent risk factor for mortality in patients with stable chronic heart failure [25]. In our study, although basal rates of glucose uptake were the same for all hearts, insulin-stimulated glucose uptake rates were reduced by 42% in chronically infarcted rat hearts and were paralleled by the 28% lower GLUT4 glucose transporter protein levels in the same hearts. This finding is similar to that observed in the obese rat heart [18] and the hypertrophied spontaneously hypertensive rat heart, which had decreased GLUT4 mRNA content and lower insulin-sensitive 2-deoxyglucose uptake [26]. However, dogs with dilated cardiomyopathy (DCM) had decreased basal and insulin-stimulated glucose uptake due to decreased GLUT4 translocation, but with normal GLUT4 protein levels [27]. In our study, GLUT4 levels may have been lower in the chronically infarcted rat heart due to increased circulating FFAs, as has been measured in dogs with DCM [27] and patients with congestive heart failure [28], probably due to increased levels of norepinephrine that activate β-receptors in adipose tissue causing lipolysis [27,28]. Such elevated levels of circulating FFAs, however, are not always observed in animal models of heart failure [29], or indeed in patients with failing hearts [30]. The negative correlation between circulating FFA concentrations and left ventricular ejection fractions in this study suggests that decreased contractile function post-infarction increased the sympathetic drive, which in turn increased the plasma FFA levels. In human subjects, elevated levels of plasma FFAs are associated with insulin resistance [31] and are believed to impair glucose transporter activity in skeletal muscle [32]. High concentrations of FFAs lower GLUT4 mRNA in cardiomyocytes [33] and lipid infusion has been associated with reduced GLUT4 mRNA in rat heart [34]. In humans, myocardial GLUT4 levels correlated negatively with fasting levels of plasma FFAs [30]. The protein levels of GLUT4 are possibly decreased through FFA stimulation of the nuclear transcription factor, peroxisome proliferator-activated receptor α (PPARα), a major switch that regulates glucose and fatty acid metabolism [35,36]. Furthermore, interstitial fibrosis increases in the viable myocardium of the infarcted rat heart, which may contribute to a reduction in myocardial GLUT4 levels. However, the area of fibrosis in infarcted hearts has been reported to be 7% compared with 4% in
sham-operated hearts [37]; hence, this would likely be a minor contribution to the decreased glucose uptake rates. Myocardial glucose uptake via GLUT4 glucose transporters is stimulated during low-flow ischemia, when the heart relies heavily on glycolytic ATP production [6,38]. Ischemia stimulates GLUT4 translocation via a different signalling pathway to that of insulin stimulation [39]. During ischemia, oxygen deprivation causes an increase in the AMP/ATP ratio, leading to an activation of AMP-activated protein kinase (AMPK). As such, AMPK acts as a molecular metabolic sensor for the cell, increasing anaerobic energy production by activating 6-phosphofructo-2-kinase (PFK-2) and promoting glucose uptake [39], increasing the presence of GLUT4 at the sarcolemma [40]. The precise target of AMPK in the GLUT4 machinery, however, has not yet been identified. Here, we found that glucose uptake during low-flow ischemia was impaired in the chronically infarcted rat heart, resulting in nearly three-fold faster loss of ATP during ischemia and 46% lower recovery of developed pressure upon reperfusion. As is typically observed in low-flow ischemia, ATP levels did not recover in any heart during reperfusion due to adenosine washout, whereas cardiac PCR levels recovered in all hearts during reperfusion, but to a greater extent in control hearts compared with infarcted hearts [17]. The greater decrease in intracellular pH during ischemia in chronically infarcted rat hearts reflected increased proton production resulting from greater ATP hydrolysis [41]. Glycogen levels were the same in all hearts prior to ischemia, indicating that differences in glycolysis from glycogenolysis did not underlie the lower functional recovery of infarcted hearts [6]. Glycogen levels were not measured at the end of the ischemic protocol, although an ischemic insult of this duration would have resulted in a complete loss of myocardial glycogen [6]. Rates of glucose uptake during a period of low-flow ischemia such as this are typically proportional to rates of lactate efflux [18], demonstrating a dependence of the heart on glucose taken up from the perfusion buffer rather than intramyocardial glycogen stores alone. The hypertrophic heart of the spontaneously hypertensive rat (SHR) has poor recovery from ischemia [42] and myocardial PCR deficiency in rats was associated with 100% mortality post-myocardial infarction [4]. It has been suggested, therefore, that the energetically compromised failing myocardium has an increased susceptibility to acute ischemic or hypoxic insult [23]. However, we have shown increased ischemic damage in the chronically infarcted rat heart probably resulting from increased fasting plasma FFA concentrations and a defect in glucose uptake. The extent of this defect in the post-prandial infarcted rat heart, when plasma FFA levels may not be significantly elevated [43], has not yet been established.

In this study, 11mmol/l glucose was the only substrate provided to the isolated hearts, although in vivo hearts use a variety of substrates for energy production, including glucose, fatty acids, lactate and ketone bodies. Thus, omitting the other substrates probably exaggerated the dependence of the hearts on glucose. Certainly, the presence of fatty acids in the buffer would have altered the response of the hearts to ischemia [17]. The availability of other substrates, however, would have decreased the changes in glucose uptake rates in response to insulin or ischemia, and so these were omitted.

During cardiac development, the primary cardiac energy source switches from glycolysis during the relatively hypoxic fetal period to long-chain fatty acid oxidation after birth [44]. During the progression to failure in animal models of pressure overload or hypertension, cardiac fatty acid β-oxidation decreases [45], with downregulation of genes for fatty acid oxidation [46] and with increased oxygen-efficient, glycolytic production of ATP: a reinduction of the foetal energy metabolic program [47]. Indeed, myocardial fatty acid metabolism is decreased in patients with idiopathic dilated cardiomyopathy [48] and is an independent predictor of left ventricular mass in human hypertensive heart disease [48]. However, increased glucose utilisation may not accompany the decreased fatty acid oxidation in the failing heart [29,49], and different models, or the severity of cardiac hypertrophy and failure, may result in differences in cardiac fatty acid and glucose metabolism [27,50,51]. The foetal shift also appears to be inconsistent with the whole-body insulin resistance [9–11], the reduced myocardial fluorodeoxyglucose (FDG) uptake and the 75% reduction in left ventricular GLUT4 mRNA levels in failing human heart [52]. Similarly, we found decreased GLUT4 protein levels in the chronically infarcted rat heart, with lower insulin-stimulated glucose uptake, that correlated with the fasting plasma FFA levels. It may be that increased glycolysis, with upregulation of glycolytic enzymes [53] and downregulation of genes for fatty acid oxidation [46] occurs when circulating FFAs do not increase [45,48].

The 28% decrease in GLUT4 protein may not have been the only reason for the 45% decrease in glucose uptake with insulin stimulation or ischemia. It is possible that there were changes in expression or activity of other proteins, such as GLUT1 or the insulin-signalling pathway, that may also have affected glucose uptake in the heart. Also, AMPK activation, which stimulates GLUT4 translocation in ischemia, could have been different in control and infarcted hearts at the end of the ischemic protocol. Taken together, further studies might reveal other defects in the ability of the infarcted heart to take up and metabolise glucose.

In conclusion, the high circulating levels of FFAs in rats with chronically infarcted hearts reflected those found in patients with congestive heart failure [28], and correlated negatively with cardiac ejection fractions and myocardial GLUT4 protein levels. Decreased myocardial levels of GLUT4 may have contributed to the reduced glucose uptake in response to insulin and during low-flow ischemia. In turn, reduced glucose uptake resulted in lower glycolytic ATP production and greater loss of ATP during ischemia and therefore impaired functional recovery during reperfusion. We conclude that the chronically infarcted rat heart is
insulin resistant and has increased susceptibility to ischemic injury, possibly owing to the raised circulating FFA concentrations.

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