

Thiazolidinedione Treatment Normalizes Insulin Resistance and Ischemic Injury in the Zucker Fatty Rat Heart

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Obesity is associated with risk factors for cardiovascular disease, including insulin resistance, and can lead to cardiac hypertrophy and congestive heart failure. Here, we used the insulin-sensitizing agent rosiglitazone to investigate the cellular mechanisms linking insulin resistance in the obese Zucker rat heart with increased susceptibility to ischemic injury. Rats were treated for 7 or 14 days with 3 mg/kg per os rosiglitazone. Hearts were isolated and perfused before and during insulin stimulation or during 32 min low-flow ischemia at $0.3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{grams wet wt}^{-1}$ and reperfusion. $\text{D}[2\text{-}^3\text{H}]\text{glucose}$ was used as a tracer of glucose uptake, and phosphorus-31 nuclear magnetic resonance spectroscopy was used to follow energetics during ischemia. At 12 months of age, obese rat hearts were insulin resistant with decreased GLUT4 protein expression. During ischemia, glucose uptake was lower and depletion of ATP was greater in obese rat hearts, thereby significantly impairing recovery of contractile function during reperfusion. Rosiglitazone treatment normalized the insulin resistance and restored GLUT4 protein levels in obese rat hearts. Glucose uptake during ischemia was also normalized by rosiglitazone treatment, thereby preventing the greater loss of ATP and restoring recovery of contractile function to that of lean rat hearts. We conclude that rosiglitazone treatment, by normalizing glucose uptake, protected obese rat hearts from ischemic injury. *Diabetes* 51:1110–1117, 2002

Obesity, a major public health problem in Western society (1), is linked to insulin resistance and type 2 diabetes, with >80% of type 2 diabetic patients being obese (2). Left ventricular hypertrophy is common in obese patients, who have an increased risk of developing cardiomyopathy that can progress to heart failure. Such abnormalities have been explained by the loading effect on the heart caused by the increased peripheral circulation in obesity (3).

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DP, developed pressure; DTT, dithiothreitol; FFA, free fatty acid; gww, grams wet weight; IRS-1, insulin receptor substrate-1; PCr, phosphocreatine; ³¹P NMR, phosphorus-31 nuclear magnetic resonance; PPAR γ , peroxisome proliferator-activated receptor- γ ; RPP, rate-pressure product; TBST, Tris-buffered saline with Tween; TG, triglyceride; TZD, thiazolidinedione.

The obese Zucker rat, a highly dyslipidemic animal model of obesity and impaired glucose tolerance, shows little evidence of abnormal cardiac function when young (3–5) but has impaired cardiac function during hypoxia at 12 months of age (4). Insulin resistance in the obese rat heart has been associated with decreased GLUT4 glucose transporter protein content and impaired GLUT4 translocation to the sarcolemma (6,7). Glucose transport plays a key role in protecting the heart during ischemia (8,9), with glycolysis being the main source of ATP (10,11). A deficient glucose transport system may limit glycolytic ATP production during ischemia and thereby increase damage, yet links between insulin resistance, decreased glucose transport, and ischemic injury in the obese rat heart have not been established.

The last decade has seen the emergence of a new class of insulin-sensitizing agent, the thiazolidinediones (TZDs), such as troglitazone and rosiglitazone, which activate peroxisome proliferator-activated receptor- γ (PPAR γ) nuclear hormone receptors (12). PPAR γ activation regulates adipogenesis (13) with increased differentiation of fat cells, improved suppression of lipolysis by insulin, and hence reduced plasma free fatty acid (FFA) concentrations (14). Abundant in adipose tissue, PPAR γ receptors are also present in lower concentrations in tissues such as heart, liver, and skeletal muscle (15–17). The TZD group of drugs may have tissue-specific effects, as their antidiabetic action has been found in mice lacking adipose tissue (18) and in isolated cardiomyocytes (15,19).

Despite the plethora of studies on adipocytes and skeletal muscle, there are few studies of the effects of TZDs on heart. GLUT4 transporter protein expression and glucose uptake were increased in isolated ventricular myocytes after 20 h incubation with troglitazone (19) or in high-fat-fed rats treated with rosiglitazone for 4 days (14). Troglitazone treatment improved recovery after ischemia in streptozotocin-induced diabetic rat hearts (20), improved left ventricular functional recovery after regional ischemia in pigs (21), and lowered triglyceride (TG) and ceramide levels in Zucker diabetic fatty rat hearts, completely preventing DNA laddering and loss of function (22). Acute and chronic treatment with rosiglitazone reduced infarct size in rats after ligation of the left anterior descending coronary artery with improved recovery of contractile function (23). These studies suggest that PPAR γ activation, whether by direct or indirect mechanisms, may

decrease insulin resistance and ischemic injury in cardiac tissue.

Here, we have tested the hypothesis that low glucose uptake and consequent decreased glycolytic ATP production during low-flow ischemia exacerbate injury in the obese Zucker rat heart. To examine the metabolic cascade that culminates in ischemic injury, $D[2-^3H]$ glucose uptake was measured during ischemia, and phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy was used to follow changes in ATP, phosphocreatine (PCr), and pH during ischemia and reperfusion. We also used rosiglitazone to test the hypothesis that restoring the capacity for glucose transport in the obese Zucker rat heart would improve cardiac insulin resistance and reduce ischemic injury. A preliminary report of this work has been presented in abstract form (24).

RESEARCH DESIGN AND METHODS

Animals. Genetically obese Zucker (*fa/fa*) rats ($n = 74$) and their age-matched lean (*fa/?*) controls ($n = 74$) (Harlan, Bicester, Oxfordshire, U.K.) were housed on a 12-h light/dark cycle at $21 \pm 2^\circ C$ and provided free access to food and water. Rats were studied at either 6 months (28 lean and 28 obese) or 12 months (46 lean and 46 obese) of age. The 6-month-old lean and obese Zucker rats were divided into two groups and given either rosiglitazone (3 mg/kg) or vehicle (H_2O 1 ml/kg) by oral gavage daily for 7 days. The 12-month-old rats were treated in a similar manner for 14 days. The University of Oxford Animal Ethics Review Committees and the Home Office (London, U.K.) approved all of the procedures performed in this study.

Heart perfusions. After rosiglitazone or placebo treatment, rats were anesthetized with a 1 ml i.p. injection of 60 mg/ml sodium pentobarbitone (Sagatal; Rhône Mérieux, Dublin, Ireland). After cessation of peripheral nervous function, blood samples were taken from the femoral artery for determination of metabolites. Blood samples were immediately centrifuged and the supernatant kept on ice for determination of glucose, FFAs, and TGs. Hearts were then quickly excised and arrested in ice-cold heparin containing Krebs-Henseleit buffer. Hearts were cannulated via the ascending aorta for retrograde Langendorf perfusion at $37^\circ C$ using modified, phosphate-free Krebs-Henseleit buffer containing 119 mmol/l NaCl, 5.4 mmol/l KCl, 1.2 mmol/l $MgSO_4$, 1.75 mmol/l $CaCl_2$, 25 mmol/l $NaHCO_3$, 0.5 mmol/l EDTA, and 11 mmol/l glucose. The buffer was continually gassed with a ratio of 95% O_2 to 5% CO_2 to give a pH of 7.40 and, because a volume of 250 ml was recirculated, was continuously filtered using an in-line prefilter, followed by 0.8- and 0.45- μm 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 Gould pressure transducer (P23dB), 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 pressures were recorded continuously using a Gould TA240s chart recorder and bridge amplifier system. Left ventricular developed pressure (DP) was calculated as the systolic pressure minus the end diastolic pressure. The rate pressure product (RPP) was the product of the DP and the heart rate in beats per min. Recovery of RPP at the end of reperfusion was expressed as a percentage of preischemic function measured 2 min before ischemia.

Ischemia and reperfusion protocol. All hearts were perfused with Krebs-Henseleit buffer containing 11 mmol/l glucose for 32 min before 32-min ischemia at 0.3 ml \cdot grams wet weight (gww) $^{-1} \cdot$ min $^{-1}$ and 32-min reperfusion at 100 mmHg constant pressure. During ischemia, the effluent from the heart was not recirculated but was collected and frozen for lactate determinations.

^{31}P NMR spectroscopy. The perfused hearts were placed into a 20-mm diameter glass nuclear magnetic resonance tube, which was inserted into a 400-MHz, 9.4T vertical wide bore super-conducting magnet (Oxford Instruments, Oxford, U.K.). The temperature of the heart was maintained at $37^\circ C$ using the variable temperature unit attached to the spectrometer. Consecutive 4-min ^{31}P NMR spectra were acquired using a Varian Inova Unity spectrometer (Varian, Palo Alto, CA) at a phosphorus resonance frequency of 161.92 MHz. Saturated spectra were acquired using a 60° pulse angle with an interpulse delay of 2.14 s. A total of 112 summed transients gave a total acquisition time of 4 min. Shimming the proton signal to a line width between 20 and 35 Hz enhanced peak resolution. The signal-to-noise ratio was increased by multiplying the ^{31}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 Lorentzian line shapes using a software program (NMRI; Tripes, St. Louis, MO). The ATP and glycogen contents were determined in other groups of lean and obese rat hearts that were frozen at the end of 30 min normal perfusion using Wollenberger clamps kept cold in liquid nitrogen. Hearts were stored at $-80^\circ C$ for the determination of ATP and glycogen concentrations. After assigning the spectrophotometrically measured ATP content to the initial β -ATP peak area, other metabolite concentrations were calculated by relating their peak areas to that of ATP, with correction for spectral saturation. Intracellular pH was estimated from the chemical shift of the inorganic phosphate (P_i) peak (δ_{P_i}) relative to that of the PCr peak using the following equation derived from titration solutions:

$$pH_i = 6.72 + \log \frac{\delta_{P_i} - 3.17}{5.72 - \delta_{P_i}} \quad (1)$$

Glucose uptake in response to insulin and during ischemia. Glucose uptake was measured in two other groups of hearts as the rate of cleavage of H^+ from glucose, as previously described (25). Hearts were perfused with 250 ml recirculating Krebs-Henseleit buffer containing 11 mmol/l glucose and $D[2-^3H]$ glucose, with an activity of 14.5 $\mu Ci/mmole$ (Amersham, Bucks, U.K.). To determine insulin response, hearts ($n = 56$) were perfused as described above and insulin was added to the buffer reservoir after 30 min, to give a final concentration of 3 units/l to ensure maximal stimulation of glucose transport. Recirculating perfusion was continued for another 30 min, and buffer samples from the reservoir were taken every 4 min throughout the protocol. The glucose used (in micromoles) was plotted against time, and the rates of glucose uptake (micromoles per gww per minute), with and without insulin, were calculated. Another set of hearts ($n = 56$) were used to determine glucose uptake during ischemia. In these experiments, buffer samples were taken immediately before ischemia to establish baseline counts, and effluent from each heart was collected over consecutive 4-min intervals during the 32 min of low-flow ischemia at 0.3 ml \cdot $gww^{-1} \cdot$ min $^{-1}$. Effluent samples were stored at $-80^\circ C$ for lactate assays.

Biochemical analyses. Plasma glucose was measured using an assay kit (Sigma, St Louis, MO), and serum FFAs were measured using the NEFA C kit (Wako Chemicals, Neuss, Germany). Plasma TGs were also determined using a kit (Randox, Co Antrim, U.K.). Frozen heart tissue was extracted using 5.6% perchloric acid, and ATP assays were performed on the neutralized extracts (26). Glycogen was extracted from the tissue using ethanol and NaOH, and the extract was assayed for glucose with glycogen calculated as micromoles of carbon-6 per gww (10). Lactate in the effluent from the hearts during ischemia was assayed as previously described (27).

Glucose transporter, insulin receptor, and insulin receptor substrate-1 protein expression. Tissue extraction and immunoblot for determination of GLUT4 and GLUT1 was performed on frozen cardiac tissue from 12 hearts (3–4 per group) from 12-month-old rats. Cardiac tissue was powdered using a stainless steel mortar and pestle under liquid nitrogen. Tissue was homogenized (Power Control Unit; zcomKinematica, Luzern, Switzerland) for 20 s in Newcastle lysis buffer containing 75 mmol/l Tris (pH 6.8), 3.8% SDS, 4 mol/l urea, and 20% glycerol. The homogenates were boiled at $98-100^\circ C$ for 5 min and centrifuged at 13,000 rpm for 5 min to remove nonlysed tissue. Protein concentrations of the supernatants were determined using an assay kit (Perbio, Cheshire, U.K.) and BSA as a standard. After adding 5% β -mercaptoethanol, the samples were boiled for 5 min at $95^\circ C$ and stored at $-80^\circ C$.

For direct immunoblotting studies, equal amounts of solubilized protein (20–50 μg per lane) were resuspended in Laemmli sample buffer containing 20 mmol/l dithiothreitol (DTT), heated at $95^\circ C$ for 5 min, and separated by 10% SDS-PAGE. The resolved proteins were electrophoretically transferred to nitrocellulose membranes using a transfer buffer containing 48 mmol/l Tris, 39 mmol/l glycine, 0.0375% SDS, and 20% methanol (pH 8.8) and a semidry transfer apparatus (BioRad, Hercules, CA). The nitrocellulose membranes were incubated in Tris-buffered saline with Tween (TBST) (0.9% NaCl, 10 mmol/l Tris, and 0.1% Tween-20) supplemented with 5% milk to reduce nonspecific binding and incubated 2 h at room temperature with anti-GLUT4 antibody (1/4,000; G.D. Holman, Bath University, U.K.) or overnight at $4^\circ C$ with anti-GLUT1 antibody (1/1,000; Biogenesis, Poole, U.K.). After the addition of the secondary antibody, the detection of signal was determined by enhanced chemiluminescence and quantified by densitometry using Qscan 32 image analysis software (Biosoft, Cambridge, U.K.).

For the insulin signaling study, the frozen cardiac tissue was homogenized as previously described (28). The protein concentration was determined using an assay kit (Pierce, Tottenham, U.K.) with BSA as standard. For direct immunoblotting, equal amounts of solubilized protein (20–70 μg per lane) were resuspended in Laemmli sample buffer containing 100 mmol/l DTT, heated at $95^\circ C$ for 5 min, and separated using 6% SDS-PAGE. After protein

TABLE 1
Morphological parameters and circulating metabolite concentrations in fed 6- and 12-month-old lean and obese Zucker rats

Rats	Age (months)	n	Morphology			Metabolites		
			Body weight (g)	Heart weight (g)	Heart/body weight ratio ($\times 10^3$)	Glucose (mmol/l)	FFA (mmol/l)	TG (mmol/l)
Lean	6	13	362 \pm 10	1.36 \pm 0.04	3.78 \pm 0.06	9.0 \pm 0.3	0.13 \pm 0.02	1.1 \pm 0.1
	12	11	505 \pm 10	1.77 \pm 0.12	3.60 \pm 0.19	9.8 \pm 0.3	0.17 \pm 0.05	1.1 \pm 0.1
Obese	6	8	568 \pm 15*	1.67 \pm 0.04*	3.08 \pm 0.06*	10.3 \pm 0.4	0.60 \pm 0.07*	3.3 \pm 0.3*
	12	11	740 \pm 12*	2.43 \pm 0.07*	3.16 \pm 0.07*	10.3 \pm 0.5	0.65 \pm 0.14*	4.4 \pm 0.6*
Lean RSG-treated	6	14	368 \pm 11	1.34 \pm 0.03	3.73 \pm 0.06	9.0 \pm 0.4	0.08 \pm 0.01	0.8 \pm 0.1
	12	15	519 \pm 18	1.90 \pm 0.12	3.69 \pm 0.15	8.7 \pm 0.2	0.13 \pm 0.01	0.7 \pm 0.1
Obese RSG-treated	6	10	565 \pm 10*	1.67 \pm 0.05*	3.09 \pm 0.07*	8.8 \pm 0.5†	0.22 \pm 0.04†	1.7 \pm 0.1†
	12	12	743 \pm 16*	2.49 \pm 0.03*	3.33 \pm 0.14*	8.8 \pm 0.4	0.31 \pm 0.06†	2.5 \pm 0.5†

Data are means \pm SE. *P* < 0.05 *vs. lean untreated, †vs. obese untreated. All statistical comparisons were made to rats of the same age. RSG, rosiglitazone.

transfer, the nitrocellulose membranes were incubated in TBST supplemented with 5% milk at 4°C overnight to reduce nonspecific binding, then either incubated for 1 h and 30 min at room temperature with anti-insulin receptor (2 µg/ml) or insulin receptor substrate-1 (IRS-1) (1/200) antibodies (Autogen Bioclear, Calne, U.K.). After several washes with TBST, the filters were incubated at room temperature with the appropriate secondary antibody for 1 h. Proteins were visualized and quantified as described above.

Statistics. Data are presented as the means \pm SE. Statistical significance was assessed using two-way ANOVA or repeated measures ANOVA and a post hoc *t* test with Bonferroni correction where appropriate (SPSS). Differences were considered significant at *P* < 0.05.

RESULTS

Physiological characteristics of lean and obese rats.

The obese Zucker rats were ~200 g heavier than lean control rats at both 6 and 12 months of age (Table 1), with no body weight changes as a consequence of rosiglitazone treatment. Heart weights were significantly increased in 6- and 12-month-old obese rats relative to lean rats. However, because there was a larger relative difference in body weights than heart weights between obese and lean rats of the same age, the heart/body weight ratios were significantly lower in obese rats. There was no significant difference in plasma glucose concentrations between fed obese and lean rats, although rosiglitazone treatment reduced plasma glucose by 15% in obese rats. Serum FFA concentrations were 4.1- to 4.4-fold higher in obese rats than their lean controls. Rosiglitazone treatment decreased FFA concentrations by 64 \pm 4 and 51 \pm 5% in the obese rats at 6 and 12 months of age, respectively. Plasma TG concentrations were three- and fivefold higher in 6- and 12-month-old obese rats, respectively, compared with their lean controls. Rosiglitazone treatment decreased plasma TG concentrations by 44 \pm 6 and 40 \pm 9% in 6- and 12-month-old obese rats, respectively. Preischemic glycogen levels, determined in separate groups of rat hearts freeze-clamped after 30 min perfusion, were the same for all hearts, irrespective of rosiglitazone treatment. At 12 months of age, myocardial glycogen contents were 9.4 \pm 0.9 and 12.4 \pm 1.9 µmol glucosyl units/gww (*n* = 3/group) in the lean and obese untreated rats, respectively, and 9.8 \pm 1.2 and 10.8 \pm 1.2 µmol glucosyl units/gww (*n* = 4/group) in the lean and obese treated rats, respectively.

Insulin-stimulated glucose uptake. Basal glucose up-

take was the same for all hearts (Fig. 1). Insulin-stimulated glucose uptake rates were normal in obese Zucker rat hearts at 6 months of age but were 34 \pm 12% lower than lean controls at 12 months of age. Thus, the obese Zucker rat hearts had developed insulin resistance between 6 and 12 months of age. Treatment with rosiglitazone restored the insulin-stimulated glucose uptake rates to normal in the 12-month-old obese rat hearts and had no effect on glucose uptake in the other heart groups.

Myocardial ischemia. Coronary flows pre- and postischemia were the same in obese and lean rat hearts (Table 2), with rosiglitazone treatment having no effect on flow rates. Heart rates were the same for all hearts and unaffected by ischemia or rosiglitazone treatment (Table 2). During ischemia, there was no evidence of contracture in any of the hearts. Recovery of left ventricular developed pressure was significantly impaired in the 12-month-old Zucker rat hearts, but rosiglitazone treatment restored recovery of developed pressure to control levels (Table 2). The preischemic RPP, a measure of contractile function, was 22% lower in all rat hearts at 12 months than at 6 months (Fig. 2). During reperfusion, recovery of contractile function in 6-month-old obese rat hearts was to 75 \pm 7% of preischemic levels, the same as their lean controls. However, contractile function in the 12-month-old obese rat hearts

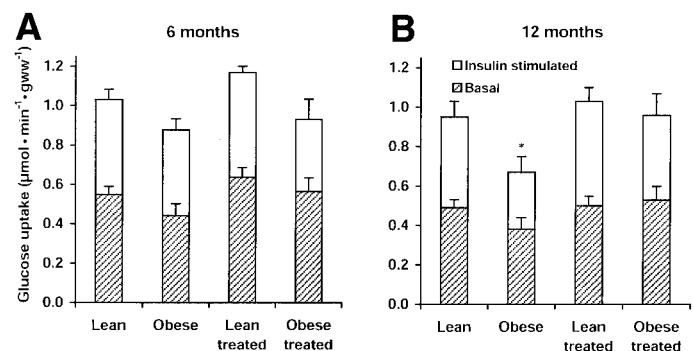


FIG. 1. Basal (▨) and 3 units/l insulin-stimulated (□) [2-³H]glucose uptake in 6-month-old (A) and 12-month-old (B) isolated Zucker rat hearts. Data are means \pm SE for *n* = 7 per group. *Significantly different from basal at *P* < 0.05.

TABLE 2

Coronary flow rates, heart rates, and developed pressures pre- and post-ischemia in 6- and 12-month-old lean and obese Zucker rat hearts

Rats	Age (months)	n	Coronary flow		Heart rate		Developed pressure	
			Pre-ischemia	Post-ischemia (ml/min)	Pre-ischemia	Post-ischemia (beats/min)	Pre-ischemia	Post-ischemia (mmHg)
Lean	6	5	23 ± 1	21 ± 1	250 ± 9	234 ± 12	150 ± 9	129 ± 7
	12	7	25 ± 1	25 ± 1	231 ± 15	235 ± 18	145 ± 8	115 ± 6
Obese	6	5	26 ± 1	26 ± 1	230 ± 14	216 ± 10	184 ± 9	148 ± 14
	12	7	29 ± 1	29 ± 1	187 ± 20	158 ± 36	168 ± 12	70 ± 17*
Lean RSG-treated	6	5	23 ± 1	23 ± 1	276 ± 13	254 ± 15	164 ± 8	128 ± 11
	12	7	24 ± 2	23 ± 2	226 ± 17	213 ± 25	144 ± 6	123 ± 6
Obese RSG-treated	6	5	27 ± 2	26 ± 2	228 ± 15	212 ± 9	169 ± 7	133 ± 15
	12	7	29 ± 1	28 ± 2	194 ± 14	178 ± 18	156 ± 3	137 ± 12†

Data are means ± SEM. $P < 0.05$ *vs. lean untreated, †vs. obese untreated. All statistical comparisons were made to rats of the same age. RSG, rosiglitazone.

was significantly lower throughout reperfusion than that of the lean controls, with the final recovery being $48 \pm 10\%$ of preischemic values compared with $76 \pm 5\%$ recovery in controls ($P < 0.05$). Rosiglitazone treatment restored recovery of myocardial function in 12-month-old obese rats to $80 \pm 7\%$, the same as the lean control rat hearts.

Myocardial energetics. The intracellular PCr concentrations were the same for all hearts during the preischemic period and rapidly fell at the onset of ischemia in all hearts (Fig. 3). During reperfusion, PCr concentrations recovered to $68 \pm 6\%$ of preischemic levels in obese hearts, but

significantly more ($103 \pm 4\%$) in lean rat hearts. Intracellular ATP concentrations were the same for all heart groups during the preischemic period, and ATP was hydrolyzed in all heart groups during ischemia, by $30 \pm 11\%$ in lean rat hearts and $69 \pm 5\%$ in obese rat hearts by the end of the ischemic period. Consequently, in obese rat hearts, ATP was significantly lower by the end of ischemia at 1.6 ± 0.2 vs. 3.1 ± 0.5 $\mu\text{mol/gww}$. ATP remained low in obese rat hearts throughout the reperfusion period, at 2.0 ± 0.2 vs. 3.0 ± 0.5 $\mu\text{mol/gww}$ in lean rat hearts over the final 16 min of reperfusion. Rosiglitazone treatment of the obese rats prevented the greater loss of ATP during myocardial ischemia and restored the recoveries of PCr and ATP to those of lean controls during reperfusion.

Myocardial glucose uptake and lactate efflux during ischemia. The total glucose uptake and lactate production during ischemia were the same for all 6-month-old rat hearts (Fig. 4), but glucose uptake was $21 \pm 9\%$ lower in 12-month-old obese compared with lean rat hearts. In parallel with glucose uptake, lactate production was $26 \pm 11\%$ lower in 12-month-old obese rat hearts during ischemia compared with lean rat hearts. Rosiglitazone treatment of obese rats increased myocardial glucose uptake and lactate efflux during ischemia to lean control rat heart rates. The intracellular pH changes during ischemia were the same for all hearts (Fig. 3).

Glucose transporter, insulin receptor, and IRS-1 protein expression. The total tissue GLUT1 transporter protein content was the same in 12-month-old obese and lean rat hearts (Fig. 5). In obese rat hearts, GLUT4 transporter total protein content was reduced by 20% ($P < 0.05$) compared with lean controls but was restored to normal by rosiglitazone treatment. Expression of insulin receptor- β subunit and IRS-1 proteins was not decreased in the 12-month-old obese rat hearts and was not altered by rosiglitazone treatment (data not shown).

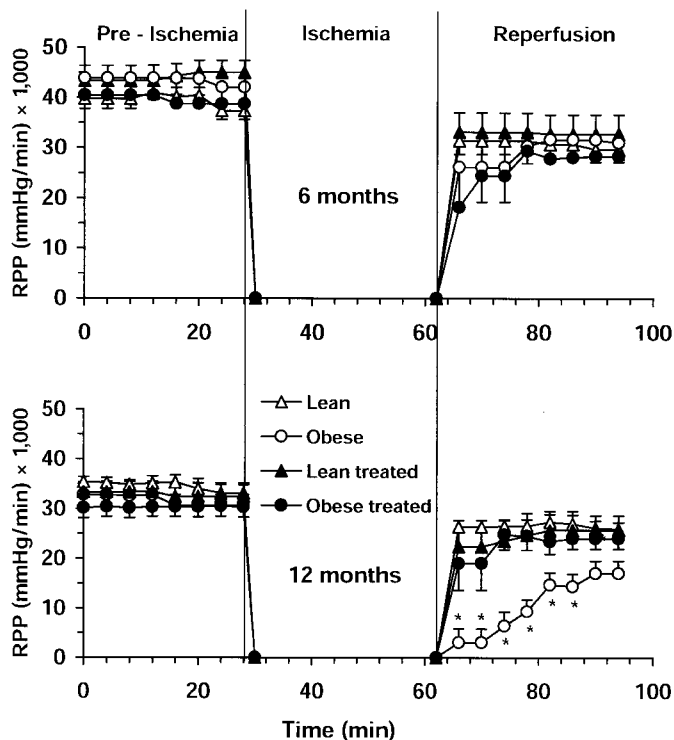


FIG. 2. Myocardial RPP, a measure of contractile function, in isolated 6-month-old (upper panel) and 12-month-old (lower panel) Zucker rat hearts during 32 min preischemia, 32 min low-flow ischemia, and 32 min reperfusion. Points are means ± SE for $n = 7$ per group. *Significantly different from the other groups at $P < 0.05$.

DISCUSSION

Human obesity is associated with left ventricular hypertrophy and increased risk of heart disease (2). We found that the obese Zucker rat hearts weighed 23 and 37% more

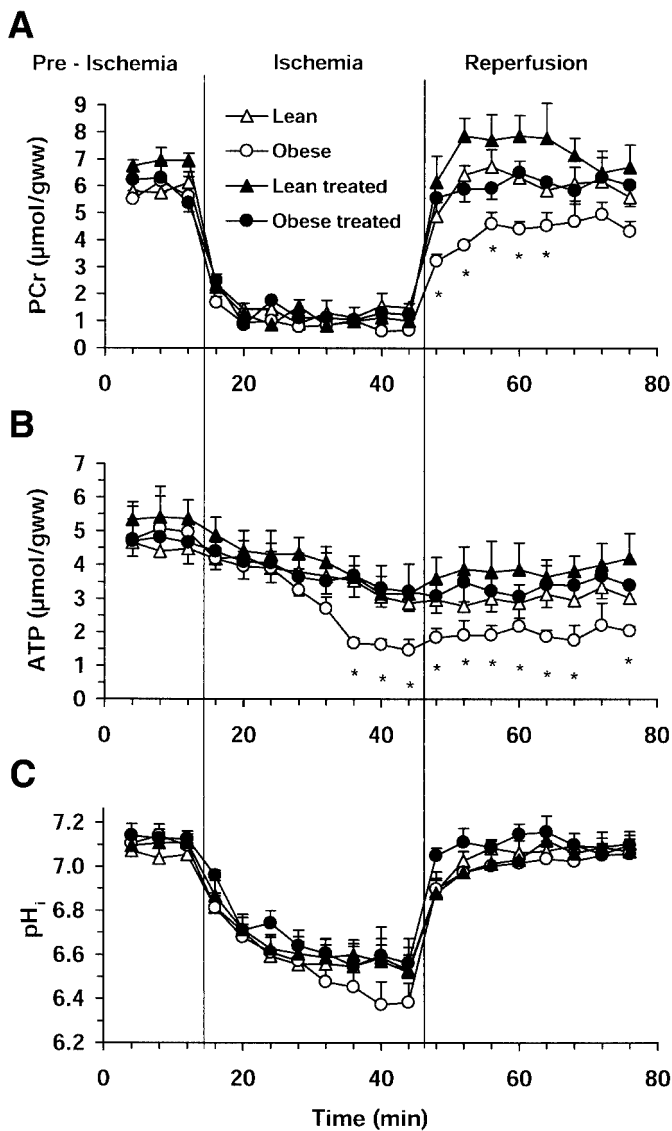


FIG. 3. Changes in PCr (A), ATP (B), and pH_i (C) in 12-month-old Zucker rat hearts during 32 min low-flow ischemia, measured using ^{31}P NMR spectroscopy. Points are means \pm SE for $n = 5$ per group. *Significantly different from the other groups at $P < 0.05$.

at 6 and 12 months, respectively, than their lean controls, but the heart weight increased relatively less than the increase in body weight. Susceptibility to ischemic injury increased with age, for all of the 6-month-old rat hearts had the same response to ischemia as the 8-month-old rat hearts (5), but the 12-month-old obese rat hearts had significantly increased ischemic injury. Rosiglitazone feeding for 14 days had no effect on rat heart or body weights and normalized ischemic injury in the 12-month-old obese rat hearts. The cellular mechanisms involved in the protective effects of TZD treatment were the focus of this study.

ATP depletion and ischemic injury. During low-flow ischemia, the heart relies on glycolysis for ATP production (10,29). There was significantly lower glucose uptake and significantly greater loss of ATP in untreated obese rat hearts during ischemia, suggesting that decreased glycolytic ATP production was associated with lower recovery of contractile function during reperfusion. Finding that

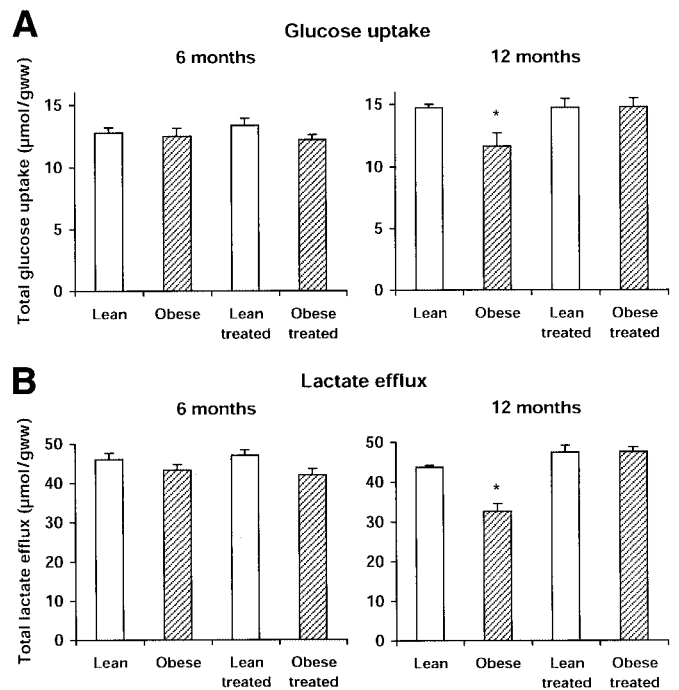


FIG. 4. Total glucose uptake (A) and lactate efflux (B) during 32 min low-flow ischemia in 6- and 12-month-old isolated Zucker rat hearts. Data are the means \pm SE for $n = 7$ per group. *Significantly different from the other groups at $P < 0.05$.

rosiglitazone treatment normalized glucose transport, the loss of ATP and the recovery of contractile function in the obese rat heart supported this association.

The greater ATP depletion in the obese rat hearts during ischemia was more than may have been expected from the lower glucose uptake alone. During low-flow ischemia, glycolytic ATP is produced not only from glucose, but also via glycogenolysis, with significant depletion of glycogen during 32 min low-flow ischemia in normal rat hearts (10,29). We did not measure end-ischemic glycogen, but decreased glycogenolysis in the obese rat hearts was indicated by the discrepancy between lactate production and glucose utilization in those hearts. Lactate production during ischemia could be accounted for by glucose plus near total glycogen utilization in the lean rat hearts but by glucose plus only 30% glycogen utilization in the untreated obese rat hearts. We have no direct evidence for decreased glycogenolysis, but Zucker obese rat hepatocytes have

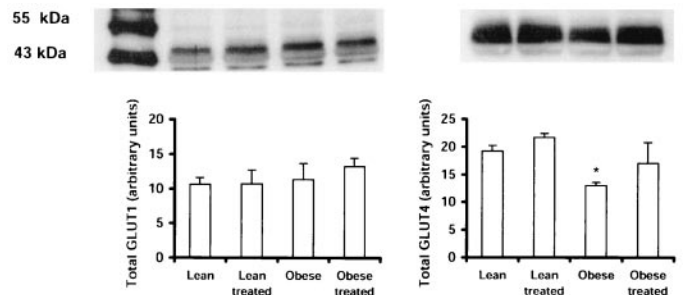


FIG. 5. GLUT1 and GLUT4 protein expression in 12-month-old Zucker rat heart. Data are the means \pm SE for $n = 4$ per group treated with rosiglitazone or $n = 3$ per untreated group. *Significantly different from the other groups at $P < 0.05$.

decreased glycogenolysis (30), and obese humans have reduced whole-body glycogenolysis (31,32).

The lack of significant differences in intracellular pH during ischemia, despite significant differences in lactate production, was caused by decreased glycolysis (producing two protons per glucose molecule) but greater ATP hydrolysis (producing three protons per ATP molecule) in obese rat hearts during ischemia (33). In other words, the production of protons by net ATP hydrolysis in untreated obese rat hearts was equivalent to the protons produced during glycolytic ATP production in the other rat heart groups.

In this study, 11 mmol/l glucose was the only substrate provided to the isolated hearts, but hearts *in vivo* use a variety of circulating substrates for energy production in addition to glucose, including fatty acids, lactate, and ketone bodies. Thus, omitting other substrates may have exaggerated the dependence of the hearts on glucose. Although glucose use is increased during ischemia, low-flow conditions provide sufficient oxygen to allow residual oxidative phosphorylation (11). Thus, the availability of other substrates may have significantly altered the response to ischemia. Indeed, we have demonstrated that the addition of palmitate to the perfusate improved recovery of contractile function in diabetic rat hearts (25). Furthermore, the availability of other substrates can also affect glucose uptake; thus, the differences seen here in the 12-month-old Zucker rats may not be apparent under *in vivo* conditions, in which circulating substrate levels also differ.

Insulin signaling and GLUT4 expression. In the untreated, obese rat hearts, significantly lower insulin-stimulated glucose transport may have been caused by defective insulin signaling, for IRS-1 protein content was reduced by 30–40% in cardiomyocytes (34) and by 60% in skeletal muscle from obese Zucker rats (35) and obese humans (36). Although we found no change in insulin receptor or IRS-1 protein expression in Zucker obese rat hearts at 12 months of age, we found reduced GLUT4 protein expression and lower glucose uptake during insulin stimulation or ischemia. Previous studies have shown decreased GLUT4 protein and lower insulin-stimulated glucose transport in obese Zucker rat hearts (6,7). In our study, the normalization of myocardial GLUT4 protein expression after rosiglitazone treatment was accompanied by normalization of glucose uptake during insulin stimulation or ischemia, suggesting a direct link between decreased GLUT4 protein expression and decreased glucose uptake. In support of this argument, different signaling pathways are involved in ischemia- and insulin-stimulated translocation of GLUT4 to the sarcolemma (37,38). Insulin-stimulated glucose uptake requires phosphatidylinositol 3-kinase activation (39,40), whereas ischemia-induced GLUT4 translocation may involve AMP kinase and/or p38 MAP kinase activation (38,41). Glucose uptake was decreased in response to both ischemia and insulin in 12-month-old obese Zucker rat hearts, which suggests that a specific defect in insulin receptor signaling may not have been involved.

Plasma fatty acids and insulin resistance. The very high circulating levels of FFAs and TGs, with normal plasma glucose concentrations, are characteristic of the

obese Zucker rat (5). Plasma insulin levels were not measured in the current study, but the development of insulin resistance in the absence of hyperglycemia is consistent with that in obese humans, in whom increased insulin levels moderate plasma glucose (42). Insulin resistance is virtually always associated with obesity, but how obesity produces insulin resistance is uncertain. One putative mechanism would be the generation of one or more metabolic messengers, such as FFAs, by adipose tissue that when released, would inhibit insulin action on muscle (42,43). The link between FFAs, obesity, and insulin resistance is well established (44); plasma FFAs inhibit insulin-stimulated glucose oxidation in a dose-dependent manner (42). However, the mechanism involved is unresolved; it may involve fatty acid inhibition of glucose phosphorylation (44) and/or glucose transport (42,43). Fatty acid inhibition of glucose transport may be via changes in the insulin-signaling cascade (43) or by direct inhibition of GLUT4 gene transcription (45). In the present study, high circulating levels of FFAs and TGs in the obese Zucker rats were normalized by rosiglitazone treatment, consistent with the known lipid-lowering, insulin-sensitizing effects of TZDs (46). Rosiglitazone treatment also normalized GLUT4 protein expression, which may have been caused by decreased FFA concentrations altering GLUT4 gene transcription (45) or via direct PPAR γ activation, as GLUT2 gene expression in pancreatic β -cells is regulated by a peroxisomal proliferator response element on the GLUT2 gene (47). Although direct effects of fatty acids or TZDs on GLUT4 gene transcription have not been reported in heart, PPAR γ receptors are found in cardiac tissue (15). An alternative, direct protective mechanism may involve PPAR γ upregulation of heme oxygenase-1 expression, shown in rat glial cells (48), as mouse hearts overexpressing heme oxygenase-1 are highly resistant to ischemia and reperfusion injury (49). However, myocardial PPAR γ gene expression was not determined in this study and, without it, the possibility of protective effects via direct PPAR γ activation has not been established.

Summary

In conclusion, 6-month-old obese Zucker rat hearts were normal with respect to insulin-stimulated glucose uptake and ischemia, but 12-month-old obese Zucker rat hearts were insulin resistant and had lower glucose uptake during ischemia than lean rat hearts, which could be explained by decreased expression of GLUT4 transporters. The lower glucose uptake was associated with greater loss of ATP during ischemia and decreased recovery of contractile function during reperfusion. Rosiglitazone treatment normalized insulin-stimulated glucose uptake and GLUT4 protein levels in the obese rat heart, normalized glucose uptake and energetic changes during ischemia, and restored recovery of contractile function. Therefore, rosiglitazone treatment protected the isolated, obese Zucker rat heart from ischemic injury.

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