Myocardial susceptibility to ischemic-reperfusion injury in a prediabetic model of dietary-induced obesity

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We assessed the myocardial susceptibility to ischemic-reperfusion injury in obese rat hearts in the absence and the presence of predicted circulating concentrations of insulin and fatty acids. Feeding rats a high-calorie diet resulted in increases in body weight, visceral fat content, cardiac hypertrophy, plasma insulin, nonesterified free fatty acid, and triglyceride concentrations. In the absence of both insulin and fatty acids in the coronary perfusate, the hearts of obese rats developed an increased infarct size (41.9 ± 1.9% for obese vs. 22.9 ± 2.3% for control, P < 0.05) and a reduced percent recovery of aortic output (4.2 ± 4.2% for obese vs. 27.7 ± 3.4% for controls, P < 0.05) after coronary artery occlusion and reperfusion. In the presence of insulin in the coronary perfusate, a cardioprotective effect was noted in both groups, an action that was greater in hearts from obese compared with control rats and which abolished the obesity-induced changes in infarct size (13.8 ± 1.2% for controls vs. 21.0 ± 1.6% for obese), and percent recovery of aortic output (60.2 ± 4.7% for controls vs. 45.7 ± 9.4% for obese). Fatty acids (0.7 mM, control; and 1.5 mM, obese) added to the coronary perfusate with in vivo concentrations of insulin dramatically increased infarct size (48.2 ± 3.1% for obese, and 37.5 ± 2.7% for control; P < 0.05 vs. without fatty acids) and decreased percent aortic output recovery (control, 10.4 ± 5.2%, and obese 7.8 ± 3.5%; P < 0.05 vs. without fatty acids) in both groups to similar values. In conclusion, in obesity, the impact of an increased susceptibility of the myocardium to ischemic-reperfusion injury on myocardial injury is likely to be overshadowed by the comparatively greater roles played by predicted increases in circulating insulin and fatty acids found in vivo. These data support the notion that adiposity per se is unlikely to be a valuable predictor of outcomes in ischemic-reperfusion injury.

insulin resistance; myocardial infarction; cardiac function

OBESITY IS A WELL-RECOGNIZED risk factor for myocardial infarction (37). Moreover, obesity and the accompanying insulin resistance may increase the susceptibility of the myocardium to ischemic-reperfusion injury as assessed ex vivo (7, 16, 24). These findings may, in part, explain some clinical studies, where worse outcomes have been reported to occur in patients with obesity after myocardial infarction and reperfusion (18, 28, 29). However, these data have not been reproduced in all studies. Indeed, myocardial ischemic-reperfusion injury may not be enhanced in obesity when assessed in vivo (31). Furthermore, not all clinical studies support the notion that obesity is associated with deleterious clinical outcomes (3, 9, 17, 18, 22, 25, 35).

An explanation for conflicting findings regarding the impact of obesity on ischemic-reperfusion injury (7, 16, 24, 31) has not been provided. One potential explanation is that obesity is associated with both hyperinsulinemia and elevated circulating fatty acid concentrations, both of which markedly modulate the extent to which myocardial injury occurs during ischemia-reperfusion (14, 15, 19, 21, 38). It is therefore possible that in obesity, the impact of circulating insulin and fatty acids on myocardial injury during ischemia and reperfusion could overshadow that effect of alterations in myocardial susceptibility to ischemic-reperfusion injury. Hence, the aim of the present study was to compare the myocardial susceptibility to ischemic-reperfusion injury in obesity in the presence versus the absence of predicted circulating concentrations of insulin and fatty acids. In this regard, to test this hypothesis, we studied an animal model of obesity recently shown by us to be more susceptible to ischemic-reperfusion injury when assessed in the absence of insulin and fatty acids (7).

MATERIALS AND METHODS

This study complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was assessed and approved by the Committee for Experimental Animal Research of the Faculty of Health Sciences of the University of Stellenbosch. Male Wistar rats weighing 200 ± 5 g received a diet containing elevated carbohydrates and fats that resembles a Western-type diet (5). The diet consists of 65% carbohydrate, 19% protein, and 16% fat compared with that of the control group receiving 60% carbohydrate, 30% protein, and 10% fat for 16 wk. Since the diet is designed to induce hyperphagia (27), the experimental group consumed a greater quantity of food and energy intake was enhanced (570 ± 23 kJ/day) compared with the control group that consumed 371 ± 18 kJ/day. To ensure that differences in micronutrient (vitamins and minerals) intake, produced by a dilution of the diet by the addition of carbohydrates and fats, did not modify the outcomes of the present study, a separate group of rats pair-fed to the micronutrient intake of the diet-fed group was also assessed. Blood pressure measurements were performed on awake, restrained rats using standard tail-cuff techniques employing a photoelectric diode to detect the tail pulse (Harvard Apparatus) at various times during the study.

Heart perfusion studies. The impact of obesity and insulin administration on myocardial infarct size and postischemic functional recovery was determined using previously described techniques (6–8).

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Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (12 mg/kg), and the hearts were rapidly excised. The hearts were placed in ice-cold buffer before being transferred to a working heart perfusion apparatus where they were retrogradely perfused with a Krebs-Henseleit buffer, equilibrated with 95% O₂-5% CO₂ at 37°C, containing (in mmol/l) 118.0 NaCl, 4.7 KCl, 1.2 MgSO₄·7H₂O, 1.25 CaCl₂, 25 NaHCO₃, 1.2 KH₂PO₄, and 10 glucose at a pressure of 100 cmH₂O. Retrograde perfusion was initiated within 45 s of excision of the heart.

To determine the impact of obesity on myocardial infarct size and reperfusion mechanical function, hearts were perfused in the working mode (preload, 15 cmH₂O; and afterload, 100 cmH₂O) and preischemic mechanical function was documented (coronary flow, aortic output, aortic diastolic and systolic pressure, and heart rate). Hearts were then retrogradely perfused for 10 min before the left anterior descending coronary artery was ligated using a silk suture to induce regional ischemia at 36.5 ± 0.5°C for 40 min. The suture was released and the heart reperfused for 30 min before assessing reperfusion mechanical function, and infarct size (Fig. 1). A reperfusion time of 30 min was selected since 30- and 120-min reperfusion periods produce similar infarct sizes in our hands (unpublished data).

To determine infarct size and reperfusion function with insulin, insulin was added to the perfusion buffer at the approximate mean circulating concentrations found in control (30 μIU/ml) and obese (50 μIU/ml) rats. Insulin perfusion was initiated 10 min before coronary artery ligation and terminated at the onset of reperfusion. Although insulin was added to the perfusion buffer, aortic output was measured 10 min after the initiation of washout of insulin. Under these circumstances, the function was measured at times when insulin was not present in the buffer and would therefore not have influenced the inotropic state of the heart. When mechanical function was assessed before and after ischemia, hearts were subjected to an insulin-free perfusion buffer. To determine infarct size and reperfusion function with fatty acids together with insulin, we perfused hearts with appropriate insulin concentrations (control, 30 μIU/ml; and obese, 50 μIU/ml) together with fatty acids (0.7 mM for control, and 1.5 mM for obese rats) prebound to bovine serum albumin (BSA) as described previously (1, 20). Fatty acids in the BSA contributed 0.3 mM to the fatty acid concentration in the buffer, and the rest of the fatty acids were added in the form of palmitate. The fatty acid concentrations employed in the present study were based on those previously described in a study to investigate the effects of high and low fatty acid concentrations on fatty acid oxidation in normal hearts (1, 20).

**Myocardial infarct size assessments.** Myocardial infarct size was determined as previously described (8). Briefly, after 30 min reperfusion of the regionally ischemic heart, the coronary artery was reoccluded at the end of the reperfusion period, and a solution of 25% Evans blue was injected into the coronary arteries to delineate the area at risk. Hearts were then frozen and cut into slices, incubated in sodium phosphate buffer containing 1% (wt/vol) triphenyltetrazolium chloride for 15 min to visualize the unstained infarcted region. Infarct and risk zone areas were determined with planimetry, and infarct area was expressed as a percentage of the area at risk.

**Myocardial norepinephrine release.** Myocardial norepinephrine release was determined as previously described (33). To assess the impact of obesity on myocardial norepinephrine release at baseline and during postischemic reperfusion, a sample of coronary effluent was collected for 1 min in prechilled containers at baseline and at regular intervals after reperfusion of the globally ischemic heart. Coronary effluent was stabilized with Na₂EDTA and HClO₄ (0.01 mol/l and 0.025%, respectively). Norepinephrine was immediately extracted from 1 ml of coronary effluent using alumina (Sigma) adsorption with a Tris buffer at pH 8.6, eluted with 0.1 M HClO₄ (40), stored at ~70°C and concentrations determined using reversed-phase, ion-exchange high-performance liquid chromatography with electrochemical detection (11). Myocardial norepinephrine release was expressed as the concentration of norepinephrine in the effluent per gram heart tissue per minute (33).

**Myocardial glycolytic flux rates.** Myocardial glycolytic flux rates were measured in normoxic hearts from control and experimental diet-fed rats. Since this measurement requires sampling from the coronary perfusate, we chose to measure these rates under normoxic conditions, since the coronary effluent collected from regionally ischemic hearts would have been the perfusate passing through nonischemic tissue. To determine the rate of myocardial glycolytic flux, the amount of H₂O released from the metabolism of 5-[³H]glucose by triosephosphate isomerase and enolase steps in the glycolytic pathway was measured. Hearts were perfused with 5-[³H]glucose under normoxic conditions and coronary effluent samples collected. To separate H₂O from 5-[³H]glucose, the perfusate was passed through an ion exchange resin column (200–400 mesh Dowex 1×4) pretreated with Na₂O₇. Samples (100 μl) were loaded on the column and eluted into scintillation vials using 0.8 ml H₂O. ³H in samples was counted using a Beckman Beta scintillation counter (2). To determine whether myocardial glycolytic flux rates were modified by the administration of insulin or insulin together with fatty acids, we repeated these experiments in separate groups of rats with insulin only (30 or 50 μIU/ml) or insulin together with fatty acids (0.7 nM for control and 1.5 mM for obese). Concentrations of insulin and fatty acids in the perfusion buffer were similar to concentrations noted to occur in vivo.

**Blood analysis.** To determine the impact of the model of obesity on circulating insulin, blood glucose, nonesterified free fatty acid, TNF-α and angiotensin II concentrations, serum samples were stored at −20°C until assays were performed. Angiotensin II was extracted using solid-phase extraction (SepPak C18 cartridges) and the plasma concentrations determined using a commercially available radioimmunoassay (Euro Diagnostica, Malmö, Sweden). TNF-α concentrations were determined by an enzyme-linked immunosorbent assay (OptEIA, Pharmingen) (7). Insulin concentrations were determined using a commercially available radioimmunoassay (Diagnostic Products). Nonesterified free fatty acid concentrations were determined using a colorimetric assay (Roche Diagnostics, Penzberg, Germany). Other blood samples were processed using standard procedures. Homeostasis model assessment was determined using the standard formula: [fasting insulin (in μIU/ml) × fasting glucose (in mmol/l)]/22.5.

**Echocardiography.** To define left ventricular structural changes associated with the model of obesity, two-dimensional-targeted M-mode echocardiography was performed using a 7.5-MHz transducer and a Hewlett-Packard Sonos 2500 sector scanner according to the American Society of Echocardiography convention and as previously described by members of our group (26). Rats were anesthetized with ketamine and xylazine (26), and echocardiography was performed blinded, and in random order, by a single observer (G. R. Norton). Two scans were obtained from each rat, and the mean values were calculated. Left ventricular end-diastolic diameter and posterior wall thickness were measured using the leading-edge technique (26).
Cardiomyocyte morphology. The presence of cardiac hypertrophy in obese rats was further assessed by measuring cardiomyocyte dimensions. Rat hearts were perfused for 10 min in the Langendorff mode. The perfusion buffer was then switched to a fixing solution containing 4% formaldehyde in a phosphate buffer (pH 7.4), and the heart was perfusion fixed for a further 5 min. Hearts were wax imbedded, stained (hematoxylin and eosin staining), and analyzed within a week. Ten slides were made of each heart, and the average myocyte size was determined using image analysis on a Zeiss Axioskop 2 (Carl Zeiss, Jena, Germany) microscope fitted with a Zeiss AxioCam (Carl Zeiss) digital camera. Average myocyte size was determined as described previously (32).

Statistical analysis. All data are presented as means ± SE. When comparisons between two groups (diet and control groups) were made, an unpaired Students t-test was performed. Comparisons between four groups (diet and control groups with and without insulin) were made with an ANOVA (two way when appropriate) and a Tukey post hoc test.

RESULTS

Characteristics of the model. Rats fed the experimental diet were ~18% heavier but had a 68% greater visceral fat mass than control diet-fed rats (Table 1). Fasting blood glucose concentrations were similar, but insulin concentrations were elevated in the diet-fed rats. The obese animals had an elevated homeostasis model assessment index compared with the controls (Table 2). Although there was a nonsignificant trend for HbAlc levels to increase in the obese rats compared with control rats, all values obtained in the experimental group were well below the threshold levels of 6% for the detection of abnormal blood glucose control, and a similar number of rats in each group had values above the upper 95% confidence intervals for the control group (50% of obese compared with 39% of control animals) (Table 2). The obese animals receiving the experimental diet were dyslipidemic with elevated triglyceride and nonesterified free fatty acid concentrations (Table 2). The obese rats also had elevated plasma angiotensin II and TNF-α concentrations (Table 2).

Cardiac remodeling. The hearts from obese rats fed the experimental diet were hypertrophied as reflected by an increased ventricular weight, ventricular weight-to-tibial length, left ventricular posterior wall thickness (despite an increased left ventricular end diastolic internal diameter), and an increased cardiomyocyte size (Table 1). The diet failed to increase systolic blood pressure during the course of the study (see Table 1 for values obtained after 16 wk on the diet).

Myocardial infarct size after ischemia-reperfusion. In the absence of insulin or fatty acids in the perfusion medium, myocardial infarct size after ischemia and reperfusion in the experimental diet-fed group was approximately double that of the controls (Fig. 2A) (22.9 ± 2.3% for the controls, and 41.9 ± 1.9% of the area at risk for the obese rats, P < 0.05). The pair-fed (lean) rats that received similar quantities of micronutrients compared with the diet-fed rats and had infarct sizes that were comparable with those of control animals (22.1 ± 1.0%).

The addition of in vivo concentrations of insulin to the perfusion buffer decreased infarct size in both the control and experimental groups and attenuated the differences noted between the groups (Fig. 2A). The exposure of the myocardium to in vivo concentrations of insulin (30 μIU/ml) in the control group reduced infarct size by ~9% of the area at risk, whereas in the heart of the obese rats, exposure to in vivo concentrations of insulin (50 μIU/ml) decreased infarct size by ~21% of the area at risk (Fig. 2A). Only in vivo concentrations of insulin (30 μIU/ml) decreased infarct size in the control animals, with higher concentrations producing no significant change in infarct size (Fig. 2A). In contrast, concentrations of insulin found in vivo in both control animals and in obese animals decreased infarct size in the experimental diet group (Fig. 2A). The 30 and 50 μIU/ml insulin concentrations produced similar effects on infarct size in obese rat hearts (Fig. 2A).

In the presence of in vivo concentrations of insulin, the addition of fatty acids to the perfusion buffer markedly increased infarct size in both control and experimental groups (Fig. 2B). In the control group, fatty acids increased infarct size by ~24% of the area at risk, whereas in the experimental group, the fatty acids increased infarct size by ~28% of the area at risk (Fig. 2B). Thus the impact of fatty acids on infarct size was quantitatively similar between the groups. Importantly, no significant differences in infarct size were noted between the control and experimental groups in the presence of in vivo concentrations of insulin together with fatty acids (Fig. 2B).

Mechanical function after ischemia-reperfusion. Preischemic and reperfusion aortic output was lower in hearts of the experimental diet group than in control animals (Fig. 3A, bottom, compared with Fig. 3A, top). Consequently, functional recovery during reperfusion was expressed as a percentage of baseline values (Fig. 3B). Importantly, percent recovery after ischemia was markedly diminished in diet-fed rats compared
with the controls (Fig. 3B). The addition of insulin to the perfusion buffer increased functional recovery in both groups (Fig. 3, A and B) and attenuated the differences in functional recovery noted between the diet and control groups (Fig. 3B). Importantly, the exposure of the control hearts to either low (30 μIU/ml, normal for control animals) or high (50 μIU/ml, normal for obese animals) concentrations of insulin caused similar increases in the percent aortic output recovery (Fig. 3B). Reperfusion aortic output doubled in the control hearts in response to insulin (30 or 50 μIU/ml) treatment. In contrast, in the hearts of the experimental diet-fed rats, a low-insulin concentration (30 μIU/ml) caused a fourfold increase in the aortic output recovery, whereas the high concentration of insulin (50 μIU/ml) caused a tenfold increase in aortic output recovery when compared with hearts perfused with an insulin free solution (Fig. 3B). At insulin concentrations of 50 μIU/ml, the percent recovery in aortic output was the same in hearts from control and diet-fed rats (Fig. 3B).

In the presence of in vivo concentrations of insulin, the addition of fatty acids to the perfusion buffer markedly decreased functional recovery, in both control and experimental groups (Fig. 4). In the control group, the decrease in functional recovery produced by fatty acids reduced aortic output by ~84%, whereas in the experimental group, the decrease in functional recovery produced by fatty acids reduced aortic output by ~82% (Fig. 4B). Thus the impact of fatty acids on functional recovery was quantitatively similar between the groups. Importantly, no significant differences in aortic output
were noted between the control and experimental groups in the presence of in vivo concentrations of insulin together with fatty acids (Fig. 4A).

Coronary effluent norepinephrine release. Coronary effluent norepinephrine concentrations increased immediately on reperfusion (Fig. 5). These concentrations declined to preischemic values within the first 10 min of reperfusion. Myocardial norepinephrine release was similar in the control and experimental rat hearts both before ischemia and during the first 10 min of reperfusion (Fig. 5).

Myocardial glycolytic flux rates. Myocardial glycolytic flux rates were reduced in the hearts of experimental animals in the absence of insulin (Fig. 6B compared with Fig. 6, A and C). Insulin (30 \(\mu\)IU/ml) included in the perfusion buffer dramatically increased glycolytic flux rates in both control and experimental hearts (Fig. 6, A and B) but did not return the values in the hearts from experimental diet-fed rats to control values. However, higher insulin concentrations (50 \(\mu\)IU/ml) increased glycolytic flux rates in the hearts of obese rats to values comparable with glycolytic flux rates noted in the hearts from control rats (Fig. 6B vs. Fig. 6, A and C).

The inclusion of fatty acids and in vivo concentrations of insulin to the coronary perfusate of either the control (Fig. 6C) or the obese (Fig. 6D) rat hearts decreased glycolytic flux rates. A much greater decline in glycolytic flux rates was noted in hearts obtained from obese rats. However, obese rat hearts had similar lower glycolytic flux rates compared with control rat hearts in the presence of fatty acids and in vivo concentrations of insulin in the coronary perfusate (Fig. 6, C and D).

DISCUSSION

The main findings of the present study are as follows. In the absence of predicted circulating concentrations of insulin and fatty acids, dietary-induced obesity resulted in an increased susceptibility of the isolated perfused heart to ischemic-reperfusion injury as reflected by the increased infarct size and a reduced mechanical functional recovery. However, in the presence of predicted circulating concentrations of insulin and fatty acids, the hearts of obese rats were equally as susceptible to ischemic-reperfusion injury as hearts obtained from nonobese rats.

**Fig. 4.** Impact of insulin and fatty acids on the hearts of control and obese rats with prediabetes on aortic output (A) and percent recovery of aortic output (B) before and after normothermic global myocardial ischemia (\(n = 6–12\) hearts/group). A, top: \#P < 0.05 vs. control + insulin. A, bottom: \#P < 0.05 vs. obese + 30 \(\mu\)IU/ml insulin. B: \#P < 0.05 vs. control + insulin or obese + insulin. Between group comparisons in A: P < 0.05 obese + insulin vs. control + insulin.

**Fig. 5.** Impact of obesity with prediabetes on coronary effluent norepinephrine release before ischemia and during the first 10 min of reperfusion (\(n = 7\) hearts/group). There were no differences between groups at each time point.
The results of the present study are in agreement with prior studies that have demonstrated an increased susceptibility of the myocardium to ischemic-reperfusion injury in nonobese animal models of insulin resistance produced by a high-fructose diet (24), in Zucker rats with clinically overt Type II diabetes mellitus (36) or insulin resistance (16) or in obese, insulin-resistant rats (7) when assessed in the absence of insulin or fatty acids. The apparent contradiction between the previously reported lack of an increase in myocardial susceptibility to ischemia-reperfusion, as assessed in vivo in obese compared with normal rats (31), is explained by the findings of the present study. In this regard, in the presence of increased insulin and fatty acid concentrations as occurs in vivo in obesity, the marked impact of these factors on myocardial injury (1, 20) is likely to overshadow the effect of obesity per se on myocardial susceptibility to ischemia-reperfusion. In contrast, as noted in the present and prior studies (7, 16, 24, 36), in the absence of insulin or fatty acids in the coronary perfusate, an enhanced ischemic-reperfusion injury is unmasked in hearts of insulin-resistant rats.

The present findings may, in part, explain the discrepant findings obtained in clinical studies assessing the impact of obesity on outcomes following myocardial infarction and reperfusion. In contrast to some studies showing worse outcomes in obese individuals (18, 28, 29), other studies have not confirmed these findings (3, 9, 17, 18, 22, 25, 35). In studies showing a worse outcome, it is possible that insulin or fatty acids concentrations may not have been excessively increased, thus unmasking the impact of obesity (18, 28, 29). In contrast, other studies may have been characterized by patients with marked increases in fatty acid and insulin concentrations, thus masking the impact of excess adiposity on ischemic-reperfusion injury (3, 9, 17, 18, 22, 25, 35). Clearly, the extent to which excess adiposity impacts on ischemic-reperfusion injury is going to be determined by a number of factors, including the extent to which sympathetic-induced changes in circulating fatty acids and insulin occurs. Importantly, as in the presence of predicted circulating concentrations of fatty acids and glucose wherein obesity was not associated with differences in ischemic-reperfusion injury, the present study supports the notion that excess adiposity per se is unlikely to be a valuable predictor of outcomes post-myocardial infarction and reperfusion.

Several studies have demonstrated an anti-ischemic cardioprotective effect of insulin in the normal rat heart whether given during ischemia and reperfusion (14, 15) or during reperfusion alone (38). Furthermore, the adverse effect of fatty acids on ischemic-reperfusion injury in normal hearts is well documented (1, 16, 20). However, the effect of fatty acids administered in the presence of insulin on myocardial tolerance to ischemia-reperfusion in obesity is unknown. This question is of importance since obesity is often associated with elevated...
circulating insulin and fatty acid concentrations. In the present study, although insulin produced a quantitatively greater beneficial effect in hearts from obese rats, the inclusion of fatty acids reduced ischemic tolerance in hearts from both lean and obese rats and the quantitative effect was similar between the groups. The overall effect of the exposure of hearts from either group to both insulin and fatty acids was a similar degree of myocardial injury and dysfunction in hearts from obese compared with hearts from lean rats.

In the present study there was an unexpected dissociation between myocardial infarct size and reperfusion mechanical function with insulin exposure in obese rat hearts. Whereas infarct size was similar between control and experimental diet groups when hearts were exposed to either 30 or 50 μIU/ml insulin concentrations, the percent recovery in the reperfusion aortic output was reduced in the obese compared with the control rat hearts when exposed to insulin concentrations noted in vivo in control rats (30 μIU/ml). Only insulin concentrations that occur in vivo in obese rats (50 μIU/ml) returned the percent reperfusion aortic output recovery in the obese rat hearts to control values. This finding is partly consistent with that of a recent study demonstrating incremental increases in reperfusion mechanical function in the hearts of diabetic mice with exposure to increasing concentrations of insulin and glucose given during low-flow ischemia, whereas increased insulin and glucose concentrations given during low-flow ischemia had no effect on reperfusion function in the hearts from control animals (13). Whether these observations reflect an excessive degree of myocardial stunning, enhanced apoptosis, or a reduced myocyte function in the insulin-resistant myocardium requires further study. The reduced baseline function noted in obese rats suggests that this may reflect an obesity-induced decrease in cardiac function ex vivo.

There is evidence to indicate that the extent of myocardial ischemic-reperfusion injury is inversely associated with myocardial glucose utilization. Indeed, a relationship between reperfusion contractile function and myocardial glucose flux rates has been noted in insulin-resistant rats (12). Moreover, insulin-stimulated myocardial glycolysis is suppressed by fatty acids (10) and, as indicated by the present and prior studies (1, 20), the exposure of the heart to fatty acids considerably increases myocardial injury in response to ischemia-reperfusion. The results of the present study suggest that myocardial injury to ischemia-reperfusion is largely driven by myocardial glucose utilization. Indeed, the extent of myocardial injury and decline in functional recovery following ischemia-reperfusion was most striking in the groups in which myocardial glycolytic flux was the lowest.

Consistent with the present study, obesity is associated with cardiac hypertrophy (23). Cardiac hypertrophy could in turn exacerbate ischemic-reperfusion injury through two potential mechanisms. First, an exaggerated uncoupling of glycolysis from glucose oxidation following an ischemic insult might explain the increased susceptibility to ischemic-reperfusion injury in the absence of insulin and fatty acids has yet to be studied. Importantly, we have previously demonstrated that the prevention of hypertrophy in the obesity model studied by us increases reperfusion mechanical function (7). This suggests that cardiac hypertrophy may indeed play an important role, possibly through the uncoupling of glycolysis from glucose oxidation. However, as indicated by the present study, this change is unlikely to impact on the degree of myocardial injury that occurs following ischemia-reperfusion in the presence of predicted concentrations of insulin and fatty acids.

With the assumption that the impact of fatty acids on myocardial injury can be alleviated through alternative approaches, the results of the present study also suggest that obesity may be a condition that is particularly susceptible to the beneficial effects of insulin during ischemia and reperfusion. However, as suggested by the present study, approaches to minimizing the deleterious impact of fatty acids on myocardial injury during ischemia and reperfusion should take precedence, before considering the potential benefits of insulin in obesity.

In conclusion, in keeping with previous studies (7, 16, 24, 36), dietary-induced obesity with insulin resistance increases the susceptibility of the ex vivo myocardium to ischemic-reperfusion injury, an effect that is nevertheless no longer apparent when the myocardium is exposed to predicted increases in circulating concentrations of insulin and fatty acids. Thus, in obesity, the impact of an increased susceptibility of the myocardium to ischemic-reperfusion injury is likely to be overshadowed by the comparatively greater role played by predicted increases in circulating insulin and fatty acids on myocardial damage during ischemia and reperfusion. These findings lend insight into the variable outcomes noted in studies assessing the impact of obesity on ischemic-reperfusion injury and support the notion that excess adiposity per se is unlikely to be a valuable predictor of outcomes in ischemic-reperfusion injury.

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