Free fatty acids, but not ketone bodies, protect diabetic rat hearts during low-flow ischemia

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Free fatty acids, but not ketone bodies, protect diabetic rat hearts during low-flow ischemia. Am J Physiol Heart Circ Physiol 280: H1173–H1181, 2001.—To determine whether the effects of fatty acids on the diabetic heart during ischemia involve altered glycolytic ATP and proton production, we measured energetics and intracellular pH (pHi) by using 31P NMR spectroscopy plus [2-3H]glucose uptake in isolated rat hearts. Hearts from 7-wk streptozotocin diabetic and control rats, perfused with buffer containing 11 mM glucose, with or without 1.2 mM palmitate or the ketone bodies, 4 mM β-hydroxybutyrate plus 1 mM acetoacetate, were subjected to 32 min of low-flow (0.3 ml·g wet wt−1·min−1) ischemia, followed by 32 min of reperfusion. In control rat hearts, neither palmitate nor ketone bodies altered the recovery of contractile function. Diabetic rat hearts perfused with glucose alone or with ketone bodies, had functional recoveries 50% lower than those of the control hearts, but palmitate restored recovery to control levels. In a parallel group with the functional recoveries, palmitate prevented the 54% faster loss of pHi during ischemia; the glucose-perfused control and palmitate-perfused diabetic hearts had significantly lower end-ischemic pHi, values that were significantly different at 6.36 ± 0.04 and 6.60 ± 0.02, respectively, but had similar functional recoveries, whereas the glucose-perfused diabetic hearts had significantly lower functional recoveries, but their pHi was 6.49 ± 0.04. We conclude that fatty acids, but not ketone bodies, protect the diabetic heart by decreasing ATP depletion, with neither having detrimental effects on the normal rat heart during low-flow ischemia.

31P nuclear magnetic resonance spectroscopy; substrate utilization; glucose uptake; myocardial energetics; glucose production; substrate oxidation; intracellular pH (pHi); metabolic control; ischemic reperfusion injury; diabetic myocardium; glucose carbohydrate metabolism; ketone bodies; free fatty acids; palmitate; glutamine; β-hydroxybutyrate; acetoacetate; streptozotocin diabetes

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heart. Parts of this work have been published in abstract form (17, 18).

METHODS

**STZ-induced diabetes.** Type I diabetes was produced in male Wistar rats (250–300 g body wt) after an injection of 50 mg/kg ip STZ (Sigma; St. Louis, MO) dissolved in 50 mM citrate, pH 4.5. Control animals were injected with vehicle. All STZ-injected animals were then given a 10% glucose solution to drink for 24 h, after which their urine was tested for glucose with the use of glucose sticks (Clinistix, Ames; Slough, UK). Only STZ-injected animals demonstrating significantly elevated urine glucose, ~95% of animals injected, were used in the experiments.

**Heart perfusions.** Seven weeks after injection, the rats were anesthetized with a 1-ml ip injection of 60 mg/ml pentobarbitone sodium (Sagatal, Rhône Mérieux; Dublin, Ireland). After cessation of peripheral nervous function, hearts were quickly excised and arrested in ice-cold heparin-containing Krebs-Henseleit buffer. Blood samples, taken from the aorta after removal of the heart, were immediately centrifuged, and the supernatant was kept on ice for determination of plasma glucose and free fatty acids. Hearts were cannulated via the ascending aorta for retrograde Langendorff perfusion at 37°C using modified, phosphate-free Krebs-Henseleit buffer containing (in mM) 119 NaCl, 5.4 KCl, 1.2 MgSO4, 2 CaCl2, 25 NaHCO3, 0.25 EDTA, and 11 glucose. For the fatty acid experiments, 3% albumin (Bovuminar reagent, fatty acid free, Intergen; Purchase, NY) was added to the buffer, with or without 1.2 mM Na palmitate (Sigma) and dialyzed against albumin-free Krebs-Henseleit buffer for 48 h before use. This concentration of palmitate was used because it reportedly causes maximal damage during ischemia-reperfusion (32). The free fatty acid concentration was quantified by using a NEFA-C kit (Wako).

**K**- and Na+ concentrations and other metabolite concentrations were calculated by relating ATP content was assigned to the initial weight ratios. After the spectrophotometrically measured ATP content was determined in another set of control and diabetic rat hearts that were frozen at the end of 30 min normal perfusion by using Wollenberger clamps kept cold with liquid nitrogen. Hearts were stored at –70°C for determination of ATP concentrations and the wet-to-dry weight ratios. After the spectrophotometrically measured ATP content was assigned 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. pHi was estimated from the chemical shift of the inorganic phosphate peak (δ31P) relative to that of the phosphocreatine (PCr) peak by using the following equation derived from titration solutions

$$pHi = 6.72 + \log \frac{\delta_{31P} - 3.17}{5.72 - \delta_{31P}}$$

**Glucose uptake in response to insulin and during ischemia.** Glucose uptake was measured as the rate of cleavage of H+ from glucose. The H+ was traced by using D-[2-3H] glucose, with the 3H released in the phoshoglucosomerase reaction (glucose 6-phosphate to fructose 6-phosphate) as H2O. Thus the amount of 3H2O in the coronary effluent was used to estimate glucose uptake (16). Hearts were perfused with 250 ml of recirculating Krebs-Henseleit buffer containing 11 mM glucose and D-[2-3H]glucose, with an activity of 14.5 mCi/mmol (Amersham; Bucks, UK). To determine insulin response in separate groups of hearts (n = 4–5 per group), insulin was added to the buffer reservoir after 30 min, to give a final concentration of 3 U/I to ensure maximal stimulation
of glucose transport. Recirculating perfusion was continued for another 30 min. Buffer samples from the reservoir were taken every 4 min throughout the protocol. The glucose used (in μmol) was plotted against time, and the rates of glucose uptake (μmol-g wet wt. -1·min -1), with and without insulin, were calculated. Other groups of hearts (n = 4–7 per group) were used to determine the effects of palmitate on glucose uptake during ischemia. In these experiments, buffer samples were taken immediately before ischemia to establish baseline counts, and effluent from the heart was collected over consecutive 4-min intervals during the 32 min of low-flow, 0.3 ml-g wet wt. -1·min -1, ischemia. Control and STZ hearts were perfused with or without fatty acids during ischemia, according to the above protocol.

Biochemical analyses. Plasma glucose was measured by using an assay kit (Sigma), and free fatty acids were measured by using the NEFA C kit (Wako Chemicals). Frozen heart tissue was extracted by using 5.6% perchloric acid, and ATP assays were performed on the neutralized extracts (31). Glycogen was extracted from the tissue by using ethanol and NaOH, and the extract was assayed for glucose with glycogen content, determined in separate groups of hearts freeze-clamped after 30 min perfusion, was two-fold higher in the diabetic rat hearts (n = 6), at 19.3 ± 2.0 μmol/g wet wt (P < 0.05), than in control rat hearts (n = 6) at 10.2 ± 0.8 μmol/g wet wt.

Statistics. Data are expressed as means ± SE. n = 6 rats per group. Plasma substrate concentrations were measured in the overnight fasted state, 7 wk after streptozotocin (STZ) injection at time animals were killed. *P < 0.05 vs. control hearts.

RESULTS

Physiological characteristics of diabetic rats. In the 7 wk after STZ injection, diabetic rat body weights did not change, whereas control rat body weights increased by ∼200 g (Table 1). Consequently, diabetic rats had body and heart weights that were 41 and 36% lower, respectively, than those of the control rats, but had similar heart-to-body weight ratios. Diabetic rats had plasma glucose levels 3.2-fold higher, free fatty acid levels 1.8-fold higher, and β-hydroxybutyric acid levels 2.6-fold higher than the control rats (Table 1). The glycogen content, determined in separate groups of hearts freeze-clamped after 30 min perfusion, was two-fold higher in the diabetic rat hearts (n = 6), at 19.3 ± 2.0 μmol/g wet wt (P < 0.05), than in control rat hearts (n = 6) at 10.2 ± 0.8 μmol/g wet wt.

Recovery of myocardial contractile function. In groups of hearts not subjected to ischemia, 90 min of aerobic perfusion with glucose as the sole substrate, decreased contractile function (RPP) by <10% (ns, not significant) in control rat hearts, but by ∼40% (P < 0.05) in diabetic rat hearts (data not shown). Thus, immediately before ischemia, contractile function of diabetic rat hearts perfused with glucose alone was significantly lower than controls (Table 2). Coronary flow rates were the same for all hearts before ischemia with 100% recovery in each heart group during reperfusion. Inclusion of albumin in the buffer lowered the recovery of the RPP by 30% (P < 0.05) in control hearts (Table 2). With glucose plus albumin, control heart functional recovery was 58%, and diabetic rat heart functional recovery was 58%, and diabetic rat heart functional recovery was 58%.

Table 1. Physiological characteristics of the control and diabetic rats studied

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>511 ± 9</td>
<td>299 ± 11*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.46 ± 0.04</td>
<td>0.93 ± 0.07*</td>
</tr>
<tr>
<td>Heart weight-to-body weight ratio</td>
<td>2.9 ± 0.1</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>15 ± 1</td>
<td>48 ± 2*</td>
</tr>
<tr>
<td>Plasma free fatty acids, mM</td>
<td>0.29 ± 0.04</td>
<td>0.51 ± 0.05*</td>
</tr>
<tr>
<td>Plasma β-hydroxybutyrate, mM</td>
<td>0.53 ± 0.16</td>
<td>1.40 ± 0.48*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats per group. Plasma substrate concentrations were measured in the overnight fasted state, 7 wk after streptozotocin (STZ) injection at time animals were killed. *P < 0.05 vs. control hearts.

Table 2. Control and diabetic rat heart coronary flow rates and contractile function before and after ischemia with EDP and recovery of RPP after ischemia

<table>
<thead>
<tr>
<th>Coronary Flows, ml/min·g wet wt. -1</th>
<th>Heart Rate, beats/min</th>
<th>EDP, mmHg (Postischemia)</th>
<th>Developed Pressure, mmHg</th>
<th>RPP × 10 -5, mmHg/min</th>
<th>% Recovery (Postischemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n Preischemia Postischemia</td>
<td>Preischemia Postischemia</td>
<td>Preischemia Postischemia</td>
<td>Preischemia Postischemia</td>
<td>Preischemia Postischemia</td>
</tr>
<tr>
<td>Control</td>
<td>7 17 ± 1 14 ± 1</td>
<td>266 ± 9</td>
<td>222 ± 13</td>
<td>23 ± 6</td>
<td>135 ± 7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>9 15 ± 1 14 ± 2</td>
<td>204 ± 16*</td>
<td>191 ± 29</td>
<td>37 ± 6*</td>
<td>122 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>6 17 ± 1 17 ± 1</td>
<td>257 ± 13</td>
<td>250 ± 18</td>
<td>22 ± 5</td>
<td>140 ± 10</td>
</tr>
<tr>
<td>Diabetic</td>
<td>9 16 ± 1 15 ± 2</td>
<td>230 ± 18</td>
<td>224 ± 18</td>
<td>19 ± 5</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>Glucose + albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5 16 ± 1 15 ± 2</td>
<td>254 ± 6</td>
<td>280 ± 25</td>
<td>3 ± 8</td>
<td>155 ± 15</td>
</tr>
<tr>
<td>Diabetic</td>
<td>4 15 ± 1 14 ± 1</td>
<td>210 ± 35</td>
<td>180 ± 32</td>
<td>36 ± 9*</td>
<td>111 ± 5*</td>
</tr>
<tr>
<td>Glucose + ketone bodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3 15 ± 1 17 ± 2</td>
<td>260 ± 21</td>
<td>222 ± 24</td>
<td>25 ± 13</td>
<td>136 ± 5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>3 17 ± 2 16 ± 1</td>
<td>212 ± 31</td>
<td>241 ± 8</td>
<td>60 ± 5*</td>
<td>128 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats per group. Function is given for 2 min before ischemia or 30 min after reperfusion. RPP, rate-pressure product; EDP, end-diastolic pressure. *Significantly different from control hearts in the same group, P < 0.05. †Significantly different from the glucose + albumin-perfused control hearts, P < 0.05.
recovery was half that of controls, at 31% of preischemic function (Fig. 1 and Table 2). The addition of palmitate or ketone bodies to the perfusion buffer did not affect the recovery of control rat hearts, which was 66–70%, nor did ketone bodies alter the recovery of diabetic hearts, which remained low at 35%. However, palmitate increased the functional recovery of diabetic rat hearts to that of control hearts, to 62% of preischemic values. During reperfusion, the EDP in the diabetic rat hearts given glucose alone or with ketone bodies, were significantly higher than in either of the control groups or in the palmitate-perfused diabetic hearts. Thus, palmitate, but not ketone bodies, decreased injury in the diabetic rat heart during low-flow ischemia.

**Myocardial energetics.** $^{31}$P NMR spectra (Fig. 2) showed that the PCr concentrations were the same in all hearts before ischemia and that PCr was hydrolyzed at the same rate to remain at 2–10 μmol/g dry wt during ischemia in all hearts (Fig. 3). However, the glucose-perfused, diabetic rat hearts had significantly lower PCr recovery during reperfusion than the palmitate-perfused diabetic rat hearts (Fig. 3 and Table 3). The concentration of ATP was the same in all hearts before ischemia at 31.5 μmol/g dry wt, but the diabetic rat hearts perfused with glucose alone had a 54% faster loss ($P < 0.05$) of ATP during ischemia (Table 3). Palmitate did not alter the rate of ATP depletion in the control rat hearts, but decreased the ATP depletion rate in the diabetic hearts to that of the controls. There was no significant increase in ATP in any of the hearts during reperfusion (Fig. 3).

**Myocardial pH**. The pH before ischemia-reperfusion was the same in all hearts (Fig. 3). However, at the end of ischemia, pH was significantly higher in hearts perfused with palmitate than in those perfused with glucose alone (Table 3). Thus diabetic rat hearts perfused with glucose alone had significantly lower recovery of contractile function and higher EDP during reperfusion, which were associated with a faster rate of ATP depletion during ischemia and lower PCr recovery during reperfusion, but were not related to the pH during ischemia. Indeed, the same functional recoveries were observed in hearts with pH values ranging from 6.36 to 6.60, suggesting that proton load did not determine recovery under these experimental conditions.

**Basal glucose uptake and insulin response.** In control rat hearts, glucose uptake rates increased from 8.1 ± 0.5 to 17.1 ± 3.9 μmol·g dry wt$^{-1}$·min$^{-1}$ ($P < 0.05$), on
stimulation with insulin. The basal glucose uptake rate in the diabetic rat hearts was significantly lower than control, at $3.5 \pm 2.5 \mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1} (P < 0.05)$, and increased to $7.8 \pm 2.4 \mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}$ on stimulation with insulin. Thus, in the presence or absence of insulin, glucose uptake rates in the diabetic rat hearts were less than half those of the controls. Contractile function did not change with the addition of insulin (data not shown).

**Myocardial glucose uptake during low-flow ischemia.** Because the pH was significantly lower in hearts perfused with glucose and was higher in all palmitate-perfused rat hearts at the end of ischemia, palmitate may have inhibited glycolysis. Consequently, the effect
of palmitate on myocardial [2,3H]glucose uptake was measured during ischemia. Glucose uptake rates were maximal at 3.4 ± 0.3 μmol·g dry wt⁻¹·min⁻¹ in the control rat hearts perfused with glucose alone (Fig. 4), but palmitate decreased uptake by 50%, to 1.7 ± 0.1 μmol·g dry wt⁻¹·min⁻¹ (P < 0.05). Glucose uptake rates were 62% lower in the diabetic rat hearts than in the glucose-perfused control hearts (P < 0.05) and were not altered by palmitate. Thus the total glucose uptake was approximately twofold higher in the control glucose-perfused rat hearts than in palmitate-perfused control and either glucose- or palmitate-perfused diabetic rat hearts (Table 3).

DISCUSSION

This work shows that, when glucose is the only substrate, the isolated diabetic rat heart has lower contractile function during preischemia, reduced glucose uptake and faster ATP depletion during ischemia, and poorer functional recovery with higher EDP during reperfusion than the control rat heart. Other groups (6, 37) have also reported low contractile function in glucose-perfused diabetic rat hearts, despite normal ATP and PCr levels, with restoration of systolic pressure to normal on the addition of hexanoate to the perfusate (4). In our experiments, addition of palmitate restored contractile function in the diabetic rat hearts to control levels after ischemia, suggesting that fatty acids were beneficial. This finding apparently contradicts reports of maximal damage occurring in rat hearts 6–8 wk after STZ injection and perfused with fatty acids during low-flow ischemia (32, 33). However, the previous study used flow rates three to four times higher than those used here, had more than double the length of ischemia, and used the working rat heart model. Paulson (32) showed that, when ischemic flow rates were adjusted for heart size, diabetic hearts perfused with palmitate had normal functional recovery, in agreement with our findings. Finally, we found that, unlike palmitate, ketone bodies were not beneficial to the diabetic heart during ischemia, probably because their oxidation may be inhibited after STZ treatment (8, 12, 14).

Cross et al. (10) and King et al. (16) showed that glucose is important for ATP production during low-flow ischemia, increasing functional recovery during reperfusion. Residual oxygen is available to the heart during low-flow ischemia, which allows limited ATP production via oxidative phosphorylation, whether the substrate be glucose or palmitate (30). Diabetic hearts have reduced glucose transporter expression, which has been attributed to the chronic lack of insulin, resulting in decreased glucose uptake (13, 38). Not only did we find 50% lower basal glucose uptake, but 50% lower insulin-stimulated glucose uptake in the diabetic rat hearts, suggesting that diabetic hearts have normal insulin signaling, but an insulin response limited by lower glucose transporter numbers.

Diabetic hearts have decreased PDH activity (36), owing to the chronic lack of insulin and increased fatty acid oxidation. Thus low glucose uptake and low PDH activity may result in a lower capacity for ATP production from glucose by both glycolysis and oxidative phosphorylation, which would make diabetic hearts more sensitive to ischemic injury. This occurred in the glucose-perfused diabetic hearts in our study; those hearts had faster ATP loss during ischemia, and higher EDPs, with lower PCr and functional recoveries during reperfusion. In the control glucose-perfused rat hearts, glucose uptake and glycolysis provided sufficient substrate for any available oxygen, the evidence for
oxidative phosphorylation being seen in the low, but measurable, PCr levels in all hearts throughout ischemia, similar to those found by Owen and co-workers (30). When added to the perfusate, palmitate was metabolized to produce the same amount of ATP via oxidative phosphorylation despite inhibiting glycolysis, as shown by decreased glucose uptake and higher pHi, with the same ATP and PCr concentrations during ischemia. It has been reported that myocytes isolated from STZ-induced diabetic rat heart have impaired glucose oxidation (7), but normal palmitate oxidation (8). We suggest that the diabetic rat hearts in our study also metabolized palmitate via β-oxidation to produce ATP, thereby restoring the recovery of heart function to control levels. Thus the detrimental effects of low glucose uptake and PDH inhibition in the diabetic rat heart may have been circumvented by palmitate β-oxidation during low-flow ischemia.

Given the protective effects of palmitate in diabetic hearts, we expected the ketone bodies, acetoacetate and d-β-hydroxybutyrate, also to be oxidized to produce ATP via residual oxidative phosphorylation. This probably occurred in the control rat hearts. However, although not detrimental, the ketones did not improve the recovery of contractile function in the diabetic hearts after ischemia. This may be a consequence of decreased activities of d-β-hydroxybutyrate dehydrogenase and 3-oxoacid CoA-transferase in STZ-induced diabetic rat heart mitochondria, depressing the oxidation of d-β-hydroxybutyrate and acetoacetate (12, 14). Indeed, Chen and co-workers (8) showed that reduced d-β-hydroxybutyrate oxidation in myocytes from STZ-induced diabetic rats, whereas the oxidation of palmitate remained similar to that of the control rat myocytes.

The circulating free fatty acid levels were 1.8-fold higher in the diabetic rats than in the controls (Table 3).

<table>
<thead>
<tr>
<th>Glucose + albumin</th>
<th>ATP depletion rate, μmol g dry wt⁻¹ min⁻¹</th>
<th>pHi</th>
<th>Glucose uptake, μmol g dry wt⁻¹</th>
<th>PCr During Reperfusion, μmol g dry wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.50 ± 0.15(4)</td>
<td>6.36 ± 0.04(4)</td>
<td>106 ± 17(5)</td>
<td>40.5 ± 6.7(4)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.77 ± 0.09*(10)</td>
<td>6.49 ± 0.04(10)</td>
<td>42 ± 20*(4)</td>
<td>33.2 ± 3.0(10)</td>
</tr>
<tr>
<td>Glucose + albumin + palmitate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.41 ± 0.10(5)</td>
<td>6.52 ± 0.04*(5)</td>
<td>52 ± 12*(5)</td>
<td>46.6 ± 3.2(5)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.51 ± 0.08(9)</td>
<td>6.60 ± 0.02*(9)</td>
<td>42 ± 5*(7)</td>
<td>53.6 ± 5.0(9)</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers of animals in each group are given in parentheses. PCr, phosphocreatine. *Significantly different from control glucose-perfused hearts, P < 0.05. †Significantly different from diabetic glucose-perfused hearts, P < 0.05.
1), yet both were lower than the 1.2 mM palmitate concentrations used during the ischemia-reperfusion protocol. We used 1.2 mM palmitate because it was the concentration used by other groups reporting (28, 29) the detrimental effects of fatty acids during ischemia-reperfusion. We rationalized that, during an infarction, the surge in catecholamine activity raises plasma free fatty acids concentrations. Similarly, the plasma β-hydroxybutyric acid concentration was 2.6-fold higher in the diabetic rats than in the controls, yet we used higher ketone body concentrations during ischemia-reperfusion. Such concentrations were used because they have functional and energetic effects similar to those of insulin in the isolated working rat heart (35).

Elevation of blood ketones to similar levels occurs after a 48-h fast (3) and almost completely reverse the mitochondrial abnormalities associated with insulin deficiency in the normal heart (35). Although high concentrations of fatty acids and ketone bodies should have a much greater impact on glucose uptake, glycolysis, and glucose oxidation than more physiologically relevant concentrations of these substrates, their impact on the tolerance to low-flow ischemia was negligible in the control rat hearts (Table 2). In the diabetic heart, fatty acids were beneficial and the ketone bodies had no impact on the recovery after ischemia.

In contrast to our results, several studies (23, 24, 32) showed that 1.2 mM palmitate has detrimental effects on the isolated rat heart during ischemia. The explanation for the opposite findings may be that the other studies used total ischemia, whereas low-flow ischemia was used here because it allowed the heart to maintain its supply of substrates, and because most cases of clinical ischemia involve a form of partial coronary artery occlusion with residual flow. One of the cellular mechanisms proposed to underlie the detrimental effects of palmitate is that it inhibits glucose utilization and thereby overall energy metabolism in the heart, the effect being more pronounced in the diabetic heart, which already has decreased glucose uptake and oxidation (32, 34). We found that palmitate did indeed inhibit glycolysis in the control rat heart, but this was not detrimental because the palmitate was oxidized to provide as much ATP as could be provided via glycolysis alone, as shown by the 31P NMR spectroscopic results. In the diabetic rat heart, palmitate oxidation had no effect on the already low glycolytic rate, but produced more ATP than glucose alone and thereby protected the heart. Of course, in total ischemia without residual oxidative phosphorylation, fatty acids would not be able to have the same protective effect.

Another hypothesis for the detrimental effects of palmitate is that it inhibits PDH activity, thereby uncoupling glucose oxidation from glycolysis, with increased proton production during reperfusion (21, 22, 25). In our study, pH was higher in all palmitate-perfused hearts during ischemia, probably due to the inhibition of glucose uptake and glycolysis, and pH was significantly lower in the glucose-perfused control rat hearts, but both heart groups had the same functional and pH recoveries during reperfusion. It may be that the proton load during low-flow ischemia was not damaging because of continued ATP production, as Cross et al. (9) showed. In the totally ischemic heart, the loss of ATP and the accumulation of protons would be considerably greater and more damaging. Yet Lewandowski and White (20) found no differences in pH recovery after zero-flow ischemia when pyruvate oxidation was stimulated with dichloroacetate, similar to our finding of the same pH changes in all hearts during early reperfusion. Consequently, our study provides no evidence for altered Na+/H+ exchange in the diabetic heart or in the presence of palmitate (15, 19, 22).

It is possible that differences in recovery after ischemia may have been caused, at least partially, by the increased lactate concentrations in the recirculating perfusion buffer, as lactate increases ischemic injury (9). The maximum lactate that could have been produced from glucose uptake and glycogenolysis was 232 μmol by the control hearts perfused with glucose alone and 124 μmol by the diabetic rat hearts. Because the recirculation buffer volume was 250 ml, the final lactate concentrations would have been 0.9 mM for the control and 0.5 mM for the diabetic rat hearts. Recent studies by Chatham and co-workers (5) showed that diabetes preferentially inhibits lactate oxidation relative to glucose oxidation. They also demonstrated that doubling the lactate concentration from 0.5 to 1.0 mM increased lactate oxidation by 50% in control rat hearts. However, in the present study, the control rat hearts had the same functional recoveries, despite a twofold difference in the calculated lactate concentrations and the diabetic rat hearts had different functional recoveries despite the same calculated lactate concentrations in the perfusion buffer, suggesting that any effects of lactate were too subtle to detect by using our experimental protocol.

In the control hearts, the recovery of contractile function in the presence of ketone bodies was significantly greater than in the presence of palmitate owing to the requirement for albumin in the palmitate buffer; albumin lowering the recovery of the RPP by 30% (P < 0.05; Table 2). It is possible that albumin increased damage during ischemia either due to leakage into the interstitial space, as a result of increased endothelial cell permeability after ischemia, or because there was preischemic shrinkage in response to increased oncotic pressure (2).

In conclusion, we have shown that palmitate was beneficial to the diabetic rat heart during low-flow ischemia, circumventing the detrimental effects of decreased glycolysis by maintaining ATP at control levels. We also found that palmitate was not detrimental to the normal heart during ischemia because, although inhibiting glucose uptake and glycolysis, the additional substrate was able to maintain the same amount of ATP as in the glucose-perfused hearts. Thus the high-serum fatty acid concentrations that occur with chronic insulin-dependent diabetes may partially compensate for decreased glucose uptake and PDH inhibition, thereby protecting the heart during low-flow ischemia,
bearing in mind that the STZ-injected rat is a model of uncontrolled diabetes. Whether this occurs in non-insulin-dependent diabetics, who make up 95% of diabetic population, remains to be determined.

The authors thank Dr. Barney Jones for help in setting up the free fatty acid perfusion protocol, Yvonne Anderson for help with the assays, and the Wellcome Trust and the British Heart Foundation for support.

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