Glucose and Palmitate Oxidation in Isolated Working Rat Hearts Reperfused After a Period of Transient Global Ischemia

Gary D. Lopaschuk, Marguerite A. Spafford, Norman J. Davies, and Stephen R. Wall

Alterations in energy substrate utilization during reperfusion of ischemic hearts can influence the functional recovery of the myocardium. Energy substrate preference by the reperfused myocardium, however, has received limited attention. Therefore, we measured oxidation rates of glucose and palmitate during reperfusion of ischemic hearts. Isolated working rat hearts were perfused with 1.2 mM palmitate and 11 mM [14C]glucose, 1.2 mM [14C]palmitate and 11 mM glucose, or 11 mM [14C]glucose alone, at an 11.5 mm Hg preload and 80 mm Hg afterload. Hearts were subjected to 60-minute aerobic perfusion or 25-minute global ischemia followed by 60-minute aerobic reperfusion. Steady-state oxidative rates of glucose or palmitate were determined by measuring 14CO2 production. In hearts perfused with glucose alone, oxidative rates during reperfusion were not significantly different than nonischemic hearts (1,008 ± 335 vs. 1,372 ± 117 nmol [14C]glucose oxidized/min/g dry wt, respectively). In the presence of palmitate, glucose oxidation was markedly reduced in reperfused and nonischemic hearts (81 ± 11 and 101 ± 15 nmol [14C]glucose oxidized/min/g dry wt, respectively). Palmitate oxidation rates were not significantly different in reperfused compared with nonischemic hearts (369 ± 55 and 455 ± 50 nmol [14C]palmitate oxidized/min/g dry wt, respectively). [14C]Palmitate was incorporated into myocardial triglycerides to a greater extent in reperfused ischemic hearts than in nonischemic hearts (26.0 and 13.8 µmol/g dry wt, respectively). Under the perfusion conditions used, palmitate provided over 90% of the ATP produced from exogenous substrates. Addition of the carnitine palmitoyltransferase I inhibitor, ethyl 2-[6-(4-chlorophenoxy)hexyl]-oxirane-2-carboxylate (Etomoxir, 10-6 M), during reperfusion stimulated glucose oxidation and improved mechanical recovery of ischemic hearts. These data demonstrate that, as under aerobic conditions, fatty acids are the preferred substrate of reperfused ischemic myocardium. However, increasing glucose oxidation during reperfusion can improve the functional recovery of the myocardium. (Circulation Research 1990;66:546–553)

Metabolic demand is an important determinant of the degree of mechanical recovery of the myocardium after a period of transient ischemia.1-4 Under aerobic conditions, fatty acids are the preferred energy substrate of the myocardium. During ischemia, however, fatty acid oxidation decreases, and a greater proportion of ATP production is derived from glucose.5,6 Energy substrate preference by the reperfused myocardium has not been well defined, and studies addressing this issue have yielded conflicting results. Positron emission tomography studies in dogs suggest a shift from predominant fatty acid utilization to predominant glucose utilization in hearts reperfused after a period of ischemia.7 However, measurement of fatty acid oxidation in swine and canine hearts reperfused after a period of ischemia suggests that fatty acid utilization is increased rather than decreased.8,9 In addition, measurement of glucose and palmitate extraction in reperfused ischemic dog hearts also indicates that fatty acids remain the preferred energy substrate of the heart.10 This contrasts with [13C]palmitate infusion studies in humans, which suggest that palmitate utilization decreases during reperfusion.11

A number of factors may explain these discrepancies. First, serum levels of fatty acids are an important determinant of myocardial substrate use5 and can increase dramatically after myocardial ischemia.12,13 Second, endogenous myocardial triglyceride and glyco-
gen stores may provide endogenous fatty acids and glucose, respectively, for oxidative metabolism.\textsuperscript{5,14} Perfusion of the myocardium with radiolabeled substrates may also result in incorporation of these exogenous substrates into endogenous pools. Other factors such as myocardial workload, hormonal influences, and neuronal input to the heart may also alter oxidative metabolism both directly and indirectly. Ideally, all of these variables should be controlled in a study of metabolic substrate use in reperfused myocardium.

In this study, we used the isolated perfused rat heart to control these variables and to quantitate myocardial energy substrate use by the heart after a period of transient global ischemia. We determined that rates of fatty acid and glucose oxidation in reperfused myocardium are similar to those in the preischemic aerobic myocardium. Furthermore, stimulation of glucose oxidation during reperfusion improved mechanical recovery. Combined with our previous work,\textsuperscript{15-17} our results suggest that depression of glucose oxidation rates in the presence of fatty acids may contribute to mechanical “stunning” in reperfused ischemic hearts.

Materials and Methods

Heart Perfusions

Male Wistar rats (200–250 g) were anesthetized with an injection of sodium pentobarbital (60 mg/kg i.p.). Hearts were subsequently removed, cannulated as Neely working hearts,\textsuperscript{18} and perfused with Krebs-Henseleit buffer, pH 7.4, gassed with 95% O\textsubscript{2}-5% CO\textsubscript{2}, containing 2.0 mM free calcium. Perfusate contained 11 mM \textsuperscript{14}C(U)glucose (500,000 dpm/ml perfusate), 11 mM \textsuperscript{14}C(U)glucose (500,000 dpm/ml) and 1.2 mM palmitate, or 11 mM glucose and 1.2 mM \textsuperscript{1-14}Cpalmitate (70,000 dpm/ml). All perfusate contained 3% bovine serum albumin previously dialyzed against 10 vol Krebs-Henseleit buffer. Palmitate, when used, was prebound to the albumin. Hearts were perfused with recirculating buffer (100 ml) at an 11.5 mm Hg preload and an 80 mm Hg afterload. The perfusion protocol involved either a 60-minute aerobic perfusion or 15-minute aerobic perfusion followed by a 25-minute period of transient no-flow ischemia and a 60-minute period of reperfusion. Etomoxir (ethy1 2-[6-(4-chlorophenyl)hexyl]oxirane-2-carboxylate) was a gift of Byk-Gulden Pharmazeutika, Konstanz, FRG. When used, it was added to the perfusate immediately before reperfusion of ischemic hearts. Etomoxir is a carnitine palmitoyltransferase I (CPT I) inhibitor in the same class of drugs as POCA (sodium 2-[5-(4-chlorophenyl)-penty1]oxirane-2-carboxylate). The product of heart rate and peak systolic pressure was used as an index of mechanical function. We have previously demonstrated that this product closely parallels cardiac output in this experimental model.\textsuperscript{16}

Oxidative rates were determined by quantitative measurement of \textsuperscript{14}CO\textsubscript{2} production by hearts perfused with either \textsuperscript{14}Cglucose or \textsuperscript{14}Cpalmitate. This procedure is used extensively in our laboratory and has been described previously in detail.\textsuperscript{15-19} Measurement of \textsuperscript{14}CO\textsubscript{2} production was determined during 60 minutes of perfusion in aerobic hearts or during the 60-minute period of reperfusion in reperfused ischemic hearts. Steady-state rates were measured between 20 and 60 minutes of perfusion in nonischemic hearts and between 20 and 60 minutes of reperfusion in ischemic hearts. Previous studies have determined that steady-state oxidative rates are achieved within 10 minutes of perfusion of nonischemic hearts with substrates labeled with \textsuperscript{14}C.\textsuperscript{18} As shown in Figures 1 and 2, steady-state oxidative rates were also achieved within 20 minutes of aerobic reperfusion of hearts subjected to a 25-minute period of transient ischemia.

Tissue Metabolite Determinations

At the end of the perfusions, hearts were freeze-clamped with Wollenberger clamps cooled to the temperature of liquid N\textsubscript{2}. Frozen ventricular tissue was weighed and powdered in a mortar and pestle cooled to the temperature of liquid N\textsubscript{2}. A portion of the powdered tissue was used to determine the dry-to-wet ratio. By use of this ratio, as well as the total frozen ventricular weight and dried atrial weight, the total dry weight of the heart was determined.

Extraction of long chain acylcarnitine and ATP was as described previously.\textsuperscript{18} These metabolites were extracted, with the aid of a mortar and pestle, from approximately 400 mg frozen tissue into 1.5 ml ice-cold perchloric acid (6% wt/vol) containing 15 mM dithiothreitol. The perchloric acid precipitate (containing long chain acylcarnitine) was separated from the acid-soluble intermediates as described previously.\textsuperscript{18} Long chain acylcarnitine was hydrolyzed to free carnitine and measured by a radiometric assay involving carnitine acetyltransferase and \textsuperscript{[3H]}acetyl coenzyme A.\textsuperscript{20}

Tissue lipids were extracted as described previously.\textsuperscript{18} Neutral lipids were separated from phospholipids by the method of Bowyer and King.\textsuperscript{21} Measurement of \textsuperscript{14}Cpalmitate incorporation into triglycerides was also determined as described previously.\textsuperscript{18,19} Myocardial triglyceride content was determined by measuring free fatty acid content of saponified triglycerides extracted from the heart.

Statistical Analysis

Data analysis was performed by a two-way analysis of variance, followed by a Newman-Keuls test or Student’s t test. Significance was set at p<0.05.

Results

Heart Function in Reperfused Ischemic Hearts

In this study, isolated working hearts were perfused with 1.2 mM palmitate and 11 mM glucose. These concentrations of fatty acids and glucose have been shown to occur in many patients after myocardial infarction.\textsuperscript{12,13} A parallel series of hearts was also
perfused with glucose as the sole carbon substrate, so that the effects of fatty acids on postischemic glucose oxidation could be determined. After the 25-minute period of transient ischemia, reperfusion of glucose-perfused hearts resulted in complete recovery of heart rate, peak systolic pressure development, and the product of heart rate and peak systolic pressure (Table 1). However, if 1.2 mM palmitate was present in the perfusate, recovery of mechanical function of reperfused hearts was significantly depressed, reflecting incomplete recovery of both heart rate and peak systolic pressure development. These results corroborate previous findings from our laboratory and others.\textsuperscript{15,17,22} As we have also previously demonstrated,\textsuperscript{15} addition of 10\textsuperscript{–6} M Etomoxir to the perfusate during reperfusion overcame the detrimental effects of fatty acids on mechanical function (Table 1). We have previously demonstrated that this beneficial effect occurs independent of changes in coronary flow and that recovery of coronary flow during reperfusion occurs in direct proportion to recovery of mechanical function.\textsuperscript{15}

### Oxidation of Glucose and Palmitate During Reperfusion of Hearts Subjected to a Period of Transient Ischemia

Glucose and palmitate oxidation rates were determined in hearts reperfused after the 25-minute period of global ischemia. Total \[^{14}\text{C}\]CO\textsubscript{2} production from \[^{14}\text{C}\]glucose was measured over the 60-minute period of reperfusion in hearts perfused in the absence or presence of 1.2 mM palmitate (Figure 1). Under both conditions, glucose or palmitate oxidative rates were not significantly different between 20 and 40 or 40 and 60 minutes of reperfusion. The presence of 1.2 mM palmitate in the perfusate resulted in a marked reduction in glucose oxidation rates. This effect is similar to the depressant effect of fatty acids on glucose oxidation in aerobic nonischemic hearts. Total \[^{14}\text{C}\]CO\textsubscript{2} production in hearts perfused with 1.2 mM \[^{14}\text{C}\]palmitate and 11 mM glucose is represented in Figure 2. Palmitate oxidation rates were higher than glucose oxidation rates at all points during reperfusion. Again, steady-state oxidative rates were not different between 20 and 40 or 40 and 60 minutes of reperfusion.

Rates of glucose and palmitate oxidation in reperfused ischemic hearts were compared with oxidation rates in nonischemic hearts. Steady-state rates (as expressed as nanomoles of \[^{14}\text{C}\]glucose or \[^{14}\text{C}\]palmitate oxidized per minute per gram dry weight) were determined between 20 and 60 minutes of reperfusion of ischemic hearts or between 20 and 60 minutes of perfusion of nonischemic hearts. As shown in Table 2, no significant difference in glucose oxidation was observed in reperfused ischemic hearts compared with nonischemic hearts. Palmitate had the same degree of depressant effect on glucose oxidation regardless of whether or not the myocardium was nonischemic or reperfused. Table 2 also shows the steady-state rates of palmitate oxidation during reperfusion of ischemic hearts. No difference in palmitate oxidative rates was seen between nonischemic and reperfused ischemic hearts.

ATP production derived from oxidation of exogenous glucose and palmitate in hearts perfused with both 11 mM glucose and 1.2 mM palmitate is shown in Table 3. ATP production from exogenous \[^{14}\text{C}\]glucose oxidation and \[^{14}\text{C}\]palmitate oxidation was calculated from the steady-state rates shown in Table 2. This calculation assumes that \[^{14}\text{C}\]palmitate is completely oxidized and processed through the tricarboxylic acid cycle. This assumption is probably valid, since we have previously demonstrated that under similar perfusion conditions \[^{14}\text{C}\]CO\textsubscript{2} production from \[^{14}\text{C(U)}\] palmitate and \[^{14}\text{C}\]palmitate are equivalent.\textsuperscript{15,18,19} In both nonischemic and reperfused ischemic hearts, the majority of ATP production was derived from palmitate. In nonischemic hearts, 93.8% of ATP was derived from palmitate and 6.2% from glucose, while...
**FIGURE 1.** Graph showing oxidation rates of 11 mM $[^{14}C]$glucose in the presence and absence of 1.2 mM palmitate in hearts reperfused after a 25-minute period of global ischemia. Hearts perfused at an 11.5 mm Hg preload and 80 mm Hg afterload were subjected to 15 minutes of aerobic perfusion followed by 25 minutes of global no-flow ischemia. Total $^{14}$CO$_2$ production from $[^{14}C]$glucose was determined between 0 and 60 minutes of reperfusion in hearts perfused in the absence (circles) or presence (triangles) of palmitate. Inset: Bar graph of oxidative rates expressed on a per minute basis. Values are the mean±SEM of at least six hearts in each group. *Significantly different from the absence of palmitate.

**FIGURE 2.** Graph showing oxidation rates of 1.2 mM $[^{14}C]$palmitate in hearts reperfused after a 25-minute period of global ischemia. Hearts perfused at an 11.5 mm Hg preload and 80 mm Hg afterload were subjected to 15 minutes of aerobic perfusion followed by 25 minutes of global ischemia. Total $^{14}$CO$_2$ production from $[^{14}C]$palmitate was determined between 0 and 60 minutes of reperfusion. Inset: Bar graph of oxidative rates expressed on a per minute basis. Values are the mean±SEM of at least six hearts in each group.
in reperfused ischemic hearts, 94.4% of ATP was derived from palmitate and 5.6% from glucose. Previous studies in our laboratory have suggested that elevated levels of fatty acid may contribute to myocardial stunning by inhibiting glucose oxidation.\textsuperscript{15,17} We also determined that the CPT 1 inhibitor Etomoxir could overcome the detrimental effect of fatty acids after ischemia. Therefore, we determined the effect of Etomoxir on glucose oxidation in reperfused hearts. Table 2 shows the effect of Etomoxir, added immediately before reperfusion, on steady-state and initial oxidation of glucose in transient ischemic hearts. In the absence of added palmitate, Etomoxir significantly increased the initial rate of glucose oxidation. Under steady-state conditions, however, this effect was not significant. In the presence of 1.2 mM palmitate, Etomoxir stimulated both initial and steady-state glucose oxidation rates. This observation supports our earlier work,\textsuperscript{17} which suggests that the beneficial effect of Etomoxir in the fatty acid–reperfused heart is a result of a stimulation of glucose oxidation.

The effect of Etomoxir on palmitate oxidation was also determined during reperfusion of ischemic hearts. Addition of Etomoxir resulted in an insignificant increase in both initial and steady-state palmitate oxidation in reperfused ischemic hearts (Table 2).

### Assumption of Fatty Acids in Myocardial Triglycerides During Reperfusion

Measurement of fatty acid oxidation using exogenous substrate labeled with \textsuperscript{14}C must allow for possible incorporation of the radiolabel into myocardial triglycerides. Similarly, endogenous triglycerides can also supply unlabeled fatty acids for oxidative metabolism. Therefore, myocardial triglyceride content and \textsuperscript{14}C-palmitate content of triglycerides were determined in hearts frozen at the end of the perfusion period. As shown in Table 3, total triglyceride content in fatty acid–perfused hearts was not significantly different between nonischemic hearts or ischemic hearts reperfused in the presence or absence of Etomoxir. Myocardial triglyceride levels in freshly excised nonperfused rat hearts range from 17 to 20 \textmu mol/g dry wt (equivalent to 54–60 \textmu mol free fatty acid as triglycerides per gram dry weight). In both nonischemic and reperfused ischemic hearts, perfu-

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#### Table 2. Glucose and Palmitate Oxidation in Nonischemic and Reperfused Ischemic Hearts

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Steady state oxidation</th>
<th>Cumulative oxidation during initial 20 min of reperfusion of ischemic heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose oxidation (nmol [\textsuperscript{14}C]glucose/ min/g dry wt)</td>
<td>Palmitate oxidation (nmol [\textsuperscript{14}C]palmitate/ min/g dry wt)</td>
</tr>
<tr>
<td>Nonischemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 mM glucose</td>
<td>1,372.5±177.4</td>
<td>...</td>
</tr>
<tr>
<td>11 mM glucose, 1.2 mM palmitate</td>
<td>101.1±14.5*</td>
<td>453.3±51.0</td>
</tr>
<tr>
<td>Ischemic reperfused</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 mM glucose</td>
<td>841.2±39.8</td>
<td>...</td>
</tr>
<tr>
<td>+ Etomoxir</td>
<td>1,431.9±400.5</td>
<td>...</td>
</tr>
<tr>
<td>11 mM glucose, 1.2 mM palmitate</td>
<td>80.8±15.0*</td>
<td>403±51.8</td>
</tr>
<tr>
<td>+ Etomoxir</td>
<td>127.2±8.8†</td>
<td>622.8±77.3</td>
</tr>
</tbody>
</table>

Hearts were perfused as described in “Materials and Methods” with 11 mM [\textsuperscript{14}C]glucose, 11 mM [\textsuperscript{14}C]glucose and 1.2 mM palmitate, or 11 mM glucose and 1.2 mM [\textsuperscript{14}C]palmitate. Etomoxir (10\textsuperscript{–6} M), when used, was added immediately before reperfusion. Values are mean±SEM of at least six hearts in each group. *Significantly different from the absence of palmitate. †Significantly different from the absence of Etomoxir.

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#### Table 3. Various Lipid Intermediates and Steady-State ATP Production From Exogenous Substrate in Hearts Perfused With Glucose and [\textsuperscript{14}C]Palmitate

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Triglyceride fatty acid content (\textmu mol free fatty acid/g dry wt)</th>
<th>[\textsuperscript{14}C]Palmitate incorporation into triglycerides (\textmu mol/g dry wt)</th>
<th>Long chain acylcarnitine (nmol/g dry wt)</th>
<th>Steady-state ATP production (\textmu mol/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>From glucose</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>63.5±8.7</td>
<td>13.8±1.6</td>
<td>1,581±264</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td>Ischemic reperfused</td>
<td></td>
<td></td>
<td></td>
<td>From glucose</td>
</tr>
<tr>
<td>No addition</td>
<td>59.7±5.4</td>
<td>26.0±2.3*</td>
<td>2,375±310</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>62.7±1.2</td>
<td>26.2±2.3*</td>
<td>2,061±336</td>
<td>4.8±0.3†</td>
</tr>
</tbody>
</table>

Hearts were perfused as described in “Materials and Methods” with 11 mM glucose and 1.2 mM [\textsuperscript{14}C]palmitate. Etomoxir (10\textsuperscript{–6} M), when used, was added immediately before reperfusion. Values are mean±SEM of at least six hearts in each group. *Significantly different from nonischemic hearts. †Significantly different from ischemic reperfused hearts perfused in the absence of Etomoxir.
sion in the presence of fatty acids did not alter total myocardial triglycerides.

Perfusion of hearts with [14C]palmitate results in a significant portion of the radiolabel being incorporated into myocardial triglycerides. After a 60-minute perfusion of nonischemic hearts, 21% of myocardial triglyceride content was comprised of [14C]palmitate (based on 3 mol fatty acid/mol triglyceride) (Table 3). In reperfused ischemic hearts, a significantly greater amount of [14C]palmitate was incorporated into triglycerides (44% of myocardial triglyceride fatty acids was now comprised of [14C]palmitate). It should be noted, however, that reperfused ischemic hearts were exposed to label for an extra 15 minutes compared with nonischemic hearts. It is unlikely, however, that this could account for the twofold increase in label incorporation into triglycerides. Addition of Etomoxir to reperfused hearts had no effect on [14C]palmitate incorporation into triglyceride.

We also measured long chain acylcarnitine levels in nonischemic and reperfused ischemic hearts (Table 3). Compared with fatty acid–perfused nonischemic hearts, levels of long chain acylcarnitine were slightly elevated in reperfused ischemic hearts. However, as we have previously demonstrated, at a concentration of 10−6 M Etomoxir, no significant decrease in long chain acylcarnitine occurred.15 This contrasts with lower doses of Etomoxir (10−9 M or 10−8 M) that cause a marked decrease in acylcarnitine levels.15,17 This lack of a decrease in acylcarnitines supports our earlier observations that the protective effect of Etomoxir is dissociated from alterations in long chain acylcarnitine levels.

Discussion

This study demonstrates that fatty acids remain the primary energy substrate oxidized by hearts reperfused after a period of transient global ischemia. No difference in either glucose or palmitate oxidation was observed in nonischemic or reperfused ischemic isolated working rat hearts. In both this experimental model and others, it has been demonstrated that myocardial fatty acid utilization decreases relative to glucose utilization during ischemia.5,6,23,24 However, our results suggest that the heart rapidly recovers the ability to oxidize palmitate if reperfused under aerobic conditions. In the presence of 1.2 mM palmitate and 11 mM glucose, we demonstrate that during reperfusion over 90% of ATP production from exogenous substrate was derived from palmitate oxidation. This high concentration of perfusate fatty acid was chosen since previous studies have demonstrated that circulating fatty acid levels can be markedly elevated in patients suffering a myocardial infarction. Circulating fatty acids can increase from 0.4 mM up to 1.2–2.0 mM, probably as a result of fasting and catecholamine stimulation of adipocyte tissue lipolysis.12,13,25–27

We also demonstrate that in the presence of high concentrations of fatty acids, glucose oxidation in reperfused myocardium is markedly depressed (Figure 1). This parallels what has previously been demonstrated in the nonischemic heart.5 Combined with our earlier work,19 the data suggest that this marked suppression of glucose oxidation may contribute to the “myocardial stunning” seen in hearts reperfused in the presence of palmitate. Furthermore, the beneficial effect of the CPT 1 inhibitor Etomoxir is correlated with its ability to stimulate glucose oxidation in the fatty acid–perfused heart (Tables 1 and 2 and Reference 15).

Another observation that can be made from these experiments is that exogenous fatty acids are incorporated to an increased extent into endogenous myocardial triglycerides in reperfused ischemic hearts. Reperfused hearts incorporated 26.0±2.3 μmol palmitate/g dry wt into triglycerides. This compared with a total of 13.8±1.6 μmol palmitate incorporated into triglycerides in nonischemic hearts. These results support what has been previously demonstrated by positron emission tomography. Schwaiger et al19 have demonstrated in dog hearts subjected to a 20-minute period of left anterior descending coronary artery occlusion that [14C]palmitate retention during the late phase of clearance was significantly elevated at 20 and 90 minutes of reperfusion. Retention of [14C]palmitate in this late phase is thought to correspond to incorporation of [14C]palmitate into endogenous lipid pools. Our data, which demonstrate high rates of radiolabel incorporation into triglycerides without an actual change in total myocardial triglycerides, may also explain some of the discrepancies obtained by studies that address substrate utilization in the reperfused heart. Investigations with positron emission tomography suggest that palmitate utilization is depressed in the reperfused ischemic myocardium. This is based on the observation that early clearance rates of fatty acid–imaging agents, such as [14C]palmitate, from reperfused myocardium are reduced to a degree not explained by flow or workload effects.7 An increased rate of incorporation of fatty acid into endogenous triglycerides could explain the delayed clearance of these imaging agents in reperfused hearts. Our data point to the need for further investigation of the relative importance of endogenous triglycerides as a source of fatty acids and for determination of factors that can alter triglyceride lipolysis in the heart.

The demonstration that fatty acids are the preferred substrate in reperfused ischemic rat hearts is consistent with other experimental studies measuring substrate oxidation or extraction by the reperfused myocardium. In the extracorporally perfused working pig heart, Liedtke et al8 observed a significant increase in fatty acid oxidation during reperfusion of ischemic hearts. This increase in fatty acid oxidation was also found by Mickle et al9 in the open-chested dog. Myears et al10 measured palmitate and glucose extraction in reperfused ischemic dog hearts and found that fatty acids remain the primary metabolic substrate of the heart, although in their studies,
myocardial utilization of palmitate was depressed compared with preischemic conditions. The differences reported between the relative proportions of fatty acids and glucose used by reperfused myocardium could be the result of any of a number of factors, including 1) the concentration of fatty acids to which the heart is exposed, 2) whether extraction or oxidation of fatty acids is being measured, 3) whether oxidative metabolism is being determined under steady-state conditions, 4) the workload of the heart, 5) the duration of ischemia, 6) the oxygen supply to the reperfused heart, and 7) the animal model being used. Regardless of these variables, and despite the quantitative differences in reported values for the proportion of fatty acid used, all of the studies described above indicate that fatty acids remain the primary substrate used by the reperfused ischemic heart.

Elevated fatty acid levels occur after an acute myocardial infarction and may contribute to the severity of ischemic injury.25–27 Postulated mechanisms for the detrimental effects of fatty acids include an increased requirement of oxygen for catabolism,3,28 an accumulation of potentially toxic intermediates of fatty acid metabolism such as long chain acylcarnitine and long chain acyl coenzyme A,23,29 or a fatty acid inhibition of myocardial glucose utilization.15 While long chain acylcarnitine and acyl coenzyme A accumulation may be important in the genesis of arrhythmias,30,31 recent studies in our laboratory15 and others32 suggest that it is not a causal factor in mediating the “mechanical stunning” that can occur in the fatty acid–perfused heart. We recently hypothesized that a marked inhibition of glucose oxidation by elevated circulating levels of fatty acids may contribute to depressed recovery of ischemic hearts.15,17 In this study, palmitate markedly depressed glucose oxidation during reperfusion. In the presence of glucose and fatty acid concentrations that are similar to serum levels seen clinically, glucose oxidation provided only 6% of total myocardial ATP production from exogenous substrates. This is significantly lower than the 30–40% of total ATP production from glucose in nonischemic hearts perfused with lower concentration of fatty acids.5,6 Theoretically, the low glycolytic flux in reperfused hearts exposed to high concentrations of fatty acid could contribute to the depressed reperfusion recovery since decreases in anaerobic glycolytic ATP production decrease the ratio of ATP produced/O2 consumed. In addition, it has been postulated that glycolytically produced ATP may be preferentially used by membrane ion pumps.33,34 Therefore, a decrease in glycolytic flux may result in a delayed reestablishment of transmembrane ion gradients during reperfusion, contributing to subsequent Ca2+ overload. We have yet to determine if the depressed recovery of fatty acid–perfused hearts is accompanied by an increase in intracellular Ca2+ accumulation.

CPT 1 inhibitors have been shown to protect the ischemic heart. Agents such as POCA,35 oxfenicine,36 2-tetraglycic acid,37 and Etomoxir15 all have a beneficial effect in the experimental setting of ischemia. This effect has been suggested to occur as a result of a lowering of myocardial long chain acylcarnitine levels.35,36 However, we have demonstrated that Etomoxir can improve recovery of fatty acid–perfused ischemic hearts without lowering long chain acylcarnitine levels.15 At low doses (10–9 M), Etomoxir markedly lowers long chain acylcarnitine levels in fatty acid–perfused hearts but does not stimulate glucose oxidation or protect the reperfused ischemic heart. This decrease is not accompanied by a decrease in palmitate oxidation, suggesting that CPT 1 inhibition can occur without a concomitant decrease in fatty acid oxidation. At a higher dose (10–6 M), Etomoxir lowers long chain acylcarnitine, stimulates glucose oxidation, and protects the reperfused ischemic heart.17 Stimulation of glucose oxidation by Etomoxir was also seen in the absence of added palmitate. This probably was a result of Etomoxir overcoming inhibition of glucose oxidation by endogenously supplied fatty acids. Myocardial triglycerides can serve as a source of fatty acids in hearts deprived of exogenous fatty acids.5 As demonstrated here, a very large dose of Etomoxir (10–6 M) no longer lowers long chain acylcarnitine levels but does stimulate glucose oxidation and does protect the reperfused ischemic heart. These results suggest that the beneficial effect of Etomoxir is due to a stimulation of glucose oxidation. The increase in glucose oxidation may occur as a result of Etomoxir partially overcoming fatty acid inhibition of pyruvate dehydrogenase, although this has yet to be determined. Stimulation of glucose oxidation may also explain the beneficial effect of CPT 1 inhibitors in other studies. To our knowledge, however, no other studies have described the effects of CPT 1 inhibition on glucose oxidation in the reperfused ischemic heart.

In summary, we demonstrate that fatty acids are the primary energy substrate oxidized by working rat hearts reperfused after a period of transient ischemia. In addition, low rates of glucose oxidation may contribute to the depressed recovery of function seen in hearts reperfused in the presence of high concentrations of fatty acids. CPT 1 inhibition appears to have a beneficial effect in the reperfused myocardium by stimulating glucose oxidation. Although the clinical importance of agents that modify fatty acid flux in humans remains unresolved, our data suggest that control of markedly elevated plasma fatty acid levels or stimulation of glucose oxidation in the reperfused ischemic myocardium may be of clinical benefit after myocardial ischemia or infarction in humans.

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