Postischemic recovery of heart metabolism and function: role of mitochondrial fatty acid transfer

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Montessuit, Christophe, Irène Papageorgiou, Isabelle Tardy-Cantalupi, Nathalie Rosenblatt-Velin, and René Lerch. Postischemic recovery of heart metabolism and function: role of mitochondrial fatty acid transfer. J Appl Physiol 89: 111–119, 2000.—Postischemic recovery of contractile function is better in hearts from fasted rats than in hearts from fed rats. In this study, we examined whether feeding-induced inhibition of palmitate oxidation at the level of carnitine palmitoyl transferase I is involved in the mechanism underlying impaired recovery of contractile function. Hearts isolated from fasted or fed rats were submitted to no-flow ischemia followed by reperfusion with buffer containing 8 mM glucose and either 0.4 mM palmitate or 0.8 mM octanoate. During reperfusion, oxidation of palmitate was higher after fasting than after feeding, whereas oxidation of octanoate was not influenced by the nutritional state. In the presence of palmitate, recovery of left ventricular developed pressure was better in hearts from fasted rats. Substitution of octanoate for palmitate during reperfusion enhanced recovery of left ventricular developed pressure in hearts from fed rats. However, the chain length of the fatty acid did not influence diastolic contracture. The results suggest that nutritional variation of mitochondrial fatty acid transfer may influence postischemic recovery of contractile function.

octanoate; palmitate; reperfusion; carnitine palmitoyltransferase I; nutritional state

THE METABOLIC SUBSTRATE used for energy production does not appreciably affect contractile performance in normal myocardium (23), but it may influence recovery of contractile function after a period of transient ischemia (11, 16, 23, 25). Specifically, a number of observations indicate that stimulation of glucose utilization results in improvement of contractile recovery (11, 16, 25). Conversely, inhibition of glycolysis is associated with aggravation of myocardial contracture and attenuation of pressure development early during reperfusion (7). Under normal physiological conditions, the nutritional state is a major determinant of the relative contribution of glucose and fatty acids to oxidative metabolism (17). Recent evidence suggests that nutritional modification of regulatory steps of glucose and fatty acid metabolism may influence the extent of myocardial injury caused by a period of transient ischemia (17, 21). In isolated rat hearts perfused with palmitate, glucose, and insulin, postischemic recovery of contractile function was improved and release of cytosolic enzymes reduced in hearts from fasted rats compared with hearts from fed rats (17). In hearts from fasted rats, palmitate oxidation was substantially higher during reperfusion than in hearts from fed rats. On the other hand, glucose oxidation was comparable in hearts from fasted and fed rats. Therefore, the possibility exists that inhibition of fatty acid oxidation during reperfusion may contribute to the impairment of contractile recovery observed in the fed state.

Decreased palmitate oxidation in perfused hearts from fed rats is associated with an increased myocardial content of malonyl-CoA during both baseline conditions and postischemic reperfusion (17). There is increasing evidence that malonyl-CoA plays an important role in the regulation of fatty acid oxidation (10). This metabolic intermediate, which is produced by carboxylation of acetyl-CoA, reduces oxidation of long-chain fatty acids by inhibition of carnitine palmitoyltransferase I (CPT I), a key regulatory enzyme of fatty acid oxidation (10, 19). Because pharmacological stimulation of the pyruvate dehydrogenase (PDH) reaction results in both enhanced myocardial content of acetyl-CoA and malonyl-CoA and decreased rate of fatty acid oxidation (19), it has been proposed that this pathway may mediate inhibition of fatty acid oxidation under conditions of carbohydrate utilization, as is the case after feeding. Recently, it has been shown that the activity of the enzyme that controls myocardial synthesis of malonyl-CoA, the acetyl-CoA carboxylase, is inhibited during postischemic reperfusion by AMP-dependent protein kinase-mediated phosphorylation, resulting in decreased myocardial malonyl-CoA content (9). This may explain the complete recovery of fatty acid oxidation in hearts from fasted rats despite postischemic stimulation of glucose oxidation (9). However, we have observed that, in hearts from fed rats, malonyl-CoA remained higher than in hearts from fasted rats (17), compatible with persistent inhibition of fatty acid oxidation at the level of CPT I after reperfusion in the fed state.
Therefore, the present study was designed to test the hypothesis that, during postischemic reperfusion, feeding-induced inhibition of CPT I limits 1) the rate of myocardial fatty acid oxidation during reperfusion and 2) the recovery of left ventricular pressure development. For this purpose, we determined in isolated rat hearts subjected to transient ischemia the effect of octanoate, which has access to β-oxidation independently of CPT I activity, on recovery of myocardial fatty acid oxidation and myocardial function.

MATERIALS AND METHODS

Animals

All procedures were conducted in accordance with institutional guidelines for animal experimentation. Experiments were performed in male OFA rats (IFFA CREDO, L’Arbresle, France) weighing 250–350 g. Rats had free access to tap water and were either fed a standard laboratory chow ad libitum or fasted for 48 h before experimentation.

Heart Perfusion

Rats were anesthetized with pentobarbital sodium (60 mg/kg ip; Nembutal, Abbott Laboratories, Chicago, IL). After thoracotomy, 1,000 IU heparin (Liquemin, Roche, Basel, Switzerland) were injected into the inferior vena cava, and the heart was quickly excised, placed in ice-cold saline, blotted, and weighed. The aorta was cannulated, and retrograde perfusion was initiated at a constant flow (10 ml min \(^{-1}\) g wet wt \(^{-1}\)) in a nonrecirculating system, including a roller pump (model IPS4, Ismatec, Glattbrugg, Switzerland). The initial perfusate was Krebs-Henseleit (KH) buffer containing (in mM) 118 NaCl, 4.0 KCl, 1.8 CaCl\(_2\), 1.4 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 25 NaHCO\(_3\), and 11 glucose. All perfusates were equilibrated with 95% O\(_2\)-5% CO\(_2\) and warmed to 37°C. The pulmonary artery was cannulated for collection of the coronary effluent. A fluid-filled latex balloon was inserted into the left ventricle via a left atriotomy and connected to a Statham P21XL pressure transducer ( Gould, Valley View, OH). At the beginning of perfusion with erythrocyte-containing medium, filling of the balloon was adjusted to give a left ventricular systolic pressure of 85 mmHg. The balloon volume was then kept constant throughout the experiment. Isovolumic left ventricular diastolic and systolic pressures were recorded continuously on a strip-chart recorder (model 2400S, Gould). Hearts were paced at 300 beats/min.

After surgical preparation, perfusion was changed to an erythrocyte-enriched KH medium (EE-KH) containing 8 mM glucose, insulin (70 mU/l; Actrapid, Novo Nordisk Pharma), and either 0.4 mM palmitate or 0.8 mM octanoate, bound to 0.4 mM albumin. Washed human erythrocytes were added to the perfusate to yield a hematocrit of 30% (4), allowing sufficient oxygen supply to the myocardium at a physiological flow rate of 2 ml min \(^{-1}\) g wet wt \(^{-1}\). EE-KH was passed through a 10-μm transfusion filter (MF10, Biotest Pharma, Dreieich, Germany) included in the perfusion system. At the end of the perfusion protocol, the heart was quickly frozen with an aluminum clamp precooled in liquid N\(_2\), powdered with a pestle in a mortar filled with liquid N\(_2\), and stored in liquid N\(_2\) for subsequent biochemical analysis.

Perfusion Protocols

Perfusion protocols are summarized in Fig. 1. The effect of substitution of 0.8 mM octanoate for 0.4 mM palmitate was assessed in hearts from either fed or fasted rats during both perfusion at a constant flow rate (nonischemic control perfusion) and during reperfusion after transient no-flow ischemia (postischemic reperfusion).

Nonischemic control perfusion. This group of hearts was perfused for 60 min at the control flow rate of 2 ml min \(^{-1}\) g wet wt \(^{-1}\) with EE-KH containing either 0.4 mM palmitate or 0.8 mM octanoate without intervention. Samples of the perfusate and the coronary effluent were withdrawn every 10 min. Radioactively labeled substrates for metabolic measurements (see Analytic Procedures) were present during the entire perfusion period.

Postischemic reperfusion. The hearts of this group were first equilibrated for 20 min with EE-KH containing unlabeled 0.4 mM palmitate. Thereafter, perfusion was stopped for 35 min. Immediately after perfusion was stopped, the coronary circulation was flushed with 3 ml of erythrocyte-free perfusate to prevent intracoronary aggregation of erythrocytes. During ischemia, the heated jacket surrounding the heart was filled with warmed KH to maintain myocardial temperature at 37°C. Hearts were then reperfused during 60 min at control flow rate (2 ml min \(^{-1}\) g wet wt \(^{-1}\)) with perfusate containing either 0.4 mM palmitate or 0.8 mM octanoate. Samples of the perfusate and of the coronary effluent were collected after 10 and 20 min of equilibration and after 5, 15, 30, 45, and 60 min of postischemic reperfusion.
Table 1. Left ventricular contractile function during control perfusion of hearts from fasted or fed rats

<table>
<thead>
<tr>
<th>LV Diastolic Pressure</th>
<th>LV Developed Pressure</th>
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<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Perfusion with 0.4 mM palmitate</td>
<td></td>
</tr>
<tr>
<td>Fasted (n = 8)</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Fed (n = 7)</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Perfusion with 0.8 mM octanoate</td>
<td></td>
</tr>
<tr>
<td>Fasted (n = 12)</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Fed (n = 12)</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg; n, no. of rats. NS, not significant.

Myocardial glycogen content was determined by the method of Keppeler and Decker (8).

Statistical Analysis

Results are presented as means ± SE. Data of myocardial metabolism and contractile function were analyzed by ANOVA for repeated measures. Cumulative release of creatine kinase and nonrepeated metabolic measurements were analyzed by factorial ANOVA, with nutritional state and chain length of fatty acid as factors. Statistical tests were conducted by using StatView II for Apple Macintosh software (Abacus Concepts, Berkeley, CA). Differences were considered significant at P < 0.05.

RESULTS

Left Ventricular Function During Control Perfusion

Left ventricular diastolic pressure and developed pressure during continuous perfusion without intervention were not influenced by the nutritional state and substitution of octanoate (0.8 mM) for palmitate (0.4 mM) (Table 1).

Effect of Substitution of 0.8 mM Octanoate for 0.4 mM Palmitate on Myocardial Metabolism During Control Perfusion

As observed previously (17), oxidation of [1-14C]-palmitate (Fig. 2) was markedly reduced in hearts from fed rats compared with hearts from fasted rats (50 ± 9 vs. 99 ± 5 nmol · min⁻¹ · g wet wt⁻¹, respectively, after 45 min; n = 3 for both groups; P < 0.01). Oxidation of [1-14C]octanoate was considerably higher than oxidation of [1-14C]palmitate and did not differ between hearts from fasted and fed rats (274 ± 37 and 274 ± 23 nmol · min⁻¹ · g wet wt⁻¹; n = 6–7).

Myocardial oxidation of [1-14C]glucose (Fig. 3) was significantly higher in palmitate-perfused hearts from fed rats (32 ± 4 nmol · min⁻¹ · g wet wt⁻¹ after 30 min; n = 3) than in the corresponding group of hearts from fasted rats (21 ± 2 nmol · min⁻¹ · g wet wt⁻¹; n = 4; P < 0.05). Substitution of octanoate (0.8 mM) in the perfusate for palmitate (0.4 mM) resulted in a marked increase in myocardial oxidation of [1-14C]glucose. Oxidation of 1-ml samples was injected into sealed flasks containing 1 mol of 2 M NaOH. CO₂ was released by addition of 3 mol of 1 M H₂SO₄ and trapped on small pieces of resin and eluted with 0.8 ml H₂O into scintillation vials.

Analytic Procedures

Hemoglobin content and oxygen saturation of hemoglobin in the perfusate and coronary effluent were measured with an oximeter (model IL-282, Instrumentation Laboratory, Lexington, KY). Po₂ and pH were measured with a blood-gas analyzer (model IL-1304, Instrumentation Laboratory). Myocardial oxygen consumption was calculated by multiplying the perfusate-coronary effluent difference of total oxygen content (hemoglobin bound and dissolved) with myocardial blood flow (4).

Results of myocardial glycogen content were analyzed by factorial ANOVA, with nutritional state and chain length of fatty acid as factors. Statistical tests were conducted by using StatView II for Apple Macintosh software (Abacus Concepts, Berkeley, CA). Differences were considered significant at P < 0.05.

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reduction of glucose oxidation in hearts from both fed rats (to 5.0 ± 1.6 nmol · min⁻¹ · g wet wt⁻¹; n = 5; P < 0.001 vs. corresponding group of hearts perfused with palmitate) and fasted rats (to 4.2 ± 0.7 nmol · min⁻¹ · g wet wt⁻¹; n = 5; P < 0.001).

Myocardial glycolytic flux was estimated on the basis of the release of [³H]H₂O from [⁵-³H]glucose. Glycolytic flux was slightly, but not significantly, lower in hearts from fasted rats compared with hearts from fed rats, in the presence of both palmitate and octanoate (Table 2). Substitution of 0.8 mM octanoate for 0.4 mM palmitate in the perfusate resulted in a moderate reduction of glycolytic flux in hearts from both fasted and fed rats, which was significant by factorial ANOVA.

Neither nutritional state nor composition of the perfusate influenced myocardial glycogen content after 60 min of control perfusion (Table 3).

**Effect of Substitution of 0.8 mM Octanoate for 0.4 mM Palmitate on Myocardial Contractile Function, Oxygen Consumption, and Enzyme Release During Postischemic Reperfusion**

**Left ventricular diastolic and developed pressure.** The time course of left ventricular diastolic pressure during baseline, ischemia, and reperfusion is depicted in Fig. 4. Hearts from both fed and fasted rats developed marked diastolic contracture during no-flow ischemia. Maximal diastolic pressure during ischemia did not differ between hearts from fasted and fed rats (48 ± 3 and 44 ± 2 mmHg, respectively), but time to maximal contracture during ischemia was slightly shorter in hearts from fed rats (26 ± 1 min; n = 20) than in hearts from fasted rats (29 ± 0.3 min; n = 22; P < 0.001).

During postischemic reperfusion with medium containing 0.4 mM palmitate, left ventricular diastolic pressure was markedly higher in hearts from fed rats (93 ± 11 mmHg after 60 min; n = 10) than in hearts from fasted rats (43 ± 11 mmHg; n = 11; P < 0.005). Substitution of octanoate (0.8 mM) for palmitate (0.4 mM) at the moment of reperfusion did not significantly modify diastolic contracture in hearts from both fed (80 ± 12; n = 11) and fasted (40 ± 8; n = 11) rats.

Figure 5 (top) displays left ventricular developed pressure before, during, and after ischemia. Postischemic recovery of left ventricular developed pressure (difference between systolic and diastolic pressure) in hearts reperfused with buffer containing 0.4 mM palmitate was significantly better if the rats were fasted before isolation of the heart (70 ± 9% of the preischemic value after 45 min) than if free access to food was allowed (17 ± 5% of the preischemic value; P < 0.001). Substitution of octanoate (0.8 mM) for palmitate (0.4 mM) in the perfusion medium during reperfusion significantly improved postischemic recovery of left ventricular developed pressure in hearts from fed rats to reach 48 ± 7% of the preischemic value. However, substitution of octanoate for palmitate did not further improve recovery of left ventricular developed pressure in hearts from fasted rats (left ventricular developed pressure 71 ± 11% of the preischemic value).

**Myocardial oxygen consumption and metabolic efficiency.** Despite incomplete recovery of contractile function, myocardial oxygen consumption recovered to preischemic values during reperfusion. Baseline (perfusion with palmitate) oxygen consumption was not different between hearts from fasted and fed rats (3.2 ± 0.1 vs. 3.0 ± 0.1 μmol·min⁻¹·g wet wt⁻¹, respectively). Fifteen minutes after reperfusion, oxygen consumption tended to be lower in hearts from fed rats (2.4 ± 0.2 μmol·min⁻¹·g wet wt⁻¹; P < 0.05 by paired t-test vs. baseline), probably reflecting increased myocyte death in this group. To obtain an index for the efficiency of oxidative metabolism in terms of contractile performance, the ratio of the rate-pressure product to oxygen consumption was calculated (Fig. 5, bottom). Efficiency was markedly reduced during postischemic reperfusion in each group. However, in palmitate-reperfused hearts, efficiency was significantly lower in hearts from fed rats compared with that in hearts from fasted rats. Substitution of octanoate for palmitate during reperfusion completely abolished the unfavorable effect of feeding on postischemic efficiency of oxidative metabolism.

**Myocardial release of creatine kinase.** During reperfusion with palmitate-containing medium, cumulative

![Graph](http://jap.physiology.org/)
myocardial release of creatine kinase into the coronary effluent was higher in hearts from fed rats than in hearts from fasted rats (Table 4). Substitution of octanoate for palmitate in the reperfusion medium did not influence myocardial release of creatine kinase.

Effect of Substitution of 0.8 mM Octanoate for 0.4 mM Palmitate on Myocardial Substrate Metabolism During Postischemic Reperfusion

Oxidation of fatty acids. Myocardial oxidation of palmitate was significantly lower in hearts from fed rats than in hearts from fasted rats (Fig. 6, top). When compared with the corresponding values during control perfusion, palmitate oxidation was not significantly different during postischemic reperfusion in hearts from both fasted (103 ± 14 and 83 ± 11 nmol·min⁻¹·g wet wt⁻¹, respectively, during control perfusion and after 30 min of reperfusion) and fed rats (55 ± 7 and 55 ± 6 μmol·min⁻¹·g wet wt⁻¹). Substitution of octanoate for palmitate (0.4 mM) or octanoate (0.8 mM) (Fig. 7). Consistent with previous observations (17) in rat hearts perfused with buffer containing 0.4 mM palmitate, myocardial oxidation of glucose was considerably higher during postischemic reperfusion (125 ± 10 μmol·min⁻¹·g wet wt⁻¹ after 15 min of reperfusion) than during control perfusion (32 ± 4 μmol·min⁻¹·g wet wt⁻¹).

Oxidation of glucose and glycolytic flux. Because glucose oxidation and glycolytic rate may influence postischemic recovery of contractile function, glucose metabolism was investigated in the groups that exhibited improvement of functional recovery with octanoate, i.e., hearts from fed rats. Glucose oxidation and glycolytic flux rate were determined in postischemic hearts from fed rats reperfused with either palmitate (0.4 mM) or octanoate (0.8 mM) (Fig. 7). Consistent with previous observations (17) in rat hearts perfused with buffer containing 0.4 mM palmitate, myocardial oxidation of glucose was considerably higher during postischemic reperfusion (113 ± 12 μmol·min⁻¹·g wet wt⁻¹ after 15 min of reperfusion vs. 5.0 ± 1.6 μmol·min⁻¹·g wet wt⁻¹ during control perfusion).

In hearts reperfused with 0.4 mM palmitate, myocardial glycolytic flux was higher 15 min after the onset of reperfusion (671 ± 109 μmol·min⁻¹·g wet wt⁻¹) compared with control perfusion (396 ± 45 μmol·min⁻¹·g wet wt⁻¹). Substitution of octanoate for palmitate did not significantly alter glycolytic flux during postischemic reperfusion (569 ± 70 μmol·min⁻¹·g wet wt⁻¹).

Determination of myocardial glycogen content at the end of the reperfusion period (Table 3) indicated that

### Table 3. Myocardial glycogen content after control perfusion and postischemic reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Perfusion With 0.4 mM Palmitate</th>
<th>Perfusion With 0.8 mM Octanoate</th>
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<tbody>
<tr>
<td></td>
<td>Fasted</td>
<td>Fed</td>
</tr>
<tr>
<td>60-min Control perfusion</td>
<td>19.2 ± 2.1 (n = 8)</td>
<td>24.0 ± 1.6 (n = 9)</td>
</tr>
<tr>
<td>60-min Reperfusion</td>
<td>4.6 ± 1.0 (n = 11)</td>
<td>4.7 ± 0.8 (n = 10)</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol glucose equivalent/g wet wt; n, no. of rats. Hearts were perfused with medium containing 8 mM glucose, 70 mU/l insulin, and either 0.4 mM palmitate or 0.8 mM octanoate.
global glycogen utilization during reperfusion was not different between groups, assuming that the glycogen levels at the beginning of reperfusion were not different between hearts from fasted and fed rats (17).

**DISCUSSION**

We have previously observed in isolated rat hearts perfused with medium containing 8 mM glucose and 0.4 mM palmitate that recovery of the myocardium after transient ischemia is lower in hearts from rats with free access to food than in hearts from fasted rats (17). This study was undertaken to determine whether feeding-induced inhibition of long-chain fatty acid oxidation is involved in the mechanisms underlying enhanced postischemic injury in hearts from fed animals. For this purpose, the effect of substitution of octanoate for palmitate on recovery of metabolism and contractile function was studied. Octanoate is a medium-chain fatty acid that crosses the mitochondrial membrane independently of CPT I and, therefore, bypasses the regulatory step most likely responsible for nutritional variations of long-chain fatty acid oxidation in the myocardium (17).

The results of this study indicate that substitution of octanoate for palmitate elicits 1) abolition of nutritional variations of fatty acid oxidation during both control conditions and postischemic reperfusion, 2) inhibition of glucose oxidation in normal myocardium but not in postischemic myocardium, and 3) improvement of recovery of left ventricular pressure development after ischemia in hearts from fed rats. The findings provide circumstantial evidence for a role of CPT I in the nutritional state-related differences in postischemic recovery of contractile function.

**Table 4. Cumulative myocardial release of creatine kinase during postischemic reperfusion of hearts from fasted or fed rats**

<table>
<thead>
<tr>
<th>Reperfusion With</th>
<th>Reperfusion With</th>
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<tr>
<td></td>
<td>0.4 mM Palmitate</td>
</tr>
<tr>
<td>Fed</td>
<td>73 ± 13</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Fasted</td>
<td>35 ± 5*</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 10)</td>
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</tbody>
</table>

Values are means ± SE given in U/g wet wt. Hearts were reperfused with medium containing 8 mM glucose, 70 mU/l insulin, and either 0.4 mM palmitate or 0.8 mM octanoate. *P < 0.01 vs. fed.

**Glucose and Fatty Acid Metabolism in Hearts From Fasted and Fed Rats During Control Perfusion**

Consistent with previous observations (17), myocardial oxidation of palmitate during control perfusion was lower by 49% in hearts from fed rats than in hearts from fasted rats. Conversely, oxidation of glucose and glycolytic flux were increased by 52 and 17%, respectively. Because a previous study's myocardial malonyl-CoA content (17) was markedly higher in hearts from fed rats, we have proposed that malonyl-CoA-mediated inhibition of CPT I may be involved in feeding-induced reduction of palmitate oxidation. Consistent with this interpretation, we have recently observed that activity
of acetyl-CoA carboxylase is increased in hearts from fed rats (unpublished observations). The observation in the present study indicating that oxidation of octanoate is not influenced by the nutritional state of the rats before the heart was harvested further supports the concept that feeding inhibits oxidation of long-chain fatty acids at the level of CPT I.

Substitution of 0.8 mM octanoate for 0.4 mM palmitate resulted in, during nonischemic control perfusion, strong inhibition of myocardial glucose oxidation that was irrespective of the nutritional state of the rats. The most likely mechanism of inhibition of glucose oxidation is an increase of the ratio of acetyl-CoA to free CoA in the mitochondrial matrix, leading to inhibition of PDH activity. In fact, production of acetyl-CoA is greatly increased by substitution of 0.8 mM octanoate for 0.4 mM palmitate. If complete degradation of the fatty acid by β-oxidation is assumed, production of acetyl-CoA in hearts from fed rats is 150% higher in the presence of octanoate than in the presence of palmitate. Octanoate also tended to reduce glycolytic flux rate in hearts from both fasted and fed rats. Inhibition of glycolysis by substitution of octanoate for long-chain fatty acid has previously been observed in smooth muscle cells (2) and in hepatocytes (18). In hepatocytes, octanoate inhibited glycolysis at the level of the 6-phosphofructokinase reaction, possibly mediated by increased production of citrate and reduced cellular fructose 2,6-bisphosphate content (18).

Effect of Substitution of 0.8 mM Octanoate for 0.4 mM Palmitate During Reperfusion on Myocardial Glucose and Fatty Acid Metabolism

Feeding-induced inhibition of palmitate oxidation was also present during reperfusion after 35 min of no-flow ischemia, although the difference between hearts from fasted and fed rats was slightly less pronounced. Kudo et al. (9) have observed that postischemic reperfusion results in rapid inhibition of the acetyl-CoA carboxylase by phosphorylation, mediated by a AMP-dependent protein kinase, leading to a drop of myocardial malonyl-CoA content. Consistent with this observation, we have previously observed that myocardial content of malonyl-CoA was lower during postischemic reperfusion than during control perfusion (17). However, values were still twice as high in hearts from fed rats during reperfusion compared with hearts from fasted rats (17). Therefore, persistent malonyl-CoA-mediated inhibition of CPT I may be responsible for the lower palmitate oxidation rate observed during reperfusion in hearts from fed rats.
The concept of persistent feeding-induced inhibition of CPT I during reperfusion is further supported by the present study, demonstrating that oxidation of octanoate, which bypasses the CPT I reaction, was not different between hearts from fasted or fed rats during the first 30 min of reperfusion. Thereafter, oxidation of octanoate slowly decreased in hearts from fed rats, presumably reflecting more extensive myocyte death by irreversible injury than in hearts from fasted rats, as evidenced by higher release of creatine kinase. In contrast to almost complete recovery of palmitate oxidation, oxidation of octanoate remained considerably lower during postischemic reperfusion compared with nonischemic control perfusion in hearts from both fasted and fed rats. This suggests that β-oxidation rate is limited in postischemic myocardium independently of CPT I. Reduction of β-oxidation capacity during reperfusion may remain unnoticed during oxidation of long-chain fatty acid, which is controlled proximally at the level of CPT I. At least two explanations may be considered. First, enzymes of intramitochondrial fatty acid oxidation may be damaged by ischemia and/or reperfusion. Second, increased glucose oxidation during reperfusion could directly inhibit fatty acid oxidation within the mitochondrial matrix. Recently, evidence has been provided suggesting that acetyl-CoA generated from glucose utilization may reduce β-oxidation of both long-chain (1, 19) and medium-chain fatty acids (1) in cardiac myocytes, possibly by inhibition of 3-ketoacyl-CoA thiolase.

The inhibition of glucose oxidation by substitution of 0.8 mM octanoate for 0.4 mM palmitate, observed during control perfusion, was completely abolished during postischemic reperfusion. This finding corroborates previous observations from our laboratory indicating that the inhibitory effect of fatty acid oxidation on glucose oxidation is attenuated during reperfusion of severely injured myocardium (23).

Because increased glucose oxidation was reduced by ruthenium red in a previous study (3), we have hypothesized that postischemic activation of glucose oxidation in severely injured myocardium is related to increased mitochondrial calcium content during reperfusion, leading to activation of the PDH complex (15). This may explain why inhibition of myocardial glucose oxidation by fatty acids oxidation is maintained during reperfusion after shorter periods of ischemia, presumably leading to either no or less increase of mitochondrial calcium content (12).

Influence of Substitution of 0.8 mM Octanoate for 0.4 mM Palmitate on Postischemic Recovery of Contractile Function

Results from our laboratory (17) and from other authors (20, 21) have shown that hearts from fed rats exhibit less recovery of contractile function during postischemic reperfusion than hearts from fasted rats. A major finding of the present study is that substitution of 0.8 mM octanoate for 0.4 mM palmitate during postischemic reperfusion markedly improves recovery of left ventricular pressure development in hearts from fed rats. This finding is consistent with the hypothesis that feeding-induced inhibition of CPT I may compromise postischemic recovery of function in hearts perfused with long-chain fatty acid alone. These observations are seemingly in contrast with observations by others indicating that fatty acid oxidation during postischemic reperfusion unfavorably influences contractile recovery by inhibition of glucose oxidation (13). However, as discussed above, in the present study, glucose oxidation was not inhibited during reperfusion by substitution of octanoate for palmitate, which is in contrast to control hearts without ischemic injury.

A number of previous reports support the view that myocardial fatty acid oxidation, concomitant with glucose oxidation, may be essential for recovery of myocardial contractile function after transient ischemia. Madden et al. (14) observed in isolated perfused rat hearts that addition of 2.4 mM hexanoate to the perfusate markedly improved postischemic recovery of contractile function compared with reperfusion with 1.8 mM palmitate alone. Furthermore, in a model of myocardial stunning in conscious dogs, intravenous administration of fatty acids during reperfusion improved functional recovery, but the beneficial effect was lost when oxefonic, an inhibitor of CPT I, was concomitantly administered (24).

The mechanism underlying the improvement of left ventricular pressure development during reperfusion by octanoate in severely injured hearts from fed rats is not known. At least two hypotheses, which are not mutually exclusive, may be considered: 1) supply of the tricarboxylic acids cycle with acetyl-CoA may be improved or 2) accumulation of potentially toxic fatty acid esters of carnitine and CoA may be reduced. Unfortunately, our results do not allow us to discriminate between these two hypothesis.

In contrast to recovery of left ventricular pressure development, aggravation of left ventricular diastolic contracture and release of creatine kinase in hearts from fed rats were not influenced by the substitution of octanoate for palmitate. This suggests that the subcellular mechanism responsible for increase of diastolic contracture and sarcolemmal disruption in hearts from fed rats is not directly related to inhibition of long-chain fatty acid oxidation. On the basis of studies using metabolic inhibition during reperfusion, it appears that the degree of postischemic contracture is more related to glycolytic flux (7) and glucose oxidation (23) early during reperfusion than to metabolism of fatty acids. In the present study, glucose oxidation, glycolytic rate, and myocardial glycogen content during reperfusion were not influenced by substitution of 0.8 mM octanoate for 0.4 mM palmitate.

Limitations

A number of limitations of this study need to be emphasized. First, 35 min of no-flow ischemia result in a mixture of reversibly and irreversibly injured myocar-
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