Substrate Competition in Postischemic Myocardium

Effect of Substrate Availability During Reperfusion on Metabolic and Contractile Recovery in Isolated Rat Hearts

Christian Tamm, Richard Benzi, Irène Papageorgiou, Isabelle Tardy, René Lerch

Abstract Normal myocardium can derive energy for contraction and relaxation from oxidative metabolism of a variety of substrates. This investigation examined the influence of substrate availability early during reperfusion on the substrate pattern of oxidative metabolism and recovery of contractile function. For this purpose, isovolumically beating isolated rat hearts, perfused retrogradely with erythrocyte-supplemented buffer containing 0.4 mmol/L palmitate and 11 mmol/L glucose, were subjected to 40 minutes of no-flow ischemia. Hearts were reperfused with medium containing selected concentrations of palmitate and glucose. The substrate pattern for oxidative metabolism was determined on the basis of myocardial release of [14C]CO2 after equilibration of the hearts during the initial 15 minutes of reperfusion with either [1-14C]palmitate or [U-14C]glucose. In continuously perfused control hearts, glucose oxidation was largely inhibited by palmitate. During postischemic reperfusion, oxidation of glucose was increased by 50% (P<.05) and 467% (P<.01) in hearts reperfused after the ischemic period with 11 mmol/L glucose plus 0.4 or 1.2 mmol/L palmitate, respectively. Oxidation of palmitate was concomitantly reduced during reperfusion at low (0.4 mmol/L) but not at high (1.2 mmol/L) palmitate concentration. Compared with hearts reperfused with medium containing 0.4 mmol/L palmitate as sole substrate, hearts reperfused with medium containing 11 mmol/L glucose with 0.4 mmol/L palmitate exhibited lower left ventricular diastolic pressure (69±5 versus 90±3 mm Hg [mean±SEM], P<.05), less release of creatine kinase (31±5 versus 59±7 U/g wet wt, P<.05), and better recovery of left ventricular pressure development (26±9 versus 6±4 mm Hg, P<.05). Omission of palmitate or increasing the palmitate concentration to 1.2 mmol/L did not significantly alter postischemic myocardial contracture and enzyme release. The findings support the view that glucose oxidation early during reperfusion may be crucial for functional recovery. The results further indicate that interaction of substrates of oxidative metabolism is altered in severely injured postischemic myocardium. Inhibition of glucose oxidation by fatty acids was partially reversed during reperfusion. (Circ Res. 1994;75:1103-1112.)

Key Words • fatty acids • glucose • metabolism • reperfusion • reperfusion injury

Normal myocardium can use a variety of metabolic substrates to derive energy for contraction and other cellular functions. In most instances, fatty acids account for the bulk of oxidative metabolism, primarily because oxidation of glucose is largely inhibited by physiologically circulating levels of fatty acids. Although substrate metabolism during postischemic reperfusion has been studied by using a variety of experimental models, the interaction between carbohydrate and fatty acid metabolism in postischemic myocardium has not been fully elucidated. Lopaschuk et al observed in isolated working rat hearts reperfused after 25 minutes of no-flow ischemia with medium containing 1.2 mmol/L palmitate and 11 mmol/L glucose that oxidation of both palmitate and glucose returned rapidly to preischemic values, indicating unaltered inhibition of glucose oxidation by fatty acids in postischemic myocardium. On the other hand, we have observed in rat hearts perfused retrogradely with medium containing 0.4 mmol/L palmitate and 11 mmol/L glucose that the contribution of glucose to oxidative metabolism was approximately doubled compared with control conditions early after the onset of reperfusion following 60 minutes of no-flow ischemia. Similarly, Myears et al have observed in dogs subjected to 60 minutes of coronary occlusion that the contribution of glucose to oxidative metabolism was increased from 12% to 25% during reperfusion with arterial blood, exhibiting an average plasma fatty acid concentration of 0.4 mmol/L. These latter results raise the possibility that inhibition of glucose oxidation by fatty acids may be attenuated in severely injured postischemic myocardium.

There is evidence to suggest that the substrate pattern of oxidative metabolism early during reperfusion may influence the severity of postischemic injury. Specifically, inhibition of glucose oxidation by fatty acids may adversely affect the recovery of reperfused myocardium, because pharmacological activation of the pyruvate dehydrogenase reaction during reperfusion by etomoxir, dichloroacetate, a high concentration of pyruvate, or the omission of fatty acids attenuated postischemic contractile dysfunction in isolated heart preparations. Conversely, inhibition of glycolysis reduced contractile recovery and aggravated contracture of postischemic myocardium.

Received December 27, 1993; accepted September 6, 1994.

From the Cardiology Center, University Hospital, Geneva, Switzerland.

Correspondence to René Lerch, MD, Cardiology Center, University Hospital, Geneva, Switzerland.

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In the present study, we sought to determine, in a model of advanced ischemic injury simulating evolving myocardial infarction, the effect of the perfusate concentration of palmitate and glucose during reperfusion (1) on the contribution of each substrate to oxidative metabolism and (2) on postischemic contracture, release of creatine kinase, and recovery of contractile function.

**Materials and Methods**

**Perfusion Technique**

Hearts of adult male ZUR:SIV rats (Tierpital Zürich, Zürich, Switzerland) were prepared and perfused as previously described. Briefly, animals weighing 250 to 350 g were fasted for 24 hours and anesthetized with diethyl ether. After intravenous injection of 1000 U heparin, the heart was rapidly excised and placed into ice-cold saline. The pulmonary and caval veins were ligated, and retrograde perfusion was initiated at constant flow in a nonrecirculating Langendorff system. Flow in the system was controlled by a calibrated roller pump (model IPS4, Ismatec SA). Perfusates were equilibrated with 95% O₂/5% CO₂ and warmed to 37°C. A thermoregulated jacket (37°C) was placed around the heart to minimize loss of temperature. A fluid-filled balloon (Hugo Sachs Elektronik) was advanced into the left ventricle via the left atrium and connected to a Statham P231D pressure transducer (Gould Inc) for continuous recording of isovolumic left ventricular pressure on a strip-chart recorder (type 2400S, Gould Instruments). The pulmonary artery was cannulated for collection of the coronary effluent. Heart rate was maintained at 280 beats per minute by atrial pacing.

**Perfusion Protocols**

During surgical preparation, hearts were perfused at a flow rate of 12 mL·min⁻¹·g wet wt⁻¹ with Krebs-Henseleit (KH) buffer containing (mmol/L) NaCl 118, KCl 4.0, CaCl₂ 2.5, KH₂PO₄ 1.4, MgSO₄ 1.2, and NaHCO₃ 25. Glucose (11 mmol/L) was added as metabolic substrate.

After completion of the preparation, the protocol consisted of three experimental phases: baseline, ischemia, and reperfusion.

**Baseline**

During the 20-minute baseline period, all hearts were perfused at a flow rate of 2 mL·min⁻¹·g wet wt⁻¹ with erythrocyte-enriched medium (EE-KH). The medium consisted of KH buffer (same composition as above) containing palmitate (0.4 mmol/L) bound to albumin (0.4 mmol/L), glucose (11 mmol/L), and insulin (70 mLU/L). Washed human erythrocytes were added to the perfusate at a hematocrit of 0.3 to ensure sufficient oxygenation at a physiological flow rate. The EE-KH was passed through a transfusion filter, included in the perfusion system, to remove microaggregates. To achieve comparable systolic loading conditions during the baseline period, filling of the left ventricular balloon was initially adjusted to provide a systolic pressure of 85 mm Hg and then kept constant throughout the experiment. Samples of the perfusate and the coronary effluent were taken 10 and 20 minutes after the onset of perfusion with EE-KH.

**Ischemia**

During the second phase, in hearts subjected to the ischemia-reperfusion protocol, perfusion was stopped for 40 minutes. Before clamping of the perfusion line, the coronary system was flushed with 2 mL erythrocyte-free standard medium containing 0.4 mmol/L palmitate plus 11 mmol/L glucose to avoid erythrocyte aggregation. During no-flow ischemia, the heated jacket was filled with warmed KH buffer to maintain temperature at 37°C.

In control hearts, without ischemia, perfusion with the standard medium (0.4 mmol/L palmitate plus 11 mmol/L glucose) was continued at a control flow rate (2 mL·min⁻¹·g wet wt⁻¹) during the 40-minute interval corresponding to the ischemic period.

**Reperfusion**

During the third phase, both control hearts and postischemic hearts were perfused for 45 minutes at the control flow rate (2 mL·min⁻¹·g wet wt⁻¹) with EE-KH medium containing one of the following substrate compositions: (1) 11 mmol/L glucose, (2) 0.4 mmol/L palmitate, (3) 0.4 mmol/L palmitate plus 11 mmol/L glucose, (4) 1.2 mmol/L palmitate, or (5) 1.2 mmol/L palmitate plus 11 mmol/L glucose. In all experiments, albumin (0.4 mmol/L) and insulin (70 mLU/L) were present in the perfusate. In experiments with glucose as the only substrate, the albumin was defatted by the method of Chen. The perfusion medium of this final phase was supplemented with trace amounts of either [1-¹⁴C]palmitate (40 μCi/L) or [U-¹⁴C]glucose (40 μCi/L) (Amersham Corp). Samples of the perfusate and the coronary effluent were taken 5, 10, 15, 30, and 45 minutes after the onset of the third phase.

At the end of the experiment, hearts were freeze-clamped and stored in liquid N₂ until analysis.

**Metabolic Measurements**

Myocardial oxygen consumption was calculated by multiplying the perfusate–coronary effluent difference of total (hemoglobin-bound and dissolved) oxygen content with myocardial blood flow. Oxidation of palmitate and of glucose (in nanomoles per minute per gram wet weight) was estimated by dividing myocardial release of CO₂-bound radioactivity (dpm·min⁻¹·g wet wt⁻¹) by the specific activity of the labeled substrate in the perfusate (in disintegrations per nanomole). Radioactivity released as ¹⁴CO₂ was determined as previously described. Myocardial release of lactate was calculated on the basis of the coronary venous-arterial concentration difference and myocardial blood flow. Lactate concentration was measured spectrophotometrically in perchloric acid–deproteinized samples of the perfusate and the coronary effluent.

For the determination of cumulative myocardial release of creatine kinase, the coronary effluent was collected during the initial 30 minutes of reperfusion, and aliquots were analyzed by a spectrophotometric assay.

For the determination of ATP and creatine phosphate, the frozen myocardium was homogenized in 0.6 mol/L ice-cold perchloric acid. ATP and creatine phosphate were determined in the neutralized supernatant by enzymatic assays.

**Statistical Analysis**

Values are expressed as mean±SEM. For parameters analyzed at one time point, one-way ANOVA was first performed to test for differences among mean values. If a difference was indicated, group means were compared by a modified t test, whereby probability values were corrected for multiple comparisons. Parameters followed over time, ANOVA for repeated measurements was first performed, which was followed (if a difference between groups was indicated) by unpaired t test with correction for multiple comparisons. Differences were considered significant at P<0.05. The relations between left ventricular diastolic pressure and metabolic parameters were assessed by linear regression analysis.

**Results**

**Effect of Substrate Composition During Postischemic Reperfusion on Recovery of Contractile Function, Enzyme Release, and Myocardial High-Energy Phosphate Content**

In the continuously perfused control hearts, isovolumic left ventricular diastolic pressure was low during the
entire experiment and was not influenced by modification of the substrate composition of the perfusate during reperfusion on left ventricular (LV) diastolic pressure. Small symbols joined by dashed lines indicate the values measured in continuously perfused control hearts. The left panel depicts the influence of 11 mmol/L glucose (glu) and 0.4 mmol/L palmitate (pal), alone or combined, during reperfusion on LV diastolic pressure. Diastolic pressure was lower when 11 mmol/L glu was present in the reperfusion medium. The right panel shows the corresponding time course for experiments with a concentration of pal of 1.2 mmol/L. The values for hearts perfused with 0.4 mmol/L pal plus 11 mmol/L glu are displayed for comparison. Diastolic pressure during reperfusion was virtually identical at high pal concentration, when compared with the corresponding values observed with medium containing 0.4 mmol/L pal. Values are mean±SEM. *P<.05 and **P<.01 vs hearts reperfused with 0.4 mmol/L pal (left panel) or 1.2 mmol/L pal (right panel) alone. Numbers in parentheses indicate the number of experiments.

Fig 1. Time courses showing the effect of substrate composition of the perfusate during reperfusion on left ventricular (LV) diastolic pressure. Small symbols joined by dashed lines indicate the values measured in continuously perfused control hearts. The left panel depicts the influence of 11 mmol/L glucose (glu) and 0.4 mmol/L palmitate (pal), alone or combined, during reperfusion on LV diastolic pressure. Diastolic pressure was lower when 11 mmol/L glu was present in the reperfusion medium. The right panel shows the corresponding time course for experiments with a concentration of pal of 1.2 mmol/L. The values for hearts perfused with 0.4 mmol/L pal plus 11 mmol/L glu are displayed for comparison. Diastolic pressure during reperfusion was virtually identical at high pal concentration, when compared with the corresponding values observed with medium containing 0.4 mmol/L pal. Values are mean±SEM. *P<.05 and **P<.01 vs hearts reperfused with 0.4 mmol/L pal (left panel) or 1.2 mmol/L pal (right panel) alone. Numbers in parentheses indicate the number of experiments.

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In continuously perfused control hearts, there was a gradual decrease of left ventricular pressure development during the final 45 minutes of the experiment (Fig 2). Left ventricular pressure development was somewhat higher in control hearts perfused with medium containing 1.2 mmol/L palmitate with or without glucose. Left ventricular pressure development was significantly reduced during postischemic reperfusion compared with the corresponding values measured in continuously perfused control hearts (P value of at least <.05 for each time point; not indicated in Fig 2). Recovery of left ventricular pressure development was particularly poor in hearts reperfused with medium containing palmitate alone at a concentration of 0.4 mmol/L (Fig 2, left panel) or at a concentration of 1.2 mmol/L (Fig 2, right panel), without a difference between the two groups. There was slight improvement of left ventricular pressure development for both palmitate concentrations when 11 mmol/L glucose was maintained in the reperfusion medium. However, the beneficial effect of glucose on left ventricular pressure development was somewhat less pronounced at the higher (1.2 mmol/L) compared with the lower (0.4 mmol/L) palmitate concentration (Fig 2, right panel).

Fig 3 depicts cumulative myocardial release of creatine kinase during the initial 30 minutes of reperfusion in postischemic hearts. Enzyme release was highest in the groups of hearts reperfused with 0.4 or 1.2 mmol/L palmitate as sole substrate. The reduction of enzyme release by the addition of 11 mmol/L glucose to the reperfusion medium was statistically significant (P<.05) for hearts reperfused in the presence of 0.4 mmol/L palmitate.

Table 1 summarizes myocardial content of ATP and creatine phosphate measured at the end of the experiment. Myocardial content of creatine phosphate was somewhat lower in control hearts perfused with 11 mmol/L glucose as sole substrate than in control hearts perfused with palmitate-containing medium, as reported previously. However, the variations of ATP content measured in continuously perfused control
hearts were statistically not significant. In postischemic hearts, myocardial contents of ATP and creatine phosphate were markedly reduced. In the hearts reperfused with medium containing palmitate, there was a tendency toward slightly, though significantly, higher myocardial ATP content, if 11 mmol/L glucose was present as cosubstrate.

**Effect of Substrate Composition During Reperfusion on Oxidative Substrate Metabolism and Release of Lactate**

Fig 4 depicts in the top panel the serial changes of myocardial oxygen consumption in hearts subjected to 40 minutes of no-flow ischemia. On reperfusion, there was rapid recovery of myocardial oxygen consumption, irrespective of the substrate composition of the perfusate, to values that were only slightly reduced compared with preischemic values (Fig 4, top panel). The bottom panel of Fig 4 displays myocardial oxygen consumption measured 15 minutes after the onset of reperfusion, together with the corresponding value measured in continuously perfused control hearts. In control hearts, the substrate composition of the perfusate did not significantly influence myocardial oxygen consumption. In postischemic hearts, recovery of myocardial oxygen consumption was higher in the group reperfused with medium containing 1.2 mmol/L palmitate plus 11 mmol/L glucose, with a reduction of mean value by only 10% compared with the value of control hearts (P=NS). Myocardial oxygen consumption did not significantly differ among the other groups of postischemic hearts.

Myocardial oxidation of palmitate, estimated on the basis of myocardial release of $^{14}$CO$_2$ from [1-$^{14}$C]palmitate, is shown in Fig 5. Values were measured after 15 minutes of equilibration with tracer-containing medium, when myocardial release of $^{14}$CO$_2$ had reached a plateau. There was only a small, statistically not significant, decrease of palmitate oxidation by the addition of 11 mmol/L glucose to control hearts perfused with medium containing 0.4 or 1.2 mmol/L palmitate.

On the other hand, increasing the palmitate concentration from 0.4 to 1.2 mmol/L increased oxidation of palmitate in control hearts perfused with both palmitate alone or with palmitate plus 11 mmol/L glucose by 21% (P<.05) and 36% (P<.05), respectively. In postischemic hearts reperfused with 0.4 mmol/L palmitate plus 11 mmol/L glucose, oxidation of palmitate was reduced by 50% (P<.001) 15 minutes after the onset of reperfusion compared with the corresponding value measured in control hearts. Recovery remained incomplete during
the 45-minute reperfusion period (data not shown). On the other hand, in hearts reperfused with 1.2 mmol/L palmitate plus 11 mmol/L glucose, recovery of palmitate oxidation was almost complete 15 minutes after the onset of reperfusion (P=NS versus control hearts).

Thus, in hearts reperfused with medium containing both palmitate and glucose, oxidation of palmitate was reduced early during reperfusion at low (0.4 mmol/L) but not at high (1.2 mmol/L) concentrations of palmitate. However, if glucose was withdrawn during reperfusion, recovery of fatty acid oxidation was incomplete in the presence of both low and high palmitate concentrations.

Myocardial oxidation of glucose, estimated on the basis of the release of 14CO2 from [U-14C]glucose, is shown in Fig 6. In continuously perfused control hearts, there existed a pronounced inverse relation between the palmitate concentration and glucose oxidation. Oxidation of glucose was reduced by 56% (P=NS) and 91% (P<.01) by the inclusion of 0.4 or 1.2 mmol/L palmitate, respectively, compared with the value measured in hearts perfused with medium containing 11 mmol/L glucose as sole substrate. During postischemic reperfusion, oxidation of glucose recovered completely in

<table>
<thead>
<tr>
<th>Substrate Composition of Perfusate</th>
<th>Control Hearts</th>
<th>Reperfused Hearts</th>
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<tbody>
<tr>
<td>Glucose (11 mmol/L)</td>
<td>5 15.6±2.2</td>
<td>6 5.5±0.4</td>
</tr>
<tr>
<td>Palmitate (0.4 mmol/L)</td>
<td>5 16.7±1.8</td>
<td>12 4.2±1.2</td>
</tr>
<tr>
<td>Palmitate (0.4 mmol/L)+glucose (11 mmol/L)</td>
<td>15 17.7±1.1</td>
<td>20 5.6±0.31</td>
</tr>
<tr>
<td>Palmitate (1.2 mmol/L)</td>
<td>5 14.9±0.8</td>
<td>7 4.6±0.4</td>
</tr>
<tr>
<td>Palmitate (1.2 mmol/L)+glucose (11 mmol/L)</td>
<td>11 18.0±0.6</td>
<td>10 6.1±0.65</td>
</tr>
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CP indicates creatine phosphate. Values are mean±SEM.

*P<.05 vs 11 mmol/L glucose; tP<.05 vs 0.4 mmol/L palmitate; §P<.05 vs 0.4 mmol/L palmitate+11 mmol/L glucose; and §§P<.05 vs 1.2 mmol/L palmitate.

Fig 4. Top, Line graph showing the time course of myocardial oxygen consumption in hearts subjected to 40 minutes of no-flow ischemia. Bottom, Bar graph showing values measured 15 minutes after the onset of reperfusion in hearts subjected to no-flow ischemia (filled bars) and the corresponding values measured in continuously perfused control hearts (open bars). Myocardial oxygen consumption was slightly to moderately reduced during reperfusion and was comparable among groups of hearts reperfused with medium containing 11 mmol/L glucose (glu), 0.4 mmol/L palmitate (pal), 0.4 mmol/L pal plus 11 mmol/L glu, or 1.2 mmol/L pal. Oxygen consumption was slightly higher in hearts reperfused with 1.2 mmol/L pal plus 11 mmol/L glu. Values are mean±SEM. *P<.05 vs 0.4 mmol/L pal; †P<.05 vs 1.2 mmol/L pal.

Fig 5. Bar graph showing the effect of substrate composition of the perfusate on myocardial oxidation of palmitate (pal), estimated on the basis of myocardial release of 14CO2 after 15-minute equilibration with [1-14C]pal. Oxidation of pal was higher in control hearts perfused with 1.2 mmol/L pal compared with the corresponding groups perfused with 0.4 mmol/L pal. During postischemic reperfusion, oxidation of pal was reduced in hearts reperfused with 0.4 mmol/L pal, 0.4 mmol/L pal plus 11 mmol/L glucose (glu), or 1.2 mmol/L pal. In contrast, oxidation of pal recovered completely in hearts reperfused with 1.2 mmol/L pal plus 11 mmol/L glu. Values are mean±SEM. **P<.01 vs 0.4 mmol/L pal; †P<.05 and ††P<.01 vs 0.4 mmol/L pal plus 11 mmol/L glu; and §§P<.01 vs 1.2 mmol/L pal.
hearts reperfused with 11 mmol/L glucose as sole substrate. In the groups reperfused with medium containing 0.4 or 1.2 mmol/L palmitate plus 11 mmol/L glucose, oxidation of glucose was significantly increased 15 minutes after the onset of reperfusion, compared with the corresponding values measured in control hearts, by 59% (P<.05) and 466% (P<.01), respectively. Subsequently, glucose oxidation decreased slowly but remained elevated compared with control hearts by 30% (P=NS) and 260% (P<.01), respectively, after 45 minutes of reperfusion (data not shown). Myocardial release of lactate measured 15 minutes after the onset of reperfusion was similar in postischemic hearts and in control hearts (Table 2).

Left ventricular diastolic pressure measured 15 minutes after the onset of reperfusion was inversely correlated with the rate of glucose oxidation (Fig 7). The corresponding relation with myocardial release of lactate was not significant.

**Discussion**

The main findings of the present study are that (1) the substrate interaction for oxidative metabolism is altered early after the onset of reperfusion in myocardium exhibiting advanced postischemic injury, with attenuation of the inhibitory effect of fatty acids on glucose oxidation, and (2) the supply of exogenous glucose early during reperfusion elicits a reduction of diastolic contracture and enzyme release.

**Influence of Substrate Availability on the Contribution of Palmitate and Glucose to Oxidative Metabolism During Reperfusion**

In normal myocardium, oxidation of glucose is inversely related to circulating levels of fatty acids. The inhibition of glucose oxidation by fatty acids is activitated by...
thought to be mediated at the level of the phosphofructokinase reaction by citrate\textsuperscript{23} and at the level of the pyruvate dehydrogenase reaction by a high ratio of acetyl coenzyme A to coenzyme A.\textsuperscript{24,25} Consistent with this concept, in the present study, addition of palmitate at a concentration of 0.4 or 1.2 mmol/L to the perfusate of continuously perfused control hearts inhibited glucose oxidation by 56% and 91%, respectively, compared with hearts perfused with medium containing 11 mmol/L glucose as sole substrate. The observed reduction of glucose oxidation by 0.4 and 1.2 mmol/L palmitate is comparable in extent to that observed by Saddik and Lopaschuk\textsuperscript{2} in working rat hearts perfused with erythrocyte-free buffer.

In control hearts, the substrate pattern did not significantly influence myocardial oxygen consumption. Because of the higher ratio between the rate of ATP production and oxygen consumption (P/O ratio) for glucose oxidation, a small decrease of oxygen consumption would be anticipated in hearts perfused with glucose as sole substrate. Such a behavior has been observed in several studies,\textsuperscript{26,27} but it is not a consistent finding.\textsuperscript{28,29} The absence of a detectable oxygen-sparing effect of fatty acid–free perfusion may be related to the fact that with glucose as sole exogenous substrate, an appreciable portion of oxidative metabolism is still attributable to the oxidation of fatty acids that originate from endogenous triglycerides.\textsuperscript{2}

In accordance with previous observations, reperfusion after 40 minutes of no-flow ischemia resulted in rapid resumption of oxidative metabolism despite persistent severe depression of contractile performance.\textsuperscript{5,12} Among groups reperfused with medium containing both palmitate and glucose, myocardial oxygen consumption increased with increasing palmitate concentration from 0.4 to 1.2 mmol/L despite comparable recovery of contractile function. The observation is compatible with lowering of the efficiency of oxidative metabolism in terms of contractile function during reperfusion in the presence of a high concentration of fatty acids, possibly by dissipation of the electrochemical potential at the inner mitochondrial membrane.\textsuperscript{28}

In the present study, inhibition of glucose oxidation by palmitate was partially reversed during postischemic reperfusion. In hearts reperfused with medium containing 11 mmol/L glucose plus 0.4 or 1.2 mmol/L palmitate, oxidation of glucose was significantly higher for both 0.4 and 1.2 mmol/L palmitate when compared with the values measured in the corresponding control group, with an increase of 59% and 466%, respectively.

The mechanisms responsible for the enhancement of glucose oxidation early during postischemic reperfusion are not apparent from the present study. At least three possibilities may be considered. First, myocyte calcium overload may lead to activation of glucose oxidation at the level of the pyruvate dehydrogenase reaction.\textsuperscript{29} The absence of concomitant stimulation of lactate release in the present study is compatible with activation of glucose oxidation at this level. Second, myocardial accumulation of adenosine during ischemia and reperfusion may lead to stimulation of glucose oxidation during reperfusion. Finegan et al\textsuperscript{10} have observed in isolated erythrocyte-free perfused rat hearts that during reperfusion after 60 minutes of low-flow ischemia, glucose oxidation was enhanced during exposure of the heart to 100 μmol/L adenosine. Third, oxidation of glucose may be stimulated by impairment of fatty acid oxidation during reperfusion.\textsuperscript{31}

Consistent with the last mentioned hypothesis, oxidation of palmitate was reduced in hearts reperfused with 0.4 mmol/L palmitate plus 11 mmol/L glucose or with palmitate (0.4 or 1.2 mmol/L) alone. The present study does not allow identification of the mechanism of reduction of fatty acid oxidation in these groups. Hearts reperfused with palmitate as sole substrate exhibited more severe myocardial injury, which complicates the interpretation of differences in metabolic measurements between groups reperfused with and without glucose (see methodological considerations). However, in hearts reperfused with medium containing 11 mmol/L glucose, which exhibited the most likely comparable degrees of irreversible injury, oxidation of palmitate recovered almost completely if palmitate concentration was increased to 1.2 mmol/L. This observation indicates that in hearts reperfused with both substrates, fatty acid oxidation is not limited by irreversible loss of capacity of the fatty acid oxidation pathway. Because activation of the pyruvate dehydrogenase reaction may inhibit carnitine palmitoyltransferase I by enhanced formation of malonyl coenzyme A,\textsuperscript{32} it is possible that if glucose is available, inhibition of fatty acid oxidation is the consequence, rather than the cause, of increased glucose oxidation.

The results of the present study agree with those reported by Myears et al,\textsuperscript{3} who observed in anesthetized dogs subjected to 60 minutes of coronary occlusion an increase of glucose oxidation associated with a decrease of fatty acid oxidation during reperfusion. On the other hand, Lopaschuk et al\textsuperscript{15} did not observe an increase of glucose oxidation during reperfusion after 25 minutes of no-flow ischemia in buffer-perfused isolated working rat hearts. The reasons for the variable behavior of glucose oxidation during reperfusion are not clear. A first possibility is that calcium-mediated activation of pyruvate dehydrogenase reaction did not occur in the study by Lopaschuk et al,\textsuperscript{5} because myocardial injury was less severe. A second possibility is that normalization of the metabolic environment is more rapid in hearts perfused with erythrocyte-free buffer because of the higher coronary flow rate required for adequate oxygen supply, potentially accelerating the washout of compounds, such as adenosine, that are possibly involved in the activation of glucose oxidation.\textsuperscript{30}

**Influence of Substrate Pattern During Reperfusion on Recovery of Contractile Function and Enzyme Release**

Several previous studies have demonstrated beneficial effects of glucose on contracture and enzyme release during low-flow ischemia.\textsuperscript{33-36} However, the possible importance of glucose availability early during reperfusion for functional recovery has only recently been recognized.\textsuperscript{5,7-9} In the present study, withdrawal of glucose at the moment of reperfusion in rat hearts reperfused with medium containing 0.4 or 1.2 mmol/L palmitate markedly aggravated diastolic contracture of the myocardium. The results are consistent with the findings of Jeremy et al,\textsuperscript{11} who observed in isolated
rabbit hearts subjected to 20 minutes of no-flow ischemia aggravation of diastolic contracture by inhibition of glucose utilization during early reperfusion by iodoacetate or deoxyglucose. Enhancement of diastolic contracture, in the present study, was associated with increased myocardial release of creatine kinase, achieving statistical significance for hearts reperfused with 0.4 mmol/L palmitate. Myocardial hypercontracture may directly contribute to sarcolemmal damage by mechanisms including mechanical disruption of the myocytes and perpetuation of subendocardial ischemia.

The mechanism of the protective effect of glucose utilization early during reperfusion is unknown. Although myocardial content of ATP tended to be slightly higher in hearts reperfused with medium containing 11 mmol/L glucose, there was no clear relation discernible between tissue content of ATP and creatine phosphate on one hand and recovery of contractile function and enzyme release on the other. Lack of correlation between recovery of high-energy phosphate compounds and myocardial injury has been attributed to the insensitivity of total myocardial content to reflect changes in either the rate of production of ATP or the content of ATP in a small compartment. It has been proposed that enhanced production of ATP in the cytosolic compartment by substrate-based phosphorylation in the glycolytic pathway, combined with removal of cytosolic inorganic phosphate by the glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglycerate kinase reaction, may prevent collapse of the cytosolic phosphorylation potential early during reperfusion. Enhancement of the cytosolic phosphorylation potential may support energy-dependent ion transport pumps at the sarcoplasmic reticulum and the sarcolemma and therefore facilitate restoration of ion homeostasis during reperfusion. In fact, there exists recent evidence indicating that glycolysis may favorably influence restoration of calcium homeostasis during reperfusion. Using the calcium indicator 5F-BAPTA and 31P nuclear magnetic resonance spectroscopy, Jeremy et al. observed in isolated rabbit hearts subjected to 20 minutes of no-flow ischemia that cytosolic calcium concentration early during reperfusion was increased five times when glycolysis was inhibited by iodoacetate.

It has been hypothesized that the protective effect of glucose may require activation of the oxidative pathway, accelerating both the clearance of protons and lactate by the pyruvate dehydrogenase reaction and the reenergization of mitochondria. Consistent with this hypothesis, in the present study left ventricular diastolic pressure during reperfusion was only poorly related to myocardial release of lactate but exhibited a significant inverse relation with glucose oxidation.

It has been suggested that the presence of a high concentration of circulating fatty acids early during reperfusion may offset the beneficial effect of glucose by inhibition of glucose oxidation at the level of the pyruvate dehydrogenase reaction. Consistent with this concept, Lopaschuk et al. observed in isolated working rat hearts perfused with medium containing 1.2 mmol/L palmitate and 11 mmol/L glucose that recovery of developed pressure after 25 minutes of no-flow ischemia was improved when palmitate was omitted in the reperfusion medium. Improvement of recovery of pressure development was also observed if palmitate was maintained in the reperfusion medium, but glucose oxidation was stimulated at the moment of reperfusion by activation of the pyruvate dehydrogenase reaction by etomoxir or dichloroacetate.

The results of the present study seemingly disagree with the observations by Lopaschuk et al. because the addition of palmitate at a concentration of 0.4 or 1.2 mmol/L did not significantly worsen diastolic contracture and enzyme release compared with hearts reperfused with medium containing 11 mmol/L glucose as sole substrate. The absence of detrimental effects of palmitate on postischemic hearts perfused with medium containing 11 mmol/L glucose may be related to the attenuation of the inhibitory effect of palmitate on glucose oxidation during reperfusion, as found in the present study. As discussed above, Lopaschuk et al. observed, in their model, that oxidation of glucose was inhibited during reperfusion to <10% of the value measured in hearts reperfused with glucose alone, when 1.2 mmol/L palmitate was present in the perfusate. Stimulation of glucose oxidation by only 50% to 25 mmol min⁻¹·g wet wt⁻¹ by etomoxir, a value that is still lower in absolute terms than glucose oxidation measured during reperfusion with palmitate-containing medium in the present study, elicited marked improvement of contractile recovery. Therefore, both studies agree in supporting the view that glucose oxidation early during reperfusion favorably influences postischemic recovery of the myocardium.

Methodological Considerations

A critique of the model has been provided previously. In contrast to earlier studies in our laboratory, in this investigation 14C-labeled substrates were added to the perfusate only during the reperfusion period to exclude distortion of estimates of oxidative metabolism during reperfusion by the contribution of 14CO₂ originating from labeled metabolites formed during ischemia.

A number of methodological limitations for the interpretation of results of the present study need to be emphasized. First, all postischemic hearts were severely and partially irreversibly injured. Although injury was identical among groups at the moment of reperfusion, it is possible that myocardial injury differed at the moment of metabolic measurements because of the variable speed of progression of irreversible injury during reperfusion, depending on the substrate composition of the reperfusion medium. Nevertheless, it is likely that injury was comparable in the three groups of hearts reperfused with glucose-containing medium, since diastolic contracture and release of creatine kinase were comparable. Second, observations do not allow the definitive distinction of whether the improvement of recovery of contractile function in hearts reperfused with glucose-containing medium is a reflection of the attenuation of reversible myocardial dysfunction or a limitation of the extent of irreversible injury. The increase of loss of creatine kinase in hearts exposed during reperfusion to palmitate alone suggests that more extensive irreversible injury contributed to reduced contractile recovery. Third, because washout of creatine kinase was not completed within the experimental period, differences in the rate of washout may
have contributed to differences in enzyme release among groups. However, because impairment of washout is likely to be most pronounced in hearts with the highest diastolic contracture, the increase of enzyme release during reperfusion without glucose could even be underestimated. Fourth, the influence of the selected experimental conditions including the nutritional state of the animals and the glucose concentration of 11 mmol/L requires further investigation.

**Implications**

The present study demonstrates that in severely injured postischemic myocardium the extent of recovery of oxidation of palmitate and glucose may be influenced by circulating substrate levels. This may contribute to the apparent variability among different studies of oxidative metabolism of fatty acids and glucose in postischemic myocardium.3-6 Furthermore, the results provide additional evidence for a pivotal role of glucose availability early during reperfusion for recovery of critically injured myocardium.8,11,44 The observed enhancement of glucose oxidation early during reperfusion, even in the presence of a high concentration of palmitate may, therefore, represent a protective response of the myocardium, potentially reducing irreversible injury.

**Acknowledgments**

This study was supported by Swiss National Science Foundation grant 32-26373.89 and the Swiss Foundation of Cardiology.

**References**


Substrate competition in postischemic myocardium. Effect of substrate availability during reperfusion on metabolic and contractile recovery in isolated rat hearts.

C Tamm, R Benzi, I Papageorgiou, I Tardy and R Lerch

Circ Res. 1994;75:1103-1112
doi: 10.1161/01.RES.75.6.1103

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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