Metabolic Oxidation of Glucose During Early Myocardial Reperfusion

Britta Renstrom, Stephen H. Nellis, and A. James Liedtke

We have previously studied the relation between long-chain fatty acid and pyruvate metabolism in reperfused myocardium and noted a rapid return of fatty acid oxidation to at least preischemic values accompanied by a marked decrease in pyruvate oxidation. The purpose of the present report is to further characterize carbohydrate metabolism during reflow by describing rates of glucose oxidation using [6-14C]glucose. Oxidative performance was determined with and without preserved fatty acid utilization; the latter condition was effected by oxfenicine, which inhibits palmitoylcarnitine transferase I. In the main protocol, two groups of working swine hearts (n=18) were perfused aerobically for 30 minutes, rendered regionally ischemic (−60 Δ%) in anterior descending coronary flow) for 45 minutes, and reperfused at control flows for a final 50 minutes of perfusion. An emulsion of Intralipid with heparin was administered systemically throughout the studies to augment serum fatty acids (average fatty acid values, 1.05±0.05 μmol/ml for both groups). Serum glucose was monitored and maintained at or about 100 mg/dl with additional infusions of glucose as needed. Oxfenicine (33 mg/kg) was administered systemically by bolus injection at time 0 and 60 minutes of perfusion in nine animals. Decreased mechanical performance, that is, stunning, during reflow was evident in both groups (−50 Δ% in regional systolic shortening, p<0.05 compared with aerobic values in the control group, and −32 Δ%, p<0.05 compared with aerobic values in treated hearts). This stunning was associated with concordant reductions in myocardial oxygen consumption during recovery (−15 Δ%, p<0.02 for the control group, and −21 Δ%, p<0.01 for the treated group). 14CO2 production from labeled glucose was strongly suppressed during preischemic perfusion in both groups, rose slightly during ischemia, and continued to rise in the oxfenicine group during reperfusion to twice the values measured in control hearts (p<0.01). These responses were contrasted with data from five additional, similarly perfused hearts that did not receive Intralipid. Reducing fatty acids twofold in the perfusate caused no major changes in glucose oxidation as compared with control hearts. Tissue glycogen was detected in both aerobic and reperfused myocardium and was unaffected by oxfenicine treatment. These data confirm previous findings and do not support an argument for increased glucose oxidation. Rather, the results support the concept of competitive inhibition of glucose and/or its intermediates by the preferred use of fatty acids. (Circulation Research 1989;65:1094–1101)

We recently characterized long-chain fatty acid and pyruvate metabolism using 14C-labeled isotopes in reperfused myocardium after moderately severe ischemia.1,2 In contrast to the time activity and scintigraphic data of positron emission tomography, which would predict a reversal of preferred substrate utilization during reflow,3 we observed the opposite. That is, through the first hour of recovery there was a rapid return of fatty acid oxidation to or above aerobic values. This was associated in separate studies with a marked decrease of pyruvate oxidation compatible with the normal dynamics of competitive substrate suppression in the presence of the more preferred fatty acids.2 However, possible concerns in these results are that the kinetics of pyruvate are not entirely representative of the generic carbohydrate pathway, that pyruvate is not subject to the same allosteric controls as glucose, or that our results of pyruvate inhibition are not explained by fatty acids but rather by rapid flux changes in endogenous glycolysis.4,5 Thus, to further characterize intermediate metabolism in reperfused myocardium, the following experiments were performed using [6-14C]glucose. Our hypothesis remains that
the preferred use of fatty acids inhibits the need for energy production derived from glucose oxidation. In the main study, the level of glucose oxidation in reperfused hearts was contrasted with that in a separate group of reperfused hearts treated with oxfenicine. This compound was given to curtail fatty acid utilization by inhibiting a key enzyme in the pathway, palmitoylcarnitine transferase. 6-8 Excess fatty acids were made available to both groups of hearts. For comparison, glucose oxidation was also characterized in a separate study in hearts not receiving excess fatty acids.

Materials and Methods

Twenty-three adolescent swine with an average weight of 49.7±0.7 kg were studied. In the main protocol, 18 animals treated with excess fatty acids were divided into two groups (control versus oxfenicine-treated). Nine animals in each group were studied for mechanical performance, and eight were treated with excess fatty acids (oxfenicine-treated). Nine animals in each group received no oxfenicine. To maintain serum fatty acid levels sufficient to compare with previous data, 1 all animals in this study also received excess free fatty acids administered as a constant infusion of 20% Intralipid (0.8 mg/kg/min, Kabi Vitrum, Alameda, California) with heparin (20,000 units initially, 20,000 units in the second hour followed by 10,000 units/hr thereafter) beginning at 0 minutes of perfusion. This was in contrast to the five animals of the separate study that received no Intralipid. The heparin schedule in this group was 20,000 units initially and 10,000 units/hr thereafter.

Indocyanine green indicator was administered (0.33 mg/ml) in accordance with earlier studies to estimate venous cross contamination and dilution in the LAD circulation. 2,10 Samples of arterial and venous blood from the LAD circuit were collected at 20, 60, and 105 minutes of perfusion to measure any unlabeled coronary blood entering the venous system of the LAD circuit. The mean dilution factors (Ks) obtained in this study were 0.91, 0.78, and 0.90 for the aerobic, ischemic, and reperfusion periods, respectively. This K value was used to correct the raw counts used in the calculation for 14CO2 production (μmol/gm dry wt/hr) from labeled glucose according to the formula:

\[
\text{14CO2 production (μmol/gm dry wt/hr)} = \frac{[A \times B] \times Q_{lad} \times 60}{[K \times ASA \times LAD dry wt]}
\]

where \(A\) is the corrected difference between venous and arterial 14CO2 (dpm/ml); \(Q_{lad}\) is the flow in the LAD bed (ml/min); and \(ASA\) (arterial specific activity of glucose in dpm/μmol) is the ratio of \((A \times B) / (C \times Q_{lad})\), where \(A\) is the concentration of infused [14C]glucose (dpm/ml), \(B\) is the infusion rate of labeled glucose (ml/min), and \(C\) is arterial glucose concentration (μmol/ml).

Metabolic and mechanical data were obtained at 10-minute intervals throughout the studies. Regional myocardial oxygen consumption (mmol/g dry wt/hr) and 14CO2 production from labeled-glucose oxidation (μmol/g dry wt/hr) were obtained together.
with serum fatty acids (μmol/ml) and glucose (μmol/ml) as described previously.\textsuperscript{9,10} The size and distribution of the combined right and LCF beds as well as the LAD bed were determined at the conclusion of each study by injecting India ink into the LAD cannula and dissecting free and weighing the appropriate ventricular myocardium. The LAD weight was used to normalize the metabolic data as indicated above.

**Analysis of Samples**

Ten milliliters of blood were taken from the LAD artery and vein at 10-minute intervals throughout the studies. Triplicate 1-ml samples of this blood were used for CO\textsubscript{2} determinations from labeled glucose. Another 1.0 ml blood was added to 1.0 ml of 0.6 M perchloric acid, centrifuged at 1,500\,g at 4° C for 10 minutes to remove precipitated protein, and analyzed by high-performance liquid chromatography (HPLC) (see below) to determine the content of glucose in the blood.

Glycogen was isolated from frozen heart tissue by KOH digestion, and ethanol precipitation was followed by enzymatic hydrolysis with 0.8 units amyloglucosidase and 30 units α-amylase according to Walaas and Walaas.\textsuperscript{11} Enzymatic assay for the determination of glucose from glycogen was replaced by that of HPLC. Glucose from the protein-free supernatant obtained from blood (see above) was determined by the identical HPLC technique (Aminex HPX-87H column, 9-μm resin particle size, 300×7.8 mm, Bio Rad, Richmond, California) using 0.01 N sulfuric acid as the mobile phase at a flow rate of 0.6 ml/min. Both glucose estimates were detected by absorbance at 193 nm.

**Protocol**

Studies were performed in intact whole-blood-perfused working swine hearts and divided into two parts. The purpose of the studies in the main protocol was to specifically determine the rate of glucose oxidation during reperfusion in the presence of excess available fatty acids with and without the addition of the fatty acid blocker, oxfenicine. These data were contrasted with those in a separate group of hearts receiving no excess fatty acids. In all animals, the LAD flow was reduced by 60% for 45 minutes to obtain moderate-to-severe ischemic conditions and then returned to aerobic levels for the final 50 minutes. Serum glucose was monitored throughout the studies. Mechanical and metabolic data were collected at 10-minute intervals. All metabolic data in the LAD bed were normalized by dry weight of LAD myocardium. Statistical comparisons of data were made within groups using paired Student's \( t \) tests and between groups using nonpaired Student's \( t \) tests. Group comparisons were made using all of the data contained within each of the three perfusion intervals, that is, aerobic, ischemic, and reperfusion. Statistical significance was defined by two-tailed probability values of less than 5%. Data are usually listed in the text by interval and reported as mean±SEM.

**Results**

Data from the main protocol are shown in Figure 1. The right coronary and LCF flows were maintained at aerobic levels throughout the perfusion studies in both groups (Figure 1A) and averaged 11.8±0.7 ml/g dry wt/min for all intervals in the control group and 12.1±0.7 ml/g dry wt/min for all intervals in the oxfenicine group (not statistically
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different between groups). LAD perfusion for the control group (Figure 1B) was held at 7.5±0.4 ml/g dry wt/min for the 30-minute aerobic period, reduced abruptly to 3.0±0.2 ml/g dry wt/min (~60%) for the next 45 minutes, and abruptly restored to preischemic values for the final 50-minute reflow. For the oxfenicine-treated group, the flow values were 7.7±0.3, 3.1±0.1, and 7.7±0.3 ml/g dry wt/min for the same perfusion intervals, respectively (not statistically different between groups). To maintain similar metabolic demands for myocardial oxygen consumption, left ventricular pressure was held at an average level of 102.1±1.5 mm Hg (all intervals in both groups, Figure 1C), and levels between groups were not statistically different. Infusions of Intralipid with heparin were begun at 0 minutes of perfusion. Thereafter, serum fatty acid values averaged 1.06±0.05 µmol/ml for all intervals in the control group and 1.03±0.05 µmol/ml for all intervals in the oxfenicine group (not statistically different between groups). Blood sugar for both groups averaged 6.3±1.3 µmol/ml with no statistical differences between groups.

Dependent mechanical variables in the two groups are depicted in Figure 2. For the control group, left ventricular maximum dp/dt (Figure 2A) showed only small changes between perfusion intervals (average aerobic values, 3,128±374 mm Hg/sec; ischemia values, 3,051±366 mm Hg/sec; reflow values, 3,203±460 mm Hg/sec with no significant difference among intervals). In the oxfenicine group left ventricular maximum dp/dt showed more variability with a slight decline with ischemia and an increase during reperfusion (average aerobic values, 2,906±332 mm Hg/sec; ischemic values, 2,473±261 mm Hg/sec; p<0.05 compared with aerobic values; reflow values, 3,395±471 mm Hg/sec; p<NS, compared with aerobic values; p<0.01 compared with ischemic values). Between groups there were no significant differences.

In regional function (Figure 2B), there was the anticipated loss of systolic shortening during ischemia in both groups. Mechanical performance recovered only incompletely during reflow, compatible with the development of myocardial stunning. For the control animals, aerobic values normalized to the initial value at 0 perfusion time averaged 94.6±3.0%; ischemic values averaged -31.9±16.9%, p<0.001; and reperfusion values averaged 47.6±17.5%, p<0.05 compared with preischemic values and p<0.002 compared with ischemic values. For the oxfenicine-treated group, systolic shortening averaged 100.7±3.1% normalized to the initial value at 0 perfusion time for the aerobic period; 15.0±11.2% for the ischemic period, p<0.001 from aerobic values; and 68.6±11.8% for the reperfusion period, p<0.05 compared with aerobic values and p<0.01 compared with ischemic values. Between groups there were no significant differences during the aerobic and reperfusion intervals. During the ischemic interval, a significant (p<0.05) retention in function was observed in the oxfenicine-treated group as compared with the control group.

The motion of the acutely ischemic myocardium was also characterized by bulging (lengthening) in early systole (Figure 2C). For the control animals, aerobic values derived from subtracting all data from the initial value at 0 perfusion time averaged
MVO$_2$ expectedly declined during ischemia and recovered somewhat during reflow (−42 $\Delta$, p<0.02) for both the control and oxfenicine-treated groups. Shifts in MVO$_2$ roughly followed those noted for regional percent systolic shortening.

Metabolic performance as estimated by regional myocardial oxygen consumption is shown in Figure 3. In both groups, oxygen consumption was reduced significantly from aerobic values during ischemia and recovered incomplete recovery during reperfusion; these findings roughly followed trends observed in mechanical function. For the control group, the average aerobic values were 1.49±0.10 mmol/g dry wt/hr; ischemic values were 0.88±0.06 mmol/g dry wt/hr, p<0.001; and reperfusion values were 1.27±0.14 mmol/g dry wt/hr, p<0.02 compared with aerobic values and p<0.01 compared with ischemic values. The oxfenicine-treated group showed similar shifts (average aerobic values, 1.59±0.11 mmol/g dry wt/hr; ischemic values, 0.91±0.04 mmol/g dry wt/hr, p<0.001; and reperfusion values, 1.25±0.07 mmol/g dry wt/hr, p<0.01 compared with aerobic values and p<0.001 compared with ischemic values). Intergroup comparisons were not statistically different during either the aerobic, ischemic, or reperfusion intervals.

Rates of glucose oxidation for both groups of hearts in the main protocol are shown in Figure 4A. Rates in both groups were trivial during the aerobic period, presumably due to the presence of and inhibition by the use of fatty acids. Oxfenicine requires approximately 30 minutes to suppress fatty acid oxidation in working aerobic hearts$^6,10$; thus, its influence was not detected early in our studies. The drug’s influence was clearly evident in the reperfusion period when glucose oxidation was twice that of untreated hearts (average aerobic values, 2.01±0.59 mmol/g dry wt/hr; ischemic values, 17.30±2.37 mmol/g dry wt/hr, p<0.001; and reperfusion values, 33.89±5.20 mmol/g dry wt/hr, p<0.001 compared with aerobic values and p<0.01 compared with ischemic values). Average aerobic values in control hearts were 8.10±3.91 mmol/g dry wt/hr; ischemic values were 14.52±1.91 mmol/g dry wt/hr, p=NS; and reperfusion values were 14.32±3.29 mmol/g dry wt/hr, p=NS compared with aerobic and ischemic values. Between groups there was no statistical separation during ischemia but a significant difference during reperfusion (p<0.01). This lower rate of glucose oxidation in the control group supports our previous findings that fatty acids regain their preferred status as the substrate of choice during recovery.

Glucose oxidation values in hearts receiving no excess fatty acids are shown in Figure 4B. Animals were otherwise identically processed to those of the main protocol. LAD flows averaged 6.5±0.7 ml/g dry wt/min, 2.6±0.3 ml/g dry wt/min, and 6.5±0.7 ml/g dry wt/min for the aerobic, ischemic, and reflow intervals, respectively. Right coronary and LCF flows averaged 12.1±0.7 ml/g dry wt/min for all intervals. Left ventricular pressure was held at 104.6±0.8 mm Hg. Serum fatty acids were 0.55±0.04 mmol/ml, and blood sugar was 8.6±0.2 mmol/ml. The CO$_2$ production data from labeled glucose in these hearts were as follows: aerobic interval, 2.61±1.80 mmol/g dry wt/hr; the ischemic interval, 11.65±3.22 mmol/g dry wt/hr, p<0.02; and the reperfusion interval, 17.09±4.69 mmol/g dry wt/hr, p<0.02 compared with aerobic values and p<0.05 compared with ischemic values. Varying serum fatty acids had no apparent major effect on glucose oxidation. Specifically, there was no significant difference in $^{14}$CO$_2$ production values at any of the perfusion intervals between the control group in the main protocol (Figure 4A) and the separate group of low fatty acid animals (Figure 4B).

Finally, tissue glycogen was surveyed in both the LAD and LCF beds from the untreated and treated hearts of the main study (Table 1). Tissue concentrations were comparable in these regions regardless of treatment. Ischemia followed by reperfusion in both groups reduced glycogen stores by approximately 46% as compared with aerobic levels. How-
ever, stores in LAD tissue were not negligible and suggested glycogen resynthesis during reflow.

Discussion

The purpose of this report was to define the rates of glucose oxidation during early myocardial reperfusion after ischemia by use of $[^{14}C]$glucose with and without the addition of the fatty acid blocker oxfenicine. The animals were fasted for 24 hours before starting the experiments to eliminate variations in serum glucose and fatty acid levels among pigs. In all animals, serum glucose level was held at or about 100 mg/dl, and additional glucose was infused throughout the perfusion trials if needed. Both groups of hearts in the main protocol received a constant infusion of fatty acids to supply the heart with an excess of preferred energy source during aerobic conditions. A third additional group of five hearts received no excess fatty acids. Indocyanine green was used to measure dilution in coronary venous blood from the LAD circulation according to the methods of previous reports. Specific activity of glucose and calculation of $^{14}$CO$_2$ production from labeled glucose were based on the formula presented in "Materials and Methods."

The extracorporeally perfused working swine heart model has been extensively critiqued previously and has not been importantly modified in these studies. The preparation has proved useful for evaluating myocardial mechanical and metabolic functions during reperfusion and, because of its deficit in preformed collateral vessels, developed reversible regional hypokinesis, that is, stunning, during reflow. In the present study, stunning was associated with correlative changes in regional myocardial oxygen consumption.

Glucose utilization in myocardium is dependent on mechanical work in aerobic conditions and on the level of oxygen delivery and washout during hypoxia and ischemia. Maximum rates of glycolysis in aerobic myocardium are dictated by the ability of

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**Table 1.** Glycogen Content Between Beds in Control and Treatment Groups of Main Study

<table>
<thead>
<tr>
<th>Perfusion beds</th>
<th>LCF myocardium (μmol/g dry wt)</th>
<th>LAD myocardium (μmol/g dry wt)</th>
<th>$p'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.2±1.6</td>
<td>17.0±1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oxfenicine</td>
<td>27.8±1.7</td>
<td>14.5±1.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LCF, left circumflex; LAD, left anterior descending; $p'$, statistical comparisons between tissue beds in either the control or treated groups; $p$, statistical comparisons between groups in either the LCF or LAD myocardium; NS, not significant.
myocardium to dispose of increased ratios of cytosolic NADH/NAD+. This increase in reducing equivalents is in turn related to the rate of hydrogen transport into mitochondria, principally accounted for by the malate-aspartate shuttle in heart muscle. With maximum stimulation, the rate of glyceraldehyde-3-phosphate dehydrogenase appears to determine the overall rate of the glycolytic pathway. In ischemia, as contrasted with hypoxia, the activity of this enzyme is ultimately curtailed by the buildup of higher tissue levels of lactate and H+ which then inhibit glycolytic flux. Kinetics of recovery of glycolysis are not completely known during myocardial reperfusion, but early evidence suggests there is rapid return to either aerobic levels or above. Nevertheless, in this latter study, Myears et al found that the increased rate of glucose utilization in reperfused hearts contributed only modestly to overall oxidative metabolism. These findings agree with those reported in the present study.

We had previously shown in two prior studies that oxfenicine treatment in ischemic myocardium partially preserved mechanical function. These findings were associated with decreases in amphiphiles (acylcarnitine and/or acyl-CoA) and increases in acetyl-CoA. Present results are in agreement with these previous reports in that systolic shortening was significantly preserved in treated as compared with untreated myocardium. This effect was not sustained during reperfusion and may relate to the natural reductions in amphiphiles that occur during reflow and that would in turn obviate and attenuate the effects of the drug.

We previously characterized the rates of fatty acid oxidation in reperfused myocardium and showed a strong preference for and aerobic use of fatty acids during reflow as estimated by 14CO2 production from labeled palmitate. We followed this with 13C]palmitate studies showing prolonged clearance times in the reflow period may reflect overall reduction in fatty acid uptake rather than a primary shift in substrate preference or selective diminution in rates of fatty acid oxidation. If so, this interpretation would also fit with our previous 14CO2 rebound data from [13C]palmitate and would again imply agreement between techniques. Moreover, past reports of [13C]palmitate studies showing prolonged clearance times in the reflow period may reflect overall reduction in fatty acid uptake rather than a primary shift in substrate preference or selective diminution in rates of fatty acid oxidation. If so, this interpretation would also fit with our previous 14CO2 rebound data from [13C]palmitate and would again imply agreement between techniques. Lastly, we evaluated the influence of excess fatty acids on glucose oxidation. Our conclusions to date are that with myocardial reperfusion there is a normal return of fatty acids as the preferred substrate of utilization with competitive inhibition of less hierarchical important carbohydrate substrates. However, all of these data were acquired in the presence of excess fatty acids, which may predispose the results heavily toward fatty acid utilization and away from glucose utilization. Thus, the hearts described in Figure 4B were tested at physiological levels of fatty acid. Reducing concentrations of perfusate fatty acids twofold failed to significantly alter glucose oxidation as judged by 18FDG uptake. In this latter study, in response to regional ischemia followed by reperfusion, pyruvate oxidation decreased by 50% from aerobic values and failed to recover at all during reflow. (In fact, it was further decreased from ischemic values.) These data as well as the present results do not support the concept of accelerated carbohydrate oxidation during early mechanical recovery. If one uses the CO2 production values as we reported for fatty acids, pyruvate, and glucose and the conversions (129 mol ATP synthesized from 1 mol palmitate; 15 mol ATP, from 1 mol pyruvate; and 38 mol ATP, from 1 mol glucose), the total contribution of pyruvate and glucose to ATP production never exceeds 13% for any of the perfusion intervals.

Such results are in seeming conflict with previous reports using positron emission tomography in which 18F2-deoxy-2-fluoro-D-glucose (18FDG) uptake was shown to be elevated at 24 hours reflow. Based on the inability of this isotope to completely trace the glycolytic and glycogen synthesis/ degradation pathways, concern was raised that estimates of glucose metabolism might be spuriously high. Strictly speaking, 18FDG traces only transmembrane exchange of glucose and hexokinase-mediated phosphorylation, both of which are accelerated in ischemia and hypoxia. Nevertheless, Schweiger et al in an abstract, reported that by using 18FDG in the immediate reperfusion period in dogs 18FDG uptake in reperfused tissues was reduced to 79±48% of control aerobic tissue. This trend is directionally compatible with our present and past observations on carbohydrate metabolism and argues for agreement between techniques. Moreover, past reports of [13C]palmitate studies showing prolonged clearance times in the reflow period may reflect overall reduction in fatty acid uptake rather than a primary shift in substrate preference or selective diminution in rates of fatty acid oxidation. If so, this interpretation would also fit with our previous 14CO2 rebound data from [13C]palmitate and would again imply agreement between techniques.

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