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 oxeficine, 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. Oxeficine (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 oxeficine 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 oxeficine 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,
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. 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 study, 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 animals, respectively, in each group were studied for metabolic function (for technical reasons one animal in the control group was deleted from metabolic analysis). In a separate group of five animals, excess fatty acids were not given, and the studies were repeated. All animals were anesthetized with pentobarbital (35 mg/kg i.v.) and intubated with controlled positive pressure ventilation using oxygen-supplemented room air. The preparation, instrumentation, and portions of the protocol used in this study have been extensively reported elsewhere. Briefly, the coronary arteries were perfused separately via an arterioarterial shunt connected extracorporeally. Blood was withdrawn from a femoral artery and returned via three low-flow perfusion pumps to the proximally cannulated right, main left, and left anterior descending (LAD) coronary arteries. Included in the LAD circuit was a 100-ml mixing chamber used to receive a constant infusion of labeled glucose (about 45 μCi/animal) for mixing with the coronary perfusate before its infusion into the myocardium. Aerobic flows were adjusted so that coronary perfusion pressures approximated arterial pressures and the venous oxygen saturation was maintained at or about 38%. In each study a venous cannula was inserted into the great cardiac vein anteriorly and used together with ports in the arterial lines to sample for gases and metabolites.

Left ventricular pressure and its maximal first derivative were measured by a high fidelity manometer-tipped pressure device (Millar Instruments, Houston, Texas) placed into the left ventricular chamber. Regional function was estimated from shortening of ultrasonic crystals placed at midmyocardial depth in the LAD and left circumflex (LCF) perfusion systems. Segment shortening was used to characterize regional contractility. Right coronary and LCF flows were held at aerobic levels throughout the experiments. In each of the 18 animals, LAD flow was maintained at aerobic levels for the first 30 minutes of perfusion, reduced by 60% for the next 45 minutes, and then returned to preischemic levels for the final 50 minutes of reflow. To set a condition of near constant myocardial oxygen demand in all hearts, blood volumes were replenished throughout the studies with 6% dextran in saline to maintain systemic pressures at or about 100 mm Hg. Serum glucose was monitored at each 10-minute sampling interval and maintained at or above 100 mg/dl with infusions of glucose if needed. [6-14C]Glucose (4.1±1.4×10^4 dpm/ml) was infused into the LAD coronary circulation at 0.5 ml/min during aerobic perfusion and reperfusion and at 0.2 ml/min during ischemia. In the nine treatment animals of the main study, a bolus of oxfenicine (33 mg/kg) was injected at time 0 and at 60 minutes of perfusion. Conversely, the nine animals in the control group received no oxfenicine. To maintain serum fatty acid levels sufficient to compare with previous data, 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. 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/g dry wt/hr) from labeled glucose according to the formula:

\[ \frac{14\text{CO}_2 \text{ production (μmol/g dry wt/hr)}}{[A^{14}\text{CO}_2 \times Q_{LAD} \times 60]/[K \times ASA \times LAD \text{ dry wt}]} \]

where Δ14CO2 is the corrected difference between venous and arterial 14CO2 (dpm/ml); QLAD is the flow in the LAD bed (ml/min); and ASA (arterial specific activity of glucose in dpm/μmol) is the ratio of (A×B)/(C×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.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 CO₂ determinations from labeled glucose. Another 1.0 ml blood was added to 1.0 ml of 0.6 M perchloric acid, centrifuged at 1,500g 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.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
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; ischemic 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...
pared 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\(^{8,10}\); 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\(\pm\)0.59 \(\mu\)mol/g dry wt/hr; ischemic values, 17.30\(\pm\)2.37 \(\mu\)mol/g dry wt/hr, \(p<0.01\); and reperfusion values, 33.89\(\pm\)5.20 \(\mu\)mol/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\(\pm\)3.91 \(\mu\)mol/g dry wt/hr; ischemic values were 14.52\(\pm\)1.91 \(\mu\)mol/g dry wt/hr, \(p=NS\); and reperfusion values were 14.32\(\pm\)3.29 \(\mu\)mol/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\(\pm\)0.7 ml/g dry wt/min, 2.6\(\pm\)0.3 ml/g dry wt/min, and 6.5\(\pm\)0.7 ml/g dry wt/min for the aerobic, ischemic, and reflow intervals, respectively. Right coronary and LCF flows averaged 12.1\(\pm\)0.7 ml/g dry wt/min for all intervals. Left ventricular pressure was held at 104.6\(\pm\)0.8 mm Hg. Serum fatty acids were 0.55\(\pm\)0.04 \(\mu\)mol/ml, and blood sugar was 8.6\(\pm\)0.2 \(\mu\)mol/ml. The \(^{14}\)CO production data from labeled glucose in these hearts were as follows: aerobic interval, 2.61\(\pm\)1.80 \(\mu\)mol/g dry wt/hr; the ischemic interval, 11.65\(\pm\)3.22 \(\mu\)mol/g dry wt/hr, \(p<0.02\); and the reperfusion interval, 17.09\(\pm\)4.69 \(\mu\)mol/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 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-
Figure 4. Graphs showing regional $^{14}$CO$_2$ production values from labeled glucose. Panel A: Two groups of the main protocol that received Intralipid. During myocardial reperfusion, glucose oxidation was over two-fold higher in the oxfenidine-treated groups as compared with the control group. Panel B: Five hearts studied at physiological levels of fatty acids (0.55±0.04 μmol/ml). Hearts in this group were perfused in a similar fashion to those of the main protocol (~60% alteration in left anterior descending arterial flow from 30-75-minute perfusion times) and maintained at similar levels of left ventricular pressure (104.6±0.8 mm Hg). Blood sugar was 8.6±0.2 μmol/ml. Glucose oxidation in this group was comparable at all perfusion intervals with those of the control group in panel A.

However, 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 oxfenidine. 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.2,10 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 previously1,6,9,10 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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<th>Table 1. Glycogen Content Between Beds in Control and Treatment Groups of Main Study</th>
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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.
ways, concern was raised that estimates of glucose oxidations (129 mol ATP synthesized from 1 mol pyruvate; 15 mol ATP, 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 [18F]2-deoxy-2-fluoro-D-glucose ([18F]FDG) 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, [18F]FDG 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 [18F]FDG in the immediate reperfusion period in dogs [18F]FDG 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 [14C]CO2 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 labeled [14C]CO2 production. These results continue to support the observations that major utilization of carbohydrates for oxidative phosphorylation during early reperfusion does not occur.

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KEY WORDS • fatty acids • glucose oxidation • oxfenicine • mechanical stunning • myocardial reperfusion
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