Distribution of Carbon Flux Within Fatty Acid Utilization During Myocardial Ischemia and Reperfusion

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

Twenty-nine intact, working pig hearts were extracorporeally perfused and divided into two study groups (16 Aerobic and 13 Ischemic/Reflow hearts). Step function, equilibrium labeling with [14C]palmitate was used to develop uptake and washout curves of radioactive fatty acid products contained in coronary effluent during either aerobic perfusion or reperfusion after ischemia (60% reduction in left anterior descending coronary flow for 30 minutes). Left anterior descending control flows were slightly overperfused in Aerobic hearts (18% higher than in Ischemic/Reflow hearts); otherwise, circumflex and right coronary flows, left ventricular pressure, and serum fatty acids and blood sugar levels were comparable between groups. As expected in Ischemic/Reflow hearts, recovery of regional systolic shortening and myocardial oxygen consumption in reperfusion was only modestly impaired (−20% and −19%, respectively, not significant and \( p < 0.011 \) compared with preischemic values, not significant from Aerobic hearts). The only significant metabolized product to be released from labeled fatty acid utilization in either group was 14CO2. A smaller fatty acid pool also was measured and accounted for by that contained in the coronary intravascular volume. We could determine no significant back diffusion of fatty acids from myocardium in either perfusion condition. Uptake time constants of the early phase of 14CO2 production also were virtually identical in both groups (19.9±3.2 versus 16.7±3.2 minutes in Aerobic and Ischemic/Reflow hearts, respectively) and strongly correlated with hemodynamics as described by heart rate. In washout studies, tissue radioactivity in the aqueous soluble and fatty acid pools declined in both study groups, and counts in complex lipids and cholesterol/cholesteryl esters remained steady, whereas those in triacylglycerols varied. Washout of 14CO2 in both groups never reached background radioactivity over a 40-minute sampling after cessation of isotope infusion into the perfusate, suggesting slow release of trapped substrate from intracellular pools, which then proceeded to fatty acid oxidation. In conclusion, these experiments have demonstrated very similar findings with respect to fatty acid uptake, storage, and release characteristics between aerobic and reperfused myocardium. We found no differences in preferred substrate utilization and oxidation as a result of reversible ischemia followed by reflow. (Circulation Research 1991;69:779–790)

Controversy exists as to the level of impairment in fatty acid metabolism and substrate utilization during early reperfusion after myocardial ischemia. In perfused pig hearts, we have shown a hierarchical preference for fatty acids with full return of fatty acid oxidation during acute reflow accompanied by inhibitions of glucose, pyruvate, and lactate oxidations.1–3 Myears et al4 similarly noted normal rates of oxidative metabolism with fatty acid substrate during reflow after 1 hour of coronary occlusion in dog hearts but reduced rates of net fatty acid uptake from the perfusate. This latter finding may not be important, because endogenous release of fatty acids from complex lipid stores is enhanced severalfold in reperfusion.5 Conversely, Schwaiger and colleagues6 reported prolonged abnormalities in fatty acid utilization after a 3-hour coronary occlusion protocol in dogs with delays in clearance of [11C]palmitate from myocardium, increases in tissue residual activity, and reduced uptake of palmitic acid lasting for up to 1 week of reperfusion. However, the data using positron-emitting isotopes may have prob-
lems with interpretation and subsequently have been criticized. Fox and coworkers described a fourfold increase in efflux from myocardium of initially extracted but nonmetabolized fatty acids in ischemia that confounded estimates of oxidative metabolism by external detection. Levels of back diffusion of fatty acids in reperfusion are at present unknown. Rosamond et al argued further that clearance rates of [1-14C]palmitate could not be extrapolated to measurements of substrate oxidation not only because of the efflux problem but also because the positron signal per se nonselectively records all events simultaneously within the utilization pathway. In addition, it has been hypothesized that complex lipids within myocytes serve as a large reservoir for fatty acids with slow turnover kinetics that cause problems with estimating rates of oxidation, even when [14C]palmitate and equilibrium labeling are used. This difficulty would be markedly exaggerated with short-lived [14C]palmitate, which would not adequately interrogate those distal portions of the utilization pathway taking up to 30 minutes to come into equilibrium. Thus, the purpose of the present studies was 1) to review further the substrate kinetics of long-chain fatty acids leading to CO2 production, with particular attention paid to the time constants of incorporation and distribution of substrate; and 2) to contrast the metabolic behavior between aerobically perfused hearts and those rendered ischemic and then reperfused.

Materials and Methods

Twenty-nine adolescent swine weighing 48.9±0.6 kg were studied. Sixteen of these animals also had been used in a separate study (unpublished data) to develop baseline characteristics of fatty acid utilization kinetics but in this study served as a control (Aerobic) group to contrast with observations acquired in a separate group (Ischemic/Reflow) during reperfusion after ischemia. Data for both studies were obtained contemporaneously. All animals were anesthetized with pentobarbital (35 mg/kg i.v.) and underwent establishment of controlled positive pressure ventilation using oxygen-supplemented room air. The preparation used in this study has been reported extensively elsewhere. Briefly, the coronary arteries were perfused separately via an arterioarterial shunt connected extracorporeally. Blood was withdrawn from a femoral artery and returned by three low flow perfusion pumps to proximally cannulated right, main left, and left anterior descending (LAD) coronary arteries. Three access ports were included in the LAD perfusion circuit. One port allowed infusion of indocyanine green directly into the perfusion circuit through a 50-ml mixing chamber. Another port was for infusion of [14C]palmitate (48±3 μCi/animal), which was mixed with the perfusate by passage of the isotope through a segment of tubing (8-ml volume) contained in series within the perfusion circuit consisting of alternating sections of small-bore (0.64-cm i.d.) and large-bore (1.3-cm i.d.) tubing. A third port was used to sample arterial blood for gases and metabolites; this blood was compared with venous blood obtained from a sampling catheter inserted anteriorly into the great cardiac vein. Left ventricular pressure and its maximal first derivative were measured by a high-fidelity, manometer-tipped pressure device (Millar Instruments, Houston, Tex.) placed into the left ventricular chamber. Regional function was estimated from myocardial shortening as measured by ultrasonic crystals placed at midmyocardial depth in the LAD perfusion system. Segment shortening was used to characterize regional contractility.

Aerobic coronary flows were adjusted so that coronary perfusion pressures approximated arterial pressures, and the venous oxygen saturations were estimated at or about 40%.

By prospectively agreed-on entrance criteria, venous saturations in Aerobic hearts were not permitted to decrease below 20% at any time in the perfusion trial. To set conditions of near constant myocardial oxygen demand in all hearts, blood volumes were replaced with 6% dextran in saline to maintain systemic pressures at approximately 100 mm Hg. Serum glucose levels were monitored continuously and maintained at 7.1±0.04 μmol/ml with intravenous infusions of dextrose. Indocyanine green indicator (0.33 mg/ml) was infused into the LAD perfusate for 5 minutes every 10 minutes at a flow rate of 1.0 ml/min during the radiolabeled perfusion period to measure the cross-contamination of any unlabeled coronary blood entering the venous system of the LAD circuit. From this, a dilution factor was calculated to correct the venous counts that then were used in the calculations. Metabolic and mechanical data were obtained at specified intervals throughout the perfusion studies. Blood samples obtained from the LAD artery and vein were used to measure blood gases and 14CO2 production from labeled palmitate. The former samples were used to calculate regional myocardial oxygen consumption (MVO2 in millimoles per hour per gram dry weight) by a previously described formula. The latter samples were centrifuged at 1,500g at 4°C for 10 minutes to obtain plasma. Blood from the femoral artery likewise was centrifuged to acquire a similar plasma fraction. These plasma samples from the LAD artery, great cardiac vein, and femoral artery were counted to measure total radiocarbon in the blood. 14CO2 production from labeled palmitate also was calculated and expressed in micromoles per hour per gram dry weight as

\[
\Delta^{14} CO_2 \times Q_{LAD} \times 60
\]

\[
\frac{ASA \times LAD \, dry \, wt}{1}
\]

where \(\Delta^{14}CO_2 = (V - A)/K\) in disintegrations per minute per milliliter. This was the difference in radioactive counts between venous (V) and arterial (A) 14CO2 in coronary perfusate. The difference in
counts was corrected by the indocyanine green dilution factor, K. Q_{\text{LAD}} is flow in the LAD bed in milliliters per minute, and ASA is the arterial specific activity of free fatty acids in disintegrations per minute per micromole, expressed as the ratio of A/B, where A is the arterial palmitate radioactivity in disintegrations per minute per milliliter, and B is the arterial palmitate concentration in micromoles per milliliter. Palmitate was measured by the method of Ho.\textsuperscript{14}

With respect to determining tissue carbon pool size for fatty acid constituents, numerical integration was used. The pool counts for CO\textsubscript{2} and fatty acids were calculated by subtracting the area under the time–activity curve for labeled product from the area beneath the equilibrium concentration level (C\textsubscript{e}) (see Figure 1). For both areas, the range of integration was from the time at which the label entered the mixing tubing (T\textsubscript{b}) until the last time sample was taken (T\textsubscript{a}). Area under the time–activity curve was determined by fitting data with a natural cubic spline.\textsuperscript{15} The spline was then integrated from T\textsubscript{b} to T\textsubscript{a} using a trapezoidal method that allowed successive approximations to the routine.\textsuperscript{16} Each estimate further subdivided the interval until the change in the area was less than or equal to 0.001%. The C\textsubscript{e} value was determined by first integrating the spline from the point at which equilibrium occurred (T\textsubscript{b}) until T\textsubscript{n}, then dividing this area by the time interval (T\textsubscript{n}−T\textsubscript{b}). The area under C\textsubscript{e} then was calculated from the product of C\textsubscript{e} and the time interval (T\textsubscript{n}−T\textsubscript{b}). The units represented on this curve are micromoles CO\textsubscript{2} or fatty acid per minute per gram dry weight, and the value at each time point is calculated using an equation similar to Equation 1. The integrated units are micromoles CO\textsubscript{2} or free fatty acid per gram dry weight, which are an index of the pool size, because the carbons defined by this integration, for the most part, do not exist as CO\textsubscript{2} or free fatty acid but instead as intermediates. The term free fatty acid mole equivalence has been used for these data. A second index (milliliters per gram dry weight) also was defined using

\[
\int_{0}^{\infty} \frac{A^{14}\text{CO}_2 \times Q_{\text{LAD}}}{A \times \text{LAD dry wt}} \, dt
\]

where A is arterial concentration of labeled palmitate in disintegrations per minute per milliliter. This index can be thought of as the volume that would be occupied by the radioactive carbons if they were found in the heart at the same concentration as they occurred in blood. This value has been termed the volume equivalence and is analogous to the distribution volume described in positron emission tomography (PET) studies.

PET studies also have demonstrated that the time–activity curves derived from reperfused myocardium have prolonged clearance times in the early phase of exponential decay after bolus administration of isotope. To provide a comparison for these studies, the uptake curves in the present experiments also were approximated by a single exponential function, and the time constants were tabulated.

At the end of the studies, tissue samples from the LAD and adjacent circumflex (LCF) beds were freeze clamped and stored at −70°C for further analysis. These samples were obtained before the infusion of isotope was discontinued. The location of the tissue perfused by the LAD artery was determined at the conclusion of each study by injection of india ink into the LAD cannula. The relation of the previously obtained tissue samples to the perfusion regions was verified. Then, LAD myocardium was dissected free from that perfused by the combined right and LCF arteries, and both perfusion systems were weighed.

Frozen tissue samples obtained from the separate perfusion beds were analyzed for metabolites and extracted with chloroform/methanol according to Bligh and Dyer.\textsuperscript{17} The organic fraction then was analyzed using a prepacked Sep-Pak silica column after the method of Hamilton and Comai\textsuperscript{18} for separation of cholesteryl esters, triacylglycerols, free fatty acids, cholesterol, and polar lipids.

**Protocol**

Studies were performed in intact, whole blood-perfused, working swine hearts that were divided into the Aerobic (n=16) and Ischemic/Reflow (n=13) groups. In all animals of both groups, right coronary and LCF flows were held at aerobic flows throughout the experiments. Within each group, separate studies were conducted to study the kinetics of labeled palmitate incorporation and washout. In the 13
hearts of the Ischemic/Reflow group, LAD flow was maintained at aerobic levels for 10 minutes of perfusion, reduced by 60% for the next 30 minutes, and then returned to aerobic levels for the final 50 minutes. Infusion of [U-14C]palmitate at 2.6×10^6 dpm/min was started 10 minutes after the conclusion of ischemia and continued for a total of 40 minutes during reflow. In five of these animals, reflow was continued for an additional 40 minutes after the palmitate infusion was terminated to create a washout period. In the 16 Aerobic animals, the LAD flow was maintained at aerobic flows throughout the studies. [U-14C]Palmitate was infused (2.4×10^6 dpm/min) at 50 minutes and was continued for the next 40 minutes. In five of these hearts, perfusion also was continued for an additional 40 minutes after infusion of labeled palmitate had been discontinued. Venous and arterial blood samples for blood gas determinations were obtained throughout the studies. Other blood samples were collected to describe the kinetics of labeled palmitate infusions. For Ischemic/Reflow hearts, venous blood samples for 14CO2 measurements were taken every 2 minutes for 10 minutes, then every 5 minutes for the next 30 minutes. Venous plasma samples were collected every 0.5 minute for 3 minutes, then every 2 minutes for the next 6 minutes, followed by every 5 minutes for the next 30 minutes. Coronary arterial blood samples for 14CO2 measurements were obtained every 5 minutes throughout the studies, and coronary arterial samples together with femoral arterial samples were collected every 10 minutes to analyze for non-14CO2 radioactivity. For the Aerobic hearts, venous blood samples for CO2 data were obtained every 2 minutes throughout the 40-minute labeling period, and venous plasma samples were collected every 0.5 minute for 3 minutes, then every 2 minutes for the rest of the 40-minute labeling period. Arterial CO2 and plasma samples together with femoral arterial samples were taken every 10 minutes for 40 minutes. During the washout period, blood was obtained at the same time intervals as described above for the Ischemic/Reflow group.

Statistical comparisons of data were made within each group using paired Student’s t tests and between groups using nonpaired Student’s t tests. Statistical significance was defined by two-tailed probability values less than 5%.

Results

Figure 2 shows coronary flows and left ventricular pressures for both Ischemic/Reflow and Aerobic groups. Venous oxygen saturations in the LAD bed at aerobic perfusions (0–10 minutes) were comparable between groups, although flow rates were slightly higher in Aerobic hearts (7.1±0.3 versus 6.0±0.3 ml/min/g dry wt, p<0.034). We do not feel this difference is physiologically significant. The LAD flow in Aerobic hearts was purposely maintained slightly higher than that required to meet the metabolic demands of the heart. This perfusion level was selected to guarantee that the tissue would not be exposed to ischemic conditions. In the Ischemic/Reflow group, LAD flow was reduced to 2.4±0.1 ml/min/g dry wt during ischemia and then acutely returned to preischemic levels at 40 minutes. Circumflex plus right coronary flows in Aerobic and Ischemic/Reflow hearts were maintained at 11.3±0.6 and 10.2±0.6 ml/min/g dry wt, respectively, throughout the experiments. Left ventricular pressure averaged 102.5±1.2 mm Hg for both groups throughout the perfusion trial and was not statistically different between groups. Serum fatty acids were 0.51±0.04 μmol/ml, and blood sugar concentrations were 7.13±0.04 μmol/ml (average of all animals in both groups). Neither substrate level was statistically different between groups.

Figure 3 shows regional mechanical and metabolic performance in the two groups. Aerobic values for both systolic shortening and myocardial oxygen consumption were steady over the course of the perfusion trials (not significant in the middle [10–40 minutes] and later [40–90 minutes] portion of the runs, as compared with the early [0–10 minutes] intervals by paired Student’s t tests). There were the expected decreases in mechanical and metabolic functions (p<0.001) in Ischemic/Reflow hearts during ischemia and partial recovery during reflow. Although MVO2 remained statistically lower during reperfusion, systolic shortening was only marginally different from aerobic values, with statistical significance at only the 50- and 70-minute time points.

Specialized studies (Table 1) were conducted to monitor radioactivity of metabolized and nonmetabolized products of labeled palmitate in coronary effluent. Blood from Ischemic/Reflow hearts was compared with blood labeled in vitro with [14C]palmitate. [14C]Palmitate was added to blood that had no radioactive material present. The amount of radioactivity added was such that the counts were similar to those of blood taken from the in vivo studies (approximately 6×10^6 dpm/ml). The distribution of radioactivity in this in vitro labeled blood then was determined in a manner identical to the approach used with the in vivo samples. These spiked standard data represent the distribution of “normal” contamination of radioactivity affected by adding fatty acids to blood constituents independent of products accumulating from myocardial metabolism. These data are compared with the Ischemic/Reflow blood (Table 1). Distribution of radioactivity in coronary venous effluent was almost totally contained in the organic or fatty acid compartments, with a small additional component noted in the aqueous soluble phase and phospholipids. The distribution was nearly identical to the pattern in the nonmetabolized spiked standard data. Indeed, the only significant metabolized product to be released from labeled fatty acid utilization in hearts was 14CO2.

Uptake and washout of fatty acids and 14CO2 are shown for two representative animals in the Ischemic/Reflow group in Figures 4 and 5 and for both groups in Table 2. Again, in the uptake studies, there
were no counts in coronary effluent in either group over and above normal background (spiked standard counts as described above) that were not accounted for in either the labeled CO$_2$ or fatty acid pools. The latter was the smaller pool in both groups, and this pool could all be contained in the coronary intravascular compartment as defined by the volume between the $[^{14}C]$palmitate infusion port and the coronary venous sampling port. Back diffusion of nonmetabolized fatty acids was estimated in five animals from each group using three different approaches. First, the difference between the free fatty acid pool during washout and the pool contained in the vascular blood volume was obtained in each group. The latter was calculated as 8 ml (the volume of the perfusate tubing) +11%×bed weight (the coronary blood volume contained in myocardial tissue weight). This difference was essentially nil, indicating that all of the free fatty acid pools in both groups could be accounted for by the combined blood volumes in the perfusion tubing and the coronary blood volumes. The level of ischemia selected in this study followed by reperfusion appeared to have no effect in exacerbating back diffusion above that in aerobic hearts. Second, the free fatty acid pools were compared between the Aerobic and Ischemic/Reflow groups and tended to be slightly smaller for the Ischemic/Reflow group (see Table 2). Finally, the levels of radioactive free fatty acid in the 20- and 40-minute samples of venous effluent during washout were compared between the two groups. Again, there was no difference between the two groups (125±60 pmol/g dry wt in Aerobic versus 56±50 pmol/g dry wt in Ischemic/Reflow hearts at 20 minutes and 42±30 versus 86±70 pmol/g dry wt, respectively, at 40 minutes). Counts in the venous effluent also were not statistically different from zero at either of the two time points for both groups.

The larger pool of radioactivity was that represented by $^{14}$CO$_2$ (Table 2). In the uptake curve analysis, there was a trend toward reduced pool size in the Ischemic/Reflow hearts, whereas equilibrium levels of $^{14}$CO$_2$ production were comparable or decreased depending on the units of measure. Interestingly, the uptake time constant for the radioactive buildup of $^{14}$CO$_2$ in coronary effluent was similar for the two groups (19.9±3.2 minutes for the Aerobic group and 16.7±3.2 minutes for the Ischemic/Reflow group). These were equal to half-times of 13.8 and 11.5 minutes, respectively. Oddly, these time constants

**Figure 2.** Controlled variables of coronary flow separately displayed as the combined circumflex (CIRC) plus right coronary (RCA) flows and left anterior descending (LAD) flows (panel A) and left ventricular pressures (LVP) (panel B) for Aerobic and Ischemic/Reflow study groups. Aerobic hearts were slightly overperfused in the LAD bed, which met statistical significance (p<0.034).
Aerobic ischemia Reflow

Figure 3. Dependent variables for both study groups describing regional mechanics (systolic shortening [SS] normalized to initial values in the left anterior descending myocardium) (panel A) and metabolic function (myocardial oxygen consumption [$MV_2$]) (panel B). Recovery of metabolic function was impaired in the Ischemic/Reflow group, but systolic shortening almost returned to that observed in Aerobic hearts.

Table 1. Distribution of Radioactivity in Coronary Venous Effluent of Ischemic/Reflow Hearts (n=5)

<table>
<thead>
<tr>
<th>Blood</th>
<th>Ischemic/ reflow (%)</th>
<th>Standard* (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous Soluble</td>
<td>7.74±1.48</td>
<td>5.70±2.55</td>
<td>NS</td>
</tr>
<tr>
<td>Organic</td>
<td>91.62±1.52</td>
<td>93.90±2.52</td>
<td>NS</td>
</tr>
<tr>
<td>Total of two phases</td>
<td>99.36±0.01</td>
<td>99.60±0.04</td>
<td>&lt;0.063</td>
</tr>
<tr>
<td>Total direct (plasma)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Lipids species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>0.11±0.02</td>
<td>0.08±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>0.07±0.04</td>
<td>0.10±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>83.38±2.87</td>
<td>83.48±2.58</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.77±0.22</td>
<td>0.54±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>1.32±0.49</td>
<td>0.68±0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Total in fractions</td>
<td>87.74±2.75</td>
<td>84.88±2.64</td>
<td>NS</td>
</tr>
<tr>
<td>Residue†</td>
<td>5.89±1.57</td>
<td>9.03±2.20</td>
<td>NS</td>
</tr>
<tr>
<td>Total organic</td>
<td>91.63±1.53</td>
<td>93.92±2.50</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Nonmetabolized blood spiked with [14C]palmitate.
†Remaining radioactivity in glass tubes.

were not significantly correlated with either the aqueous or lipid phase fractions of tissue radioactivity or the myocardial oxygen consumption in either group. Conversely, the time constants of $^{14}$CO$_2$ release in both groups were inversely correlated with heart rate (Figure 6) according to the expression Time Constant=57.1–0.25×heart rate; $r=0.62$, $p<0.0004$ (all animals included from both groups).

In separate studies in 10 animals, washout curve analysis also was performed (Figure 5, Table 2). Exponential decay of counts was similar between groups but different between labeled constituents.
FIGURE 4. Labeled fatty acid (FFA) and $^{14}$CO$_2$ uptake measurements in left anterior descending coronary venous effluent in representative hearts from the Ischemic/Reflow group. Interpolated areas (gray hatched areas) are the estimated pool sizes of each constituent. Insert: Expanded time scale to show the small size of the FFA pool. The configuration of these two curves was quite similar to those found in Aerobic hearts.

Fatty acids quickly reached background levels of radioactivity after cessation of $[^14]$C]palmitate infusions, whereas $^{14}$CO$_2$ did not. This trend is compatible with the concept that labeled substrate is still present in intracellular fatty acid pools, probably complex lipids, and after discontinuance of labeled infusions is slowly released to enter fatty acid oxidation and $^{14}$CO$_2$ production.

In a final comparison, distribution of radioactivity in tissue was compared between groups in both uptake and washout protocols (Table 3). In uptake studies in Aerobic hearts, counts between lipid and aqueous soluble phases were almost evenly divided (52% and 48%, respectively), whereas in Ischemic/Reflow hearts, counts were increased in the lipid fraction (66%). Generally, there were no major statistical differences between groups among the various fractions and lipid species in either the uptake or washout data. Triacylglycerol radioactivity tended to be higher in the Ischemic/Reflow group, and the aqueous soluble phase radioactivity was slightly smaller as measured by the volume equivalency method. In washout studies, counts in complex lipids and cholesterol/cholesteryl esters in both groups remained relatively fixed, with more variability noted in the triacylglycerol compartment, particularly in Aerobic hearts. Conversely, counts in the aqueous soluble fraction and tissue fatty acid compartment dramatically declined with washout in both groups.

These latter two constituents correlated closely with the $^{14}$CO$_2$ uptake pool in both groups (Figure 7) and probably represent the same material.

**Discussion**

There has been renewed interest in the mechanical and metabolic behavior of reperfused myocardium, in part stemming from the development of clinical salvaging techniques to reperfuse injured ischemic myocardium in patients with acute coronary events. Stunning,19-21 an impairment in mechanical recovery after reperfusion in the absence of obvious histological damage, has been variously explained to result from problems with ATP resynthesis or compartmentalization, calcium overload, or free radical injury.22-28 Metabolism in reflow is not completely understood, and there is possible disagreement as to the rank of substrate preference between fatty acids and glucose in the reperfused condition.1-4,29-34 With respect to fatty acid metabolism, differences in interpretation may result from the choice of techniques used to measure rates of utilization and oxidation. The PET technique, essentially a pulse labeling technique, has provided evidence that $^{11}$C-tracer clearance and decay from bolus injection of positron-labeled fatty acids is substantially depressed early after reperfusion35 and recovers only slowly.6 Conversely, other studies using $[^14]$C]palmitate and equi-
librium labeling have shown that fatty acid oxidation is not depressed.\textsuperscript{1-4}

More than one hypothesis is available to explain this discrepancy. One possibility, although not shown in our study, is that the \textsuperscript{1}C-tracer is removed from the heart by nonmetabolic means, such as back diffusion, that might be enhanced under some conditions of reflow. If the rates of metabolic and nonmetabolic turnover are similar, the deconvolution of the time–activity curve to separate the two would be difficult. In short, the time–activity curve might be misinterpreted based on nonmetabolic events. Alternatively, because the time required to dissipate radioactivity in a pool depends not only on the rate of removal but also on the size of the pool, an increase in the distribution volume could prolong the time–activity curve described by PET, and in so doing, skew the interpretive results. Neither event would bias the data defined by equilibrium labeling.

On the other hand, some problems in analysis are common to both techniques. The time–activity curves acquired by PET represent transmural information reflecting heterogeneously perfused myocardium. The venous samples obtained in this study also are derived transmurally, but the heterogeneity of perfusion is diminished in pig hearts because of the deficit in preformed collateral circulation.\textsuperscript{36,37} The consequence of this heterogeneity on the measured time constants is different for the two techniques. Time–activity curves from PET represent tissue retention of radioactivity and thus would over-represent cells with high retention or slow metabolism. Conversely, myocardial efflux of \textsuperscript{14}CO\textsubscript{2} as determined in this study primarily reflects behavior in the most metabolically active cells.

A final problem deals with radioactive labeling of intracellular lipid pools and its effect on quantitating fatty acid oxidation. This study has demonstrated by washout curve analysis a prolonged release of \textsuperscript{14}CO\textsubscript{2} after termination of infused \textsuperscript{13}C-palmitate. We interpreted this to reflect the reintroduction of labeled substrate into the fatty acid utilization pathway from temporary storage in complex lipids, particularly triacylglycerols. Because this pool size may be quite large, the release kinetics of isotope are complex and prolonged (beyond our 40-minute sampling interval) and may account for a 20\% error in estimating \textsuperscript{14}CO\textsubscript{2} production based on equilibrium labeling. PET studies also have recognized a late phase in the time–activity curves, which has been attributed to tracer deposition in the endogenous lipid pool but which has been ignored as an element of metabolic activity. Analysis by PET would be even more compromised because of the short half-life of the \textsuperscript{13}C-tracer and the difficulties in adequately labeling all intracellular lipid pools homogeneously within the allotted time constraints of the isotope.
TABLE 2. Estimated CO₂ and Free Fatty Acid Pool Sizes and Equilibrium Values: Uptake and Washout Curve Analysis

<table>
<thead>
<tr>
<th></th>
<th>CO₂</th>
<th>Equilibrium</th>
<th>Free fatty acids</th>
<th>Equilibrium</th>
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<tbody>
<tr>
<td></td>
<td>Pool</td>
<td></td>
<td></td>
<td>Pool</td>
</tr>
<tr>
<td></td>
<td>(μmol/g</td>
<td>(ml/g</td>
<td>(μmol/min/g</td>
<td>(ml/min/g</td>
</tr>
<tr>
<td></td>
<td>dry wt)</td>
<td>dry wt)</td>
<td>dry wt)</td>
<td>dry wt)</td>
</tr>
<tr>
<td>Uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic (n=16)</td>
<td>4.77±0.91</td>
<td>17.6±2.85</td>
<td>0.44±0.08</td>
<td>1.58±0.23</td>
</tr>
<tr>
<td>Ischemic/Reflow (n=13)</td>
<td>2.77±0.41</td>
<td>9.33±1.34</td>
<td>0.30±0.04</td>
<td>0.99±0.13</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.075</td>
<td>&lt;0.022</td>
<td>NS</td>
<td>&lt;0.041</td>
</tr>
<tr>
<td>Washout</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic (n=6)</td>
<td>3.92±1.07</td>
<td>11.4±3.15</td>
<td>0.04±0.004</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>Ischemic/Reflow (n=5)</td>
<td>2.84±0.54</td>
<td>9.16±2.28</td>
<td>0.03±0.01</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

p values indicate statistical comparisons of data between Aerobic and Ischemic/Reflow hearts. Units are volume equivalence and mole equivalence of free fatty acids (see text).

The following data were observed in this study. Metabolic analysis of the coronary effluent virtually precludes the existence of significant nonmetabolic routes of release of radioactivity in reflow after the moderate levels of ischemia imposed in these experiments. Although back diffusion cannot be categorically eliminated, the magnitude of this release, if present, must be trivial and would occur only during capillary transit. This should not obscure the rapid phase of the uptake curve. There was a modest decrease in the free fatty acid pool in Ischemic/Reflow hearts. This reduction could represent either a decline in blood volume or an alteration in fatty acid uptake by the sarcolemma, perhaps as a residual consequence of the ischemic stress.

The tissue fraction and pool sizes expressed as volume equivalents in our results are analogous to the distribution volume described by PET. Our studies revealed modest changes in these pool sizes. The pool size measured from 14CO₂ efflux decreased, as did the tissue fraction composed of the aqueous soluble phase and free fatty acids. This same tissue fraction was rapidly lost during washout. These findings would not explain the prolonged clearance rates of the rapid exponential decay portion of the time–activity curve described by PET in reflow. The PET data would imply a shift to a larger pool size, not a smaller one as found in these studies. The triacylglycerol tissue fraction in Ischemic/Reflow hearts did evince a trend toward larger values, but this pool manifests slow turnover kinetics and is thought to influence the late phase of time–activity curves.

These studies provide further evidence that the oxidation of fatty acid is not depressed during reperfusion. Although there is a trend toward decreased 14CO₂ production after equilibrium in the Ischemic/Reflow hearts, the significant decrease in 14CO₂ and free fatty acid efflux expressed as a volume/flow equivalent is most likely an indication of the higher LAD flow in Aerobic hearts. This ratio represents the venous flow required to contain venous radioactivity if the venous concentration was equal to arterial concentration. As such, it is a function of perfusion.

![Figure 6](http://circres.ahajournals.org/attachment/6372470/787)
TABLE 3. Distribution of Radioactivity in Tissue in Aerobic (n=16) and Ischemic/Reflow (n=13) Hearts

<table>
<thead>
<tr>
<th></th>
<th>Aerobic (n=10)</th>
<th>Ischemic/Reflow (n=7)</th>
<th>p'</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake</td>
<td>(µmol/g dry wt)</td>
<td>(ml/g dry wt)</td>
<td>(µmol/g dry wt)</td>
<td>(ml/g dry wt)</td>
</tr>
<tr>
<td>Lipid fraction</td>
<td>4.90±1.18</td>
<td>19.5±3.04</td>
<td>7.87±1.99</td>
<td>25.1±4.68</td>
</tr>
<tr>
<td>Aqueous soluble</td>
<td>4.46±0.61</td>
<td>18.8±1.45</td>
<td>4.01±0.33</td>
<td>14.3±1.79</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>0.04±0.008</td>
<td>0.14±0.02</td>
<td>0.04±0.01</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>2.59±0.31</td>
<td>10.9±0.89</td>
<td>6.01±1.90</td>
<td>18.6±4.90</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.33±0.12</td>
<td>1.24±0.38</td>
<td>0.28±0.08</td>
<td>0.93±0.26</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.31±0.11</td>
<td>1.20±0.30</td>
<td>0.34±0.05</td>
<td>1.15±0.08</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>1.70±0.80</td>
<td>6.25±2.26</td>
<td>1.2±0.13</td>
<td>4.31±0.68</td>
</tr>
<tr>
<td>Washout</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid fraction</td>
<td>9.14±3.97</td>
<td>23.5±8.21</td>
<td>7.90±2.78</td>
<td>20.5±4.50</td>
</tr>
<tr>
<td>Aqueous soluble</td>
<td>1.43±0.27*</td>
<td>4.00±0.66*</td>
<td>0.98±0.13*</td>
<td>3.00±0.42*</td>
</tr>
<tr>
<td>Cholesterol esters</td>
<td>0.04±0.01</td>
<td>0.11±0.02</td>
<td>0.03±0.01</td>
<td>0.08±0.03</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>7.04±3.76</td>
<td>17.5±8.01</td>
<td>6.07±2.57</td>
<td>15.1±4.22</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.08±0.02</td>
<td>0.22±0.06†</td>
<td>0.10±0.02</td>
<td>0.29±0.08†</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.28±0.11</td>
<td>0.75±0.22</td>
<td>0.24±0.08</td>
<td>0.62±0.12*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>1.70±0.26</td>
<td>4.92±0.84</td>
<td>1.47±0.27</td>
<td>4.52±0.76</td>
</tr>
</tbody>
</table>

*p' values indicate statistical comparisons of free fatty acid equivalent data between Aerobic and Ischemic/Reflow heart; p values indicate statistical comparisons of volume equivalent data between Aerobic and Ischemic/Reflow heart. Units are volume and mole equivalence of free fatty acids (see text).

* p<0.05, † p<0.1 between washout and uptake experiments.

The surprising aspect of this study was that the time constants were insensitive to ischemia and reperfusion. These time constants should be comparable to those measured from PET time–activity curves, and their insensitivity to altered perfusion states was unexpected. Several explanations are possible. The ischemic stress imposed in our studies may be less severe than that reported with the use of PET. However, at least one PET study in dogs 35 did use comparable ischemic times but with complete coronary occlusion. Although our experiments were performed with only a partial occlusion, such a restriction would provoke at least moderate insult given the absence of collateral flow in this heart model. Impairments in both mechanical and metabolic functions were noted during the ischemic interval, which continued to compromise oxygen consumption during reperfusion. Another possibility is that the two techniques, because they are interrogating cells with different metabolic activities (see above), are not measuring the same biological information from the heterogeneously perfused myocardium. The assumption of comparability of time constants might then be fallacious. A final concern deals with heart rate and its relation with the time constants of 14CO2 release. This relation was not dependent on oxygen consumption and suggested some nonmetabolic interaction that directly related to hemodynamic performance. Perhaps this determinant is influencing the two techniques differently, and the time–activity curves de-

![Figure 7](http://circres.ahajournals.org/) Correlation of calculated 14CO2 tissue pool size estimates from uptake curve analysis in both Aerobic and Ischemic/Reflow hearts, with tissue counts of radioactivity measured directly from the aqueous soluble fraction and tissue free fatty acids (FFA). The correlation was significant and linear, suggesting the two parameters may be measuring the same pool.
rived from PET should be reexamined with a focus on heart rate.

In summary, the present data do not support a major metabolic alteration in fatty acid metabolism in reflow and are confirmatory of previous data acquired in this heart model. Under the conditions of flow restriction in these studies, fatty acid oxidation was not significantly impaired in reperfusion but rather closely approximates metabolic behavior defined in aerobic hearts.

Acknowledgments


References


**KEY WORDS** • fatty acids • reperfusion • coronary effluent • radioactivity • stunned myocardium
Distribution of carbon flux within fatty acid utilization during myocardial ischemia and reperfusion.
S H Nellis, A J Liedtke and B Renstrom

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