Quantitative Assessment of Prolonged Metabolic Abnormalities in Reperfused Canine Myocardium

Denis B. Buxton, PhD; Freny Vaghaiwalla Mody, MD; Janine Krivokapich, MD; Michael E. Phelps, PhD; and Heinrich R. Schelbert, MD

Background. Prolonged metabolic abnormalities have been demonstrated previously in postischemic myocardium, including relative increases in glucose uptake and abnormal fatty acid kinetics. However, quantitative metabolic information is limited, and the time course of changes in MVO$_2$ in postischemic myocardium is unknown. To address these issues, chronically instrumented dogs were studied serially over 1 month after transient left anterior descending coronary artery (LAD) occlusion, using positron emission tomography.

Methods and Results. Dynamic imaging protocols were used in conjunction with tracer kinetic models to quantify blood flow and metabolic rates. Myocardial sectors were defined as normal, predominantly reversibly injured, and infarct-containing, based on occlusion blood flow images and postmortem histochemistry. Myocardial blood flow and metabolism were homogeneous at baseline. During LAD occlusion for 3 hours, myocardial blood flow in reversibly injured and infarct-containing sectors (determined with $^{13}$NH$_3$) was decreased to 46% and 23%, respectively, of blood flow in normal tissue. MVO$_2$, determined with [1-1$^{13}$C]acetate, was decreased less than myocardial blood flow, consistent with increased oxygen extraction in the ischemic tissue. After reperfusion, blood flow normalized rapidly in reversibly injured tissue but remained depressed in infarct-containing sectors. Regional myocardial function, assessed by two-dimensional echocardiography, was severely depressed during occlusion and did not improve significantly until 1 week after reperfusion. MVO$_2$ remained depressed after reperfusion in both reversibly injured and infarct-containing sectors, did not improve from occlusion levels until 1 week after reperfusion, and remained significantly depressed 1 month after reperfusion even in reversibly injured sectors; [1-1$^{13}$C]palmitate kinetics were also abnormal in postischemic tissue. As reported previously, glucose metabolic rates were increased relative to baseline in normal but not in postischemic tissue 3 hours after reperfusion. Subsequently, glucose metabolism tended to be higher in postischemic relative to normal myocardium.

Conclusions. The results demonstrate decreased oxidative metabolism in postischemic tissue, with concomitant abnormalities in palmitate kinetics and glucose metabolism. Oxidative metabolism and function demonstrated a parallel recovery with time. (Circulation 1992;85:1842–1856)

KEY WORDS • ischemia • reperfusion • metabolism • positron emission tomography

In recent years, it has become increasingly apparent that the functional abnormalities observed in stunned myocardium (reversibly injured myocardium reperfused after a transient ischemic injury) are accompanied by metabolic abnormalities. Increased glucose metabolism has been demonstrated in reperfused relative to normal myocardium 24 hours after reperfusion$^{1-4}$; however, early after reperfusion (0–3 hours), no such increase is observed.$^{5-7}$ The increase in glucose uptake in reperfused myocardium 24 hours after reperfusion reflects at least in part enhanced nonoxidative glucose utilization.$^{3}$ Qualitative evaluation of glucose utilization in chronically instrumented dogs demonstrated that glucose metabolism remained significantly higher in postischemic myocardium relative to remote normal myocardium 1 week after reperfusion.$^{2}$ Quantitative measurements in chronically instrumented dogs demonstrated that relative enhancement of glucose metabolism in postischemic myo-

From the Division of Nuclear Medicine and Biophysics (D.B.B., M.E.P., H.R.S.), Department of Radiological Sciences; the Division of Cardiology (F.V.M.), Department of Medicine, Wadsworth Veterans Administration Medical Center; the Division of Cardiology (J.K.), Department of Medicine, UCLA School of Medicine, University of California; and the Laboratory of Nuclear Medicine, Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles.

Supported in part by the Director of the Office of Energy Research, Office of Health and Environmental Research, Washington, DC; by research grants HL-29845 and HL-33177, National Institutes of Health, Bethesda, Md.; by an investigative group award by the American Heart Association, Greater Los Angeles Affiliate, Inc.; and by a Grant-in-Aid from the American Heart Association, with funds contributed in part by the American Heart Association, Greater Los Angeles Affiliate, Inc. The Laboratory of Biomedical and Environmental Sciences is operated for the US Department of Energy by the University of California under contract DE-FC03-87ER60615.

Address for correspondence: Denis B. Buxton, PhD, Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024-1721.

Received August 6, 1991; revision accepted December 18, 1991.
cardium was observed only when glucose metabolism in normal myocardium was low.1

Depressed oxygen consumption has also been demonstrated in reperfused myocardium, both acutely4,8–10 and 24 hours after reperfusion,4 although it should be noted that others have found normal11 or even increased12 oxygen consumption in postischemic myocardium. Conflicting results have also been published on fatty acid metabolism in postischemic myocardium. Results with positron emission tomography (PET) and [1-14C]palmitate, based on analysis of tissue time–activity curves, have been interpreted to indicate decreased oxidation and increased esterification of fatty acid in postischemic myocardium both acutely13 and in chronic preparations.2 Lopaschuk et al7 found increased esterification of labeled fatty acid in postischemic myocardium, but oxidation, although tending to be decreased, was not significantly depressed. Liedtke et al10 found normal or increased fatty acid oxidation in acutely reperfused myocardium but decreased fatty acid oxidation 4 days after reperfusion in chronically instrumented pigs.14 Thus, the time course of changes in fatty acid utilization after reperfusion remains unclear.

The aim of this study was to take advantage of the noninvasive capabilities of PET to perform serial regional quantitative measurements of flow, oxygen consumption, glucose metabolism, and qualitative assessment of fatty acid metabolism. This capability for serial studies allows the time course of changes in these parameters in response to regional ischemia and reperfusion to be examined and compared with functional measurements made with two-dimensional echocardiography. In addition, in vitro histological staining was used to divide sectors at risk during occlusion into those containing predominantly reversibly injured tissue and those containing significant amounts of infarcted tissue. The glucose metabolism and microsphere data from this study have been presented in detail elsewhere; the present study focuses on myocardial oxidative metabolism and its relations to substrate metabolism, blood flow, and regional function.

Methods

Animal Instrumentation

Nine adult mongrel dogs weighing 24–35 kg (mean, 28.2±3.2 kg) were studied. Animals were anesthetized with sodium thiamylal (Surital) 2 mg/kg i.v. and were ventilated with room air after endotracheal intubation. Anesthesia was maintained with further increments of sodium thiamylal as needed. Under sterile conditions, the heart was exposed via a left thoracotomy, and a small incision was made in the pericardial sac. Tygon tubes were inserted into the left atrium and descending aorta to inject microspheres, withdraw arterial reference blood samples, and monitor aortic blood pressure. The chest was then closed, evacuating the pneumothorax by continuous suction. The Tygon tubes were passed subcutaneously to the back of the dog and brought out through the skin between the scapula. Antibiotic therapy (ampicillin 0.6 g daily) was maintained for 3 days.

Study Protocol

Animals were studied with PET at baseline, during occlusion, and after reperfusion.

Baseline study. The animals were allowed to recover from surgery for approximately 1 week before being reanesthetized and studied with PET and 13NH3 to measure flow. One hour later, a [1-14C]acetate study was performed to measure oxygen consumption, followed 1–1.5 hours later by [1-14C]palmitate to assess fatty acid metabolism. Finally, 1–1.5 hours after injection of [1-14C]palmitate, [2-18F]fluorodeoxyglucose (FDG) was used to measure glucose metabolic rate. Two-dimensional echocardiography was performed to obtain baseline functional measurements. A wooden cradle was constructed to allow reproducible repositioning of the dog. Careful comparison of transmission scans was used to ensure accurate axial repositioning.

Occlusion. One week after the control study, the animals were reanesthetized and the left carotid artery was exposed. A 7F catheter was positioned under fluoroscopic guidance at the ostium of the left anterior descending coronary artery (LAD) and a 3F Fogarty catheter was advanced through the guiding catheter into the LAD distal to the first diagonal branch. The balloon was then inflated with a mixture of saline and contrast material, and the guiding catheter was disengaged. The animal was placed in the wooden cradle and positioned in the tomograph for imaging. Two-dimensional echocardiography was used to confirm the existence of wall motion defect. Myocardial blood flow (MBF) was determined with 15NH3 90 minutes after occlusion of the LAD. Radiolabeled microspheres were also injected at this time for flow measurement. Forty-five minutes later, MVO2 was measured with [1-14C]acetate; occlusion MVO2 was not obtained in three dogs because of technical problems.

Reperfusion. Three hours after occlusion of the LAD, the balloon catheter was slowly deflated and both the balloon and guiding catheters were removed. A bolus of lidocaine (50 mg) was administered intravenously before reperfusion, followed by drip infusion of a saline solution containing lidocaine (1 mg/min). Procanamide (300 mg) was also administered intravenously over 5 minutes immediately before reperfusion; [1-14C]acetate and [1-14C]palmitate were then used in turn to measure oxygen consumption and fatty acid metabolism. The order of the two tracers was alternated between dogs; injections were performed approximately 0.5–1 hour and 2–2.5 hours after reperfusion. Radiolabeled microspheres were given between these two tracers, and function was measured with two-dimensional echocardiography. Imaging of the heart with FDG to measure glucose metabolism was performed approximately 3.5–4 hours after reperfusion.

Follow-up studies. Twenty-four hours, 1 week, and 1 month after occlusion, animals were anesthetized again and studied with 13NH3, [1-14C]acetate, [1-14C]palmitate, and FDG with dynamic PET. Flow measurements with radiolabeled microspheres and functional measurements with two-dimensional PET were performed at each time point. One dog died of noncardiac causes (hemorrhage) before the 1-month study.

Postmortem examination. After the 1-month study, the animals were killed by intravenous injection of saturated potassium chloride while under deep anesthesia. The heart was excised and sliced into 1-cm-thick slices perpendicular to the long axis of the left ventricle. The slices were stained with triphenyltetrazolium chlo-
ride (TTC) to detect scarred and necrotic tissue\(^ {15}\) and were then cut into segments of approximately 1.5–2 g, which were subdivided into subepicardial and subendocardial halves for measurement of microsphere activity in a well counter.

**Positron Emission Tomography**

**Image acquisition.** PET was performed with a multiplane whole-body tomograph. Four dogs were studied on an ECAT III tomograph (CTI, Knoxville, Tenn.), which provides three imaging planes, and five on a 931 tomograph (CTI), which gives 15 imaging planes. When using the 931 tomograph, two adjacent slices were added to give an effective slice thickness of approximately 13 mm, similar to that of the three-slice ECAT III (approximately 12 mm). After positioning of the animal, transaxial transmission images were acquired for subsequent correction for photon attenuation. Images were reconstructed with a Shepp-Logan filter, rolled off at 0.48 cm\(^{-1}\).

**Image Processing**

Cross-sectional images of the left ventricle were analyzed with a semiautomated edge detection routine, which automatically defines the endocardial and epicardial borders after manual assignment of ellipses to approximate myocardial inner and outer boundaries.\(^ {16}\) The routine also divided myocardium into eight sectors of 45° each. An additional small region of interest was placed in the center of the left ventricular blood pool for measurement of the arterial input function.\(^ {17}\) For ECAT 911 studies, two or three cross-sectional planes were analyzed; for ECAT 931 studies, four cross-sectional planes were analyzed. Time–activity curves were then generated for each sector and the blood pool from the serial images. Correction for loss of counts caused by the partial volume effect was performed assuming a uniform wall thickness of 10 mm, with correction factors based on phantom calibration experiments. Time–activity curves were also corrected for physical decay of tracer.

**Myocardial blood flow.** For measurement of MBF, \(^ {13}\)NH\(_3\) (15 mCi) was injected intravenously as a bolus spread over 30 seconds, with simultaneous onset of injection and image acquisition. Twelve images of 10-second duration, two of 60 seconds each, and one of 900 seconds were acquired for a total of 19 minutes. MBF was quantified by application of a two-compartment tracer kinetic model\(^ {18}\) to time–activity curves obtained after intravenous administration of \(^ {13}\)NH\(_3\). The model also fits for spillover of activity from blood pool to myocardium. The first 90 seconds of data were used to derive the quantitative measurements, thus minimizing effects of recirculation of plasma metabolites.

**Myocardial glucose utilization.** Measurements of myocardial glucose utilization with FDG in these animals and glucose metabolic rate data analyzed by compartmental analysis using nonlinear regression have been reported previously.\(^ {1}\) For this study, myocardial glucose utilization was quantitated by Patlak analysis of regional time–activity curves\(^ {19}\) rather than the parameter fitting used previously\(^ {1}\) to allow inclusion of additional sectors in which nonlinear regression did not converge. The early linear portion of the Patlak plot was used to minimize effects of \(k_s\) (the rate constant for dephosphorylation of FDG-6-PO\(_4\)) on the glucose metabolic rate. A lumped constant of 0.67 was assumed to correct for differences in transport and phosphorylation rates for glucose and FDG.\(^ {20}\)

**Myocardial oxygen consumption.** Myocardial oxygen consumption was estimated using [1-\(^ {13}\)C]acetate. Myocardial [1-\(^ {13}\)C]acetate activity has been demonstrated to clear biexponentially after initial uptake from the vasculature, and the early rapid clearance phase has been shown to correlate linearly with regional M\(\text{Vo}_2\) over a wide range of conditions.\(^ {8,21–23}\) Experiments in perfused hearts suggest that the rapid exponential of tracer clearance represents oxidation via the tricarboxylic acid cycle of cycle intermediates and amino acid pools in rapid equilibrium with cycle intermediates (glutamate and aspartate), whereas the slower exponential represents equilibration with tissue glutamine.\(^ {24}\)

Six images of 30 seconds, eight of 60 seconds, six of 120 seconds, and six of 180 seconds were acquired for a total of 26 images and 41 minutes. Myocardial time–activity curves were corrected for spillover using the fraction of blood pool activity appearing in the left ventricular myocardium during the first 30-second image before injection, when left ventricular tissue activity is assumed to be minimal. This fraction of the blood pool, obtained for each sector, was subtracted for each time point of the subsequent time–activity curve.

Weighted (1/\(y\)) biexponential fitting of the regional time–activity curves was then used to obtain rate constants for the rapid and slower phases of tracer clearance. The rate constant \(k_1\) for the faster exponential was then multiplied by a constant derived from the relation between \(k_1\) and M\(\text{Vo}_2\) in validation experiments\(^ {8}\) to convert the rate constant to M\(\text{Vo}_2\). To obtain the relative contributions of the two exponentials, they were extrapolated back to the time of peak myocardial activity, and the intercept of the slower exponential was expressed as a fraction of the estimated peak activity, given by the sum of the two exponentials. The sum of the two exponentials at the time of peak myocardial activity was taken as the acetate uptake.

**Fatty acid metabolism.** The clearance of activity from myocardium after intravenous administration of [1-\(^ {13}\)C]palmitate is also biexponential; the rapid and slower phases have been attributed to direct oxidation and esterification, respectively.\(^ {25,26}\) It should be noted, however, that because oxidation of [1-\(^ {13}\)C]palmitate occurs by production of [1-\(^ {13}\)C]acetate units, the [1-\(^ {13}\)C]palmitate time–activity curves must contain an additional component reflecting the slower exponential of [1-\(^ {13}\)C]acetate clearance. The slow clearance component thus reflects the sum of lipid pool turnover esterification and equilibration of label with tissue glutamine. Myocardial time–activity curves obtained after intravenous administration of [1-\(^ {13}\)C]palmitate were corrected for spillover of activity from blood pool to tissue and subjected to weighted biexponential fitting as described previously for [1-\(^ {13}\)C]acetate. Six images of 30 seconds, eight of 60 seconds, six of 120 seconds, and six of 300 seconds were acquired for a total of 26 images and 53 minutes. The peak activity and relative contributions of the two exponentials were also determined as described for [1-\(^ {13}\)C]acetate.
Microsphere Blood Flow

Regional MBF was determined with microspheres during occlusion and 2 hours, 24 hours, 1 week, and 1 month after reperfusion as described by Heymann et al.27 Results of the microsphere flow measurements have been published previously.1

Tissue Classification

Myocardial sectors were classified as normal, predominantly reversibly injured, or infarct-containing. Classification was based on the occlusion blood flow study with \(^{13}\)NH\(_3\), which was used to identify the area at risk by visual inspection, and the postmortem TTC stain, which identified scarred and necrotic myocardium. The method used has been illustrated schematically previously.1 Postmortem myocardial slices were aligned with \(^{13}\)NH\(_3\) images, visually using landmarks such as papillary muscles, right ventricular junctions, and mitral valve plane. Sectors with reduced flow during occlusion but no necrosis were classified as reversibly injured; sectors containing necrotic tissue were classified as infarct-containing. It should be recognized that sectors defined as reversibly injured may be contaminated by signal from necrotic tissue, as discussed in more detail elsewhere (see "Methodological Considerations"). It should also be emphasized that infarct-containing sectors usually demonstrated only subendocardial infarction and thus represent a mixture of infarcted and reversibly injured tissue in the same sector. The percentage of sector area that did not stain with TTC in sectors defined as infarct-containing was 38±20% (range, 5–80%). In two dogs, no TTC-negative tissue was found, indicating absence of gross necrosis. Sectors containing the posterior papillary muscle, which have a greater wall thickness than other sectors, were omitted from analysis to reduce partial volume correction errors arising from the assumption of a constant 10-mm wall thickness. The “template” of sector assignments was then applied to the serial images, matching image planes as closely as possible. After calculation of metabolic rates for individual sectors, mean values for each tissue type were calculated for each study.

Myocardial Function

Myocardial function was assessed by two-dimensional echocardiography at each time point during the study. Short-axis two-dimensional echocardiograms were obtained at the midventricular level using a Hewlett-Packard or ATL MK 600 imaging system. Images were stored on videotape, digitized using a microprocessor, and displayed using a continuous-loop format, allowing direct comparison of images. The location, extent, and severity of wall motion abnormalities were determined visually by one observer without knowledge of the PET results. Wall motion was graded as normal (0), mildly hypokinetic (1), severely hypokinetic (2), akinetic (3), or dyskinetic (4). The inner circumference of the left ventricle and the percentage of the circumference demonstrating abnormal wall motion were measured at end diastole. The wall motion score was defined as the average wall motion grade in the myocardial segment with abnormal wall motion. The wall motion index was obtained by multiplying the mean wall motion score for the abnormal segment by the percentage of the myocardial short-axis circumference affected.

Hemodynamic and Metabolic Data

Aortic pressure and electrocardiographic data were monitored continuously throughout each study. Two arterial blood samples were withdrawn from the aorta during each study for determination of plasma glucose, lactate, and free fatty acid concentrations by standard enzymatic methods.

Data Analysis

Values are presented as mean±1 SD. Average values were calculated for each dog for each of the three tissue types, and these results were then combined to give group results with results from each dog equally weighted. For statistical analysis, comparisons between two groups were made using Student’s \(t\) test for paired or unpaired data as appropriate. Comparisons between three or more groups were made by ANOVA followed by Dunnett’s modified \(t\) test. Wall motion data were analyzed by nonparametric methods using Friedman’s test to determine whether significant differences between wall motion at different times existed, followed by the Wilcoxon signed pairs test with Bonferroni correction to determine differences between individual time points. Regression analysis used nonweighted least-squares analysis. A probability value of less than 0.05 was considered significant.

Results

Plasma substrate and hemodynamic data in these experimental animals have been published previously.1 Plasma glucose was decreased during occlusion and 3 hours after reperfusion, relative to baseline study. Twenty-four hours after reperfusion, plasma free fatty acid levels were elevated, probably reflecting the fact that the animals did not eat after the occlusion/reperfusion study and were thus in a fasted state. A concomitant decrease in plasma lactate concentrations was found at this time. No other significant alterations in plasma substrate concentrations were found.

Mean heart rates were similar at all study times. Systolic blood pressure was depressed 24 hours after reperfusion, relative to baseline study, but the rate–pressure product was not significantly decreased.

Myocardial Wall Motion

Myocardial function was assessed by two-dimensional echocardiography at each time point during the study. The results are summarized in Figure 1. Wall motion was severely depressed in the LAD territory during occlusion, as evidenced by both the mean wall motion score and the wall motion index in the affected area. Neither parameter improved 2 hours after reperfusion; thereafter, progressive recovery of wall motion occurred over the following month, although significant improvement was not found until 1 week (mean wall motion score) or 1 month (wall motion index) after reperfusion. The percentage of the myocardial circumference affected decreased from 35±12% during ischemia to 15±14% 1 month after reperfusion (\(p<0.001\)), indicating normalization of function in the less severely ischemic
being retained presumably as triglyceride. Delayed clearance of activity from $[1-1^1C]$palmitate in postischemic tissue was observed in some studies at this time (results not shown). Uptake of $^{18}$FDG in the reperfused tissue remained low relative to normal myocardium 3–4 hours after reperfusion.

Twenty-four hours after reperfusion, flow was improved in the anterior wall as indicated by the $^{13}$NH$_3$ flow image and the initial $[1-1^1C]$acetate image. Clearance of tracer after administration of $[1-1^1C]$acetate remained slower in the anterior wall, and a similar pattern of tracer clearance was also observed with $[1-1^1C]$palmitate, with decreased initial uptake and decreased tissue washout. Uptake of $^{18}$FDG was markedly enhanced in the reperfused region at this time.

One month after reperfusion, myocardial blood flow, oxygen consumption, and substrate metabolism were largely homogeneous. Postmortem TTC staining demonstrated the absence of gross myocardial necrosis in this example.

Myocardial Blood Flow

Regional MBF throughout the study, measured with $^{13}$NH$_3$ and dynamic PET, is shown in Figure 3. Blood flow was homogeneous at baseline. During occlusion, blood flow was reduced to 46±14% of that in normal tissue in sectors subsequently found to have been predominantly reversibly injured; the flow reduction was significantly greater (to 23±16% of control) in sectors that were later found to contain significant infarction.

Twenty-four hours after reperfusion, flow in reversibly injured sectors increased to 83±18% of that in normal sectors, whereas flow in infarct-containing sectors remained more severely depressed at 56±28% relative to normal sectors. One week and 1 month after reperfusion, flow in reversibly injured tissue was normal (90±15% and 87±17% of normal, respectively) but remained depressed in infarct-containing sectors (65±21% and 67±27%, respectively).

Myocardial Acetate Kinetics and Oxygen Consumption

At baseline, uptake, clearance kinetics, and the distribution of tracer between the two exponentials were regionally homogeneous after intravenous administration of $[1-1^1C]$acetate. However, regional uptake and clearance of $[1-1^1C]$acetate were abnormal during occlusion and reperfusion of the LAD. Representative time–activity curves obtained during ischemia and 1–2 hours after reperfusion are illustrated in Figure 4 and demonstrate that the slope of the first exponential was decreased in ischemic and postischemic tissue. Regional $\text{MVO}_2$ was calculated from rate constants obtained from the regional time–activity curves by using a previously determined constant.$^8$ Regional $\text{MVO}_2$ throughout the study is illustrated in Figure 5. $\text{MVO}_2$ was homogeneous at baseline but was significantly decreased in ischemic relative to normal tissue during occlusion: to 67±12% in reversibly injured tissue and to 53±26% in infarct-containing sectors. No improvement was observed in regional $\text{MVO}_2$ 1–2 hours or 24 hours after reperfusion either in reversibly injured or infarct-containing sectors, but subsequently, progressive normalization of $\text{MVO}_2$ was observed in both tissue types. However, $\text{MVO}_2$...
remained depressed at 1 month at 90±6% and 71±24%, respectively.

Apparent initial uptake of [1-13C]acetate was decreased in ischemic and posts ischemic tissue. Myocardial acetate uptake in normal, ischemic, and posts ischemic tissue as a function of time is shown in Figure 6A. The initial distribution of tracer is flow dependent because [1-13C]acetate is avidly extracted. This is reflected in the similarity between acetate uptake and MBF measured with 13NH3 (Figure 3). Relative acetate uptake in posts ischemic sectors, normalized to acetate uptake in normal sectors, correlated closely with measurements of MBF with 13NH3 in posts ischemic tissue, normalized to MBF in normal tissue \( (y = 0.82x + 11.9; r = 0.93, \text{SEE}=0.042, p<0.001) \).

The relative size of the slower exponential was also altered by ischemia and reperfusion. Figure 6B demonstrates that the contribution of the slower exponential was small and homogeneous at baseline, averaging approximately 20% of total uptake. During ischemia, this increased to 37±6% in reversibly injured sectors and 55±19% in infarct-containing sectors. Subsequently, in reversibly injured tissue, the relative size of the second exponential decreased to 28±5% at 1–2

**Figure 2.** Myocardial images obtained at the mid–left ventricular level in a dog during occlusion and reperfusion. The dog is lying on its right side and viewed from the tail, with the posterior wall to the right, anterior wall to the left, and interventricular septum below. Images from [1-13C]acetate (AC) and [1-13C]palmitate (CPA) studies show an image immediately after blood pool clearance (early) and a second image from the end of the rapid clearance phase (late). Each image is scaled to its own maximum to facilitate visualization of activity in the late image. Top panel: Occlusion and reperfusion. During occlusion, the 13NH3 blood flow image demonstrates a defect in the anterior wall (arrow). The occlusion AC images (upper row) demonstrate the flow-dependent delivery of AC (early) with slower clearance of tracer from the ischernic region relative to normally perfused tissue (late). In reperfusion, the early blood flow–dependent AC image demonstrates improved blood flow to the previously ischemic region; clearance remains slower relative to normal myocardium. CPA uptake is depressed in postischernic tissue in the early image; clearance is fairly homogeneous in this example (late). Fluorodeoxyglucose (FDG) uptake is depressed in the previously ischemic tissue. Middle panel: One day after reperfusion; 13NH3 image shows improved blood flow in postischernic tissue. Clearance of AC (yellow arrow) and CPA (blue arrow) is delayed in the posts ischemic tissue (late images). The same region displays increased FDG uptake (gray arrow). Bottom panel: One month after reperfusion. Blood flow, uptake, and clearance of AC and CPA, and FDG uptake are largely homogenous.

**Figure 3.** Distribution of [1-13C]acetate in the dog heart 1 hour after injection. The dog is lying on its right side and viewed from the tail, with the posterior wall to the right, anterior wall to the left, and interventricular septum below. Each image is scaled to its own maximum to facilitate visualization of activity in the late image. The distribution of acetate uptake is shown in the anterior wall (arrow). The 13NH3 blood flow image demonstrates a defect in the anterior wall (arrow). The occlusion AC images (upper row) demonstrate the flow-dependent delivery of AC (early) with slower clearance of tracer from the ischemic region relative to normally perfused tissue (late). In reperfusion, the early blood flow–dependent AC image displays improved blood flow to the previously ischemic region; clearance remains slower relative to normal myocardium. CPA uptake is depressed in postischernic tissue in the early image; clearance is fairly homogeneous in this example (late). Fluorodeoxyglucose (FDG) uptake is depressed in the previously ischemic tissue. Middle panel: One day after reperfusion; 13NH3 image shows improved blood flow in postischernic tissue. Clearance of AC (yellow arrow) and CPA (blue arrow) is delayed in the posts ischemic tissue (late images). The same region displays increased FDG uptake (gray arrow). Bottom panel: One month after reperfusion. Blood flow, uptake, and clearance of AC and CPA, and FDG uptake are largely homogenous.

**Figure 4.** Relative uptake of 13NH3 in the dog heart 1 hour after injection. The dog is lying on its right side and viewed from the tail, with the posterior wall to the right, anterior wall to the left, and interventricular septum below. Each image is scaled to its own maximum to facilitate visualization of activity in the late image. The distribution of acetate uptake is shown in the anterior wall (arrow). The 13NH3 blood flow image demonstrates a defect in the anterior wall (arrow). The occlusion AC images (upper row) demonstrate the flow-dependent delivery of AC (early) with slower clearance of tracer from the ischemic region relative to normally perfused tissue (late). In reperfusion, the early blood flow–dependent AC image displays improved blood flow to the previously ischemic region; clearance remains slower relative to normal myocardium. CPA uptake is depressed in postischernic tissue in the early image; clearance is fairly homogeneous in this example (late). Fluorodeoxyglucose (FDG) uptake is depressed in the previously ischemic tissue. Middle panel: One day after reperfusion; 13NH3 image shows improved blood flow in postischernic tissue. Clearance of AC (yellow arrow) and CPA (blue arrow) is delayed in the posts ischemic tissue (late images). The same region displays increased FDG uptake (gray arrow). Bottom panel: One month after reperfusion. Blood flow, uptake, and clearance of AC and CPA, and FDG uptake are largely homogenous.
Figure 3. Bar graph shows time course of myocardial blood flow measured with $^3$H$_2$ and dynamic positron emission tomography at baseline and during left anterior descending coronary artery occlusion and reperfusion. Closed bars, normal myocardium; shaded bars, reversibly injured tissue; open bars, infarct-containing sectors. *p<0.001 vs. normal sectors; **p<0.01 vs. normal sectors; #p<0.05 vs. reversibly injured sectors.

hours after reperfusion and normalized by 1 month but remained elevated in infarct-containing sectors at 42±21% 1 month after reperfusion.

Myocardial Oxygen Extraction

The chemical extraction of oxygen by myocardium was estimated by dividing MVO$_2$, determined from regional $[1^{-13}]$C acetate kinetics, by MBF, determined by microspheres (Figure 7). Oxygen extraction in normal sectors increased significantly in reversibly injured and infarct-containing sectors during LAD occlusion, but 2 hours after reperfusion, no significant differences in oxygen extraction were found between tissue types. In contrast, oxygen extraction was decreased in reversibly injured tissue 24 hours and 1 week after reperfusion but had normalized by 1 month after reperfusion. No significant alterations in oxygen extraction were found in infarct-containing sectors after reperfusion. Similar results were obtained when oxygen extraction was estimated using MBF determined with dynamic PET, using $^3$H$_2$ (results not shown).

Relation Between VO$_2$ and Wall Motion

It may be observed from Figures 1 and 5 that the time courses of recovery of regional wall motion and MVO$_2$ were generally similar. To compare these two parameters more directly, the mean MVO$_2$ of the area of

Figure 4. Plots show myocardial $[1^{-13}]$C acetate tissue time–activity curves. Panel A: During left anterior descending coronary artery occlusion; panel B: 2 hours after reperfusion. Closed circles, normal tissue; open circles, reversibly injured tissue; closed squares, infarct-containing sectors; k$_1$, mean rate constant for the rapid exponential determined regionally for each sector type.
FIGURE 6. Bar graphs. Panel A: Regional uptake of [1-11C]acetate; panel B: relative size of the slower clearance exponential in normal, reversibly injured, and infarct-containing sectors. Closed bars, normal myocardium; shaded bars, reversibly injured tissue; open bars, infarct-containing sectors. *p<0.05 vs. normal myocardium; **p<0.05 vs. normal tissue.

decreased occlusion blood flow at a midventricular level was normalized to MVO_2 in normal sectors and plotted as a function of the regional myocardial wall motion index. Figure 8 shows the significant relation between these two parameters during occlusion and over the following period of reperfusion (p<0.001).

Kinetics of [1-11C]Palmitate

The rate constant $k_1$ for the rapid clearance phase was homogeneous at baseline, but, as has been reported previously, after reperfusion, $k_1$ was decreased in postischemic sectors (Figure 9A). Again, progressive recovery of $k_1$ was observed over the following month. The decrease in $k_1$ for palmitate was similar to that observed for $k_1$ for acetate and hence, to the decrease in MVO_2.

The relative size of the slower phase of tracer clearance from myocardium was also increased for palmitate in postischemic tissue (Figure 9B), again in agreement with previous studies. Early (2 hours) after reperfusion, differences between normal and postischemic sectors were small compared with subsequent studies, which is similar to results in acute reperfusion studies. Twenty-four hours after reperfusion, the relative size of the second exponential was reduced in normal myocardium, probably reflecting increased oxidative fatty acid utilization in response to elevation of the plasma free fatty acid concentration. In postischemic tissue, the size of the second phase remained elevated relative to normal tissue and continued to show a small but significant elevation 1 month after reperfusion in infarct-containing sectors. Overall, the relative size of the first exponential in normal myocardium displayed a significant positive correlation with the plasma fatty acid concentration (percentage of first exponential=50+29.7×[plasma free fatty acid]; $r=0.46, p<0.01$), whereas no significant relation was found in postischemic tissue.

To investigate the relation between palmitate oxidation and MVO_2, the ratio of $k_1$ for palmitate to that for acetate was determined. The ratio averaged 0.75 at baseline; no significant changes in this ratio were observed, with the exception of a significant increase in reversibly injured myocardium 24 hours after reperfusion to 1.05±0.14, compared with 0.85±0.14 in normal sectors ($p<0.002$; results not shown). A significant correlation was observed between the rate constants for the two tracers in normal, reversibly injured, and infarct-containing sectors ($r=0.67, p<0.001$; results not shown). However, the slope was significantly less than unity (0.47; SEE=0.061, $p<0.001$). No significant differences were found between the slopes of the relations for the three tissue groups.

Glucose Metabolic Rate

The effects of reperfusion after 3 hours of regional ischemia on glucose metabolic rate (MRGlc) in these studies have been described previously. For the present study, MRGlc was estimated using Patlak analysis, allowing inclusion of additional sectors in which fitting with the five-parameter model was unsatisfactory. No significant differences in MRGlc between the two methods were found except 24 hours after reperfusion, when MRGlc values obtained by Patlak fitting were approximately 70% of those obtained using the five-parameter model. At baseline, MRGlc varied considerably from dog to dog, probably reflecting dietary variability. Three hours after reperfusion, MRGlc was elevated in normal
remote myocardium relative to the baseline study, but no increase in MRGlc was observed in postischemic tissue. Twenty-four hours after reperfusion, MRGlc decreased in normal myocardium, probably reflecting a switch to fatty acid metabolism. The increase in fatty acid oxidation, demonstrated by the increase in the relative size of the first exponential of tracer clearance after [1-13C]palmitate administration, reflects the fasted state of the dogs at this time, because they did not eat overnight after the occlusion and reperfusion; however, the decrease in glucose metabolism was attenuated in reversibly injured myocardium, leading to an elevated MRGlc relative to normal remote myocardium. Subsequently, no significant differences in MRGlc were found between tissue types. MRGlc was lower in all tissue types 1 day after reperfusion relative to baseline and tended to remain depressed relative to baseline.

**Glucose Extraction**

Changes in the chemical extraction of glucose were estimated by dividing the regional myocardial MRGlc by MBF, determined with 13NH3. PET-derived flows were used to minimize the effect of partial volume on glucose extraction estimates, because underestimation of tissue activity caused by the partial volume effect should be similar for both the flow and metabolic tracers. Glucose extraction was homogeneous at rest but significantly increased in postischemic relative to normal myocardium 1 day after reperfusion, returning toward normal over the course of the following month (Figure 10A). Interestingly, the difference between remote and postischemic myocardial glucose extraction reflected a prolonged decrease in glucose extraction in normal remote myocardium relative to the baseline study rather than an increase in postischemic myocardium. Early (3 hours) after reperfusion, flow was not measured with 13NH3; however, by using microsphere blood flow, glucose extraction was significantly decreased in postischemic tissue, from 0.83±0.4 µmol/ml in normal tissue to 0.40±0.14 and 0.39±0.24 µmol/ml in reversibly injured and infarct-containing tissue, respectively (p<0.005).

**Relation Between Glucose Metabolic Rate and MVO2**

The MRGlc's obtained by Patlak analysis of FDG studies were normalized to MVO2 to give the oxygen equivalence of glucose, assuming a value of 6 mol of oxygen used per mole of glucose oxidation. The values obtained thus represent an upper limit on the contribution of glucose to overall substrate oxidation; in reality, the actual contribution will be less because some glucose will be converted to lactate, some to glycogen, and minor amounts will enter other pathways such as the pentose phosphate pathway. Baseline rates were homogeneous, with a mean value of approximately 60% (Figure 10B). Three hours after reperfusion, MRGlc/MVO2 tended to be higher in normal tissue relative both to the baseline study and to postischemic tissue, but in neither case was the difference significant.

Twenty-four hours after reperfusion, MRGlc/MVO2 decreased significantly in normal tissue relative to baseline, reflecting elevated plasma fatty acid levels and a switch to myocardial fatty acid utilization. MRGlc/MVO2 did not decrease in postischemic tissue, however, and was thus increased relative to normal tissue. MRGlc/MVO2 remained elevated in postischemic relative to normal.
tissue 1 week after reperfusion but was no longer significantly increased by 1 month after reperfusion.

**Relation Between Glucose Metabolic Rate and Palmitate Kinetics**

In normal tissue, a significant correlation was observed between the relative size of the second exponential of tracer clearance after [1-13C]palmitate administration and glucose metabolic rate, which was normalized to myocardial oxygen consumption (MRGlc/MVO2). A plot of the percentage of second phase as a function of percentage of MRGlc/MVO2 was described by \( y=22+0.31x \) (\( r=0.53, p<0.0025 \)). Thus, when MRGlc was low, and hence fatty acid was the main oxidative substrate, the contribution of the second exponential approached 20%, in agreement with the value found by using [1-13C]acetate as tracer; this suggests that the contribution of esterification to the second exponential became minimal, with all label entering the tricarboxylic acid cycle and equilibrating with amino acid pools. In postischemic tissue, the relation between the relative size of the second exponential and normalized glucose metabolism was not significant (\( y=43.5+0.12x; r=0.33, {NS} \)), and the slope of the regression line was significantly less than that for normal remote tissue (\( p<0.05 \)). When the postischemic tissue results were divided into early (2 hours to 1 week after reperfusion) and late (1 month after reperfusion) time points, a significant increase in the slope of relation between the relative size of the second exponential and normalized glucose metabolism was found, increasing from 0.094 (SEE=0.061) to 0.439 (SEE=0.162; \( p<0.05 \)).

**Discussion**

Previous studies in this laboratory have demonstrated prolonged metabolic abnormalities in postischemic myocardium, including a relative increase in FDG uptake and abnormal [1-13C]palmitate kinetics.1-4 These studies have now been expanded, taking advantage of advances in PET technology to obtain quantitative flow and metabolic data. These advances include 1) the improved spatial and temporal resolution of current generation tomographs, reducing the spillover of activity between adjacent regions, and permitting quantification with dynamic imaging; and 2) the use of [1-13C]acetate, which was recently validated as a quantitative tracer for measurement of regional MVO2.8,23,28

**Methodological Considerations**

The experimental model used in this study, a 3-hour occlusion in closed-chest dogs, was selected to be clinically relevant, leading to a spectrum of myocardial injury including both reversible injury and a varying extent of necrosis. Necrosis was largely confined to the subendocardial layer. To reduce the effect of the variability of injury on the metabolic parameters measured, myocardial sectors at risk during occlusion were divided into those containing only reversibly injured tissue and those containing significant amounts of necrotic and scar tissue on the basis of gross postmortem TTC staining. Infarct-containing sectors thus represent a heterogeneous tissue population containing varying degrees of necrosis and viable cells. Limitations of this classification technique include the difficulty of ensuring exact registration of TTC-stained myocardial slices with PET-derived images, which leads to the possibility that some necrotic tissue may be included in sectors classified as reversibly injured. The comparison of transaxial image planes with short-axis tissue slices makes accurate correlation with the postmortem examination more difficult; however, because the region of most interest, the LAD territory, is adjacent to the right ventricle, visualization of the right ventricle and right ventricular blood pool facilitates localization of the anterior wall and septum with the short-axis slices. Microinfarctions not observable by the gross separation of tissue on the basis of TTC staining may also have occurred. Morphological changes in the infarcted tissue, with condensation of scar and hypertrophy of surviving tissue, are also likely.29 In addition, limitations in the spatial resolution of the tomographs used will still result in spillover of activity from sector to sector, thus contaminating the signal from reversibly injured sectors by adjacent infarct-containing (and normal) sectors and visa versa; thus, some sectors defined as reversibly injured are likely to contain a small contribution from infarcted myocardium. However, this method does al-
low the separation of sectors containing significant necrosis from those with minimal necrosis and thus gives better spatial resolution than metabolic measurements based on arteriovenous sampling.

A further assumption made in this study was the use of a constant recovery coefficient for tissue activity based on a uniform wall thickness of 10 mm. Underestimation of tissue activity caused by wall motion abnormalities has been demonstrated previously.\textsuperscript{30} Count recovery was decreased 36\% during ischemia, when wall thickening was replaced regionally by paradoxical bulging.\textsuperscript{30} This loss in count recovery will be less severe in the present study, which used newer tomographs with higher resolution. However, it is clear that count loss caused by wall motion abnormality will tend to reduce apparent blood flow and glucose metabolic rates in posts ischemic myocardium. It should be noted that \( k_t \) for \([1-\text{C}]\)acetate (and hence, estimates of \( \text{MVO}_2 \)) and \([1-\text{C}]\)palmitate will not be affected by underestimation of tissue activity caused by decreased wall thickening in posts ischemic tissue because the constant factor will not affect the exponential clearance rate.

Glucose metabolic rates were calculated assuming a fixed lumped constant of 0.67\%; the estimates obtained thus assume that the lumped constant did not differ in posts ischemic tissue and was not altered in response to changes in plasma substrates and effectors. Experiments performed both in vivo\textsuperscript{36} and in vitro\textsuperscript{31} have demonstrated the stability of the lumped constant in all except extreme nonphysiological conditions. In addition, it has recently been shown in the isolated perfused rat heart that the lumped constant was unchanged in reperfused myocardium and in fasting.\textsuperscript{32} Patlak analysis of FDG time–activity curves was used in the present study in contrast to the five-parameter fitting used in the detailed analysis of the FDG data published previously; this allowed additional sectors to be included in which fitting by nonlinear regression was unsatisfactory. Results obtained by the two methods correlated well (MRGlc by five-parameter fit = 0.96 × MRGlc Patlak + 0.14; \( r = 0.93 \)). The reasons for the nonzero intercept have not been explored but may reflect increasingly significant effects of \( k_2 \), the dephosphorylation rate constant, on the accuracy of the Patlak analysis at low MRGlc despite fitting the early linear portion of the Patlak plots when the effects of \( k_4 \) are minimized.

Also of potential concern is the contribution of infiltrating inflammatory cells to the metabolic changes observed, because leukocytes use anaerobic glycolysis for most of their energy needs. Although this issue has not been addressed directly, FDG uptake was not found to correlate well with deposition of \([11\text{C}]\)In-labeled leukocytes in dogs subjected to two hours of LAD occlusion and 4 hours of reperfusion;\textsuperscript{33} increased FDG uptake occurred predominantly in areas of posts ischemic viable tissue, whereas leukocyte accumulation occurred predominantly in necrotic areas, including patchy necrosis. Elevated FDG uptake in posts ischemic tissue has also been demonstrated in the absence of necrosis, when leukocyte accumulation is much attenuated;\textsuperscript{34} after a 20-minute occlusion in closed-chest dogs, when no necrosis is found;\textsuperscript{35,35} and in less severely ischemic dogs with longer occlusion periods in which necrosis is absent.\textsuperscript{33} The quantitative measurement of oxygen consumption with \([1-\text{C}]\)acetate has been validated over a wide range of conditions, including changes in substrate supply, ischemia, and acute reperfusion in the first 3 hours after a 20-minute LAD occlusion.\textsuperscript{8,23,36} However, the method has not been validated in reperfusion after more severe ischemia, when subendocardial infarction occurs, or at later times after reperfusion. Biochemical measurements in isolated hearts suggest that the relation between \( k_t \) for acetate and \( \text{MVO}_2 \) would be modified by changes in the sizes of tissue pools of glutamate and aspartate, which are in equilibrium with tricarboxylic acid intermediates.\textsuperscript{34} Although alterations in the relation between \( k_t \) and \( \text{MVO}_2 \) might be expected to be most apparent early after reperfusion, the validity of the method at later times will need to be confirmed by invasive methods.

**Myocardial Blood Flow**

Measurement of MBF with \( ^{13}\text{N} \text{H}_3 \) and PET demonstrated an apparent decrease in MBF in reversibly injured tissue 1 day after reperfusion, with no subsequent significant flow deficit. Because microsphere measurements of MBF in these dogs demonstrated that flow was not significantly depressed in reversibly injured sectors 2 hours or 24 hours after reperfusion,\textsuperscript{3} this apparent flow deficit is likely to reflect decreased count recovery caused by wall motion abnormality and the partial volume effect.\textsuperscript{30} Microsphere blood flow was also normal after reperfusion in the subepicardium of infarct-containing sectors, whereas blood flow remained depressed in the subendocardium of infarcted tissue, reflecting the largely subendocardial nature of the injury in this model.\textsuperscript{1} This is consistent with the greater degree of flow reduction in the subendocardium, in which MBF was reduced to 18\% of control compared with the value of 46\% of control found in the subepicardium of infarct-containing sectors.\textsuperscript{1}

**Metabolic Measurements**

The results obtained in this study demonstrate that \( \text{MVO}_2 \) remained depressed in posts ischemic tissue for prolonged periods after reperfusion. This continued depression of \( \text{MVO}_2 \) occurred despite the rapid normalization of MBF in reversibly injured sectors, indicating that oxygen extraction was depressed in this predominantly stunned tissue.

One to 2 hours after reperfusion, \( \text{MVO}_2 \) in reversibly injured and infarct-containing sectors was decreased to 69\% and 48\%, respectively, of levels in normal tissue. The MRGlc in normal tissue was increased relative to baseline, whereas MRGlc in posts ischemic tissue at this time was low relative to normal remote tissue, in agreement with other studies demonstrating the absence of preferential glucose utilization in posts ischemic myocardium early after reperfusion.\textsuperscript{5,6,10} Glucose metabolism was also decreased at this time after normalizing for relative flow using the regional initial uptake of \([1-\text{C}]\)acetate (in the absence of \( ^{13}\text{N} \text{H}_3 \) flow measurements). Palmitate kinetics were consistent with regional fatty acid oxidation being decreased in parallel with oxygen consumption in posts ischemic myocardium, with increased esterification of palmitate indicated by increased relative size of the second exponential.

The absence of an increase in glucose metabolism 3 hours after reperfusion contrasts with results obtained 24 hours after reperfusion, when reversibly injured
sectors displayed a significant increase in MRGlc relative to normal remote tissue, and glucose extraction was increased in postischemic relative to normal tissue. Oxidation was the primary fate of long-chain fatty acids at this time, as indicated by the tracer tissue kinetics after \([1-{^1}C]\)palmitate injection. The relative size of the second exponential was close to 20% in normal remote tissue, indicating esterification of palmitate to be very low; as esterification decreases and an increasing fraction of \(^{13}C\) activity enters the tricarboxylic acid cycle as \([1-{^1}C]\)acetate, tissue tracer kinetics will tend toward the kinetics seen with direct injection of \([1-{^1}C]\)acetate, in which the second exponential accounts for \(-20\%\) of the tracer clearance. These changes in substrate metabolism reflect a switch to fatty acid oxidation in response to elevated plasma fatty acid levels. However, in postischemic tissue, the relative size of the slower palmitate clearance phase remained elevated, consistent with continued esterification.

One week after reperfusion, the general metabolic pattern was similar to 24 hours after reperfusion. MVo2 and fatty acid oxidation, although improved from 1 day after reperfusion, remained depressed in postischemic relative to remote tissue, with concomitant elevation of fatty acid esterification. Whereas glucose uptake in postischemic tissue was not increased overall at this time,\(^1\) this probably reflects in part underestimation of glucose uptake in hypokinetic myocardium caused by the partial volume effect, because glucose extraction calculated with \(^{13}NH_3\)-derived blood flow measurements, in which partial volume effects on flow and glucose metabolism will tend to cancel out, was elevated. In contrast, using microsphere blood flows to calculate glucose extraction, no significant difference was found between normal and postischemic myocardium;\(^1\) the difference between the two methods is likely to reflect the partial volume effect and possibly misalignment of images with in vitro tissue data. MVo2 had not normalized completely 1 month after reperfusion either in infarct-containing or reversibly injured sectors.

To obtain estimates for the contribution of glucose metabolism to overall oxidative metabolism, regional MRGlcs were normalized to regional MVo2 estimated from acetate \(k_c\). Assuming the size of the glycogen pool to be constant during the imaging period, this figure will give an upper estimate for the contribution of glucose metabolism to overall oxidative metabolism, ignoring the production of lactate and other pathways. The oxidative equivalence of glucose, MRGlc/MVo2, was quite high during the baseline study, accounting for 60% of oxidative metabolism. Three hours after reperfusion, MRGlc/MVo2 tended to be increased in normal remote myocardium, although this difference did not reach statistical significance and could account for 100% of oxidative metabolism. Because time–activity curves obtained with \([1-{^1}C]\)palmitate were consistent with continuing fatty acid oxidation at this time, this finding may suggest increased incorporation of glucose into glycogen in normal myocardium; of note is the qualitative demonstration of depressed glycogen content in remote myocardium during LAD occlusion in open-chest dogs.\(^{37}\) Increased lactate output has also been reported from nonischemic as well as postischemic tissue after reperfusion,\(^{37}\) which could also contribute to the high MRGlc.

MRGlc/MVo2 in normal remote myocardium fell to 25% of MVo2 24 hours after reperfusion. A similar value (\(-27\%)\) can be derived from arteriogenous sampling data in open-chest dogs 24 hours after reperfusion.\(^3\) The increase in MRGlc/MVo2 in postischemic tissue at this time has been shown to reflect largely increased nonoxidative glucose metabolism.\(^3\) It is unclear at present whether the continued elevation of MRGlc/MVo2 in postischemic tissue 1 week after reperfusion represents continued nonoxidative glucose utilization or a switch in substrate preference from fatty acid to glucose. It should be noted that partial volume effects will tend to cause an underestimation of MRGlc/MVo2 in postischemic tissue, because, as discussed above, estimates of MRGlc will be affected by count loss, whereas estimates of MVo2 will not.

A number of lines of evidence are consistent with abnormalities in the relation between fatty acid oxidation and glycolytic flux in reperfused myocardium. In normal myocardium, the fraction of palmitate oxidized, as indicated by the relative size of the rapid clearance phase, correlated positively (this study) and the MRGlc correlated negatively\(^1\) with the plasma free fatty acid concentration. In postischemic tissue, neither relation was significant, suggesting that switching between glucose and fatty acid metabolism in response to extracellular signals is abnormal in postischemic tissue. In addition, the proportion of palmitate entering esterification correlated with MRGlc/MVo2 in normal tissue but not in postischemic tissue overall; however, by 1 month after reperfusion, the relation between these parameters returned to normal. Increases in MRGlc in postischemic relative to normal tissue were observed only when MRGlc in normal tissue was low, again consistent with decreased suppressibility of glycolytic flux in postischemic tissue.\(^1\) Similar results have been found using in vitro tissue counting of FDG tissue activity in dogs studied 24 hours after a 20-minute LAD occlusion.\(^{35}\) Although the abnormalities in regulation of glycolysis are unknown at present, phosphofructokinase (PFK) represents a key regulatory step linking fatty acid oxidation and glycolytic flux. Because PFK is believed to be stimulated by increases in cytosolic calcium,\(^{38}\) the increased calcium transients reported in acutely stunned ferret heart\(^{39}\) suggest one possible mechanism for stimulation of glycolytic flux in postischemic myocardium. However, the status of calcium transients at later times after reperfusion in vivo is unknown at present.

The decrease in MVo2 early after reperfusion in reversibly injured sectors was relatively small, averaging approximately 35%, despite severe dysfunction. This result is similar to the decrease in regional MVo2 in regional dyskinesia induced by intracoronary infusion of lidocaine\(^{40}\) or cardioplegia solution,\(^{41}\) when MVo2 was reduced regionally to 55–67% of that in normal myocardium. This paradoxically high MVo2 of dyskinetic myocardium may be related to passive stretch, because ventricular unloading decreased MVo2 in the dyskinetic segment to approximately 25% of normal.\(^{40,4}\) The generation of residual systolic work in postischemic myocardium, despite severe dyskinesia, has also been demonstrated.\(^42\) The time courses for the recovery of regional MVo2 and of function, assessed by two-dimensional echocardiography, were similar, and a modest
correlation was found between recovery of the two parameters. A closer correlation between these two parameters would not necessarily be expected. Wall motion assessment by two-dimensional echocardiography gives only a partial measurement of overall myocardial functional energy expenditure, neglecting factors such as changes in wall stress. Inaccuracies in registration of echocardiograms and PET images may also decrease the correlation.

Palmitate time-activity curves were consistent with decreased fatty acid oxidation and increased esterification in postischemic tissue. The increase in the relative size of the second exponential was small early (1–2 hours) after reperfusion. This may reflect increased back-diffusion of nonmetabolized palmitate from postischemic tissue, which is not distinguished kinetically from tracer oxidation. Increased back-diffusion has been demonstrated in ischemic and hypoxic myocardium.25,43,44

The effects of reperfusion on the first exponential k1 of tracer clearance have been reported to be more severe for [1-14C]palmitate than for [1-13C]acetate,45 possibly implying preferential inhibition of fatty acid oxidation in reperfused myocardium. However, a comparison of the rate constants for palmitate and acetate in the present study did not show any greater decrease in k1 for the long-chain fatty acid at any time point. In fact, k1 palmitate/k1 acetate was increased relative to baseline 24 hours after reperfusion; the reason for this increase is unclear. Caution should be exercised in comparing k1 for the short- and long-chain fatty acids, because the extent of back-diffusion of [1-13C]palmitate in postischemic tissue, particularly in chronic situations, is currently unknown. Changes in the distribution volumes of the tracers may also complicate this issue.

The finding of sustained metabolic abnormalities in postischemic tissue 1 month after reperfusion is interesting. By this time, necrotic tissue has been largely replaced by scar tissue, and thus metabolism in infarct-containing sectors reflects metabolism of reversibly injured cells, mostly in the subepicardium. Comparison of results in the predominantly reversibly injured sectors and infarct-containing sectors reveals a similar metabolic pattern, with decreased oxygen consumption, abnormal palmitate kinetics, and increased glucose extraction. The results are consistent, with the surviving myocardium in infarct-containing sectors being more severely injured and recovering more slowly than the tissue in reversibly injured sectors, reflecting the greater degree of ischemia during occlusion.

The decreased MBV2 in postischemic tissue found in this study could reflect a functional deficit in the reperfused tissue or a limitation of function by decreased oxidative capacity, and the results obtained cannot distinguish between these possibilities. Bush et al46 performed LAD occlusions for 2 hours or 4 hours in conscious dogs, and found that, for myocardial segments with moderate to severe segmental dysfunction during occlusion, functional recovery was poorer at 1 month after reperfusion in dogs subjected to the longer occlusion despite a similar degree of necrosis; they hypothesized that a continued metabolic dysfunction could be responsible. Ventricular remodeling could contribute to the metabolic abnormalities observed in postischemic tissue; in rats, myocyte hypertrophy in the peri-infarct zone was shown to lead to increased oxygen diffusion distances,47 which could lead to metabolic abnormalities caused by decreased oxygen tension.

The results obtained in this study can be extrapolated to the clinical situation of acute myocardial infarction with salvage of tissue at risk by reperfusion of the occluded vessel. Oxidative metabolism would be expected to be depressed and glucose metabolism enhanced early after reperfusion in such patients. Preliminary reports suggest that this is the case; several studies have demonstrated depressed MVo2 in the risk zone in acute myocardial infarction. In the absence of reperfusion, no improvement was found in oxidative metabolism,48 but after thrombolysis, a progressive recovery of MVo2 was observed.49 In patients studied early (2–7 days) after acute myocardial infarction, myocardial segments at risk were divided into a group with enhanced FDG uptake relative to MFB and a group with concordant decreases in MFB and metabolism.50 Although MVo2 was decreased in both groups relative to remote tissue, enhanced FDG uptake was associated with higher MVo2 relative to segments with concordant decreases in MFB and FDG uptake. At follow-up (70–140 days), wall motion and MVo2 improved in the group with previously enhanced FDG uptake, and FDG uptake normalized. In contrast, no changes in function or metabolism were found in the group previously displaying concordant decreases in MFB and FDG uptake. Thus, while in the clinical setting of acute myocardial infarction, recovery of tissue is likely to be complicated further by varying degrees of residual stenosis, preliminary studies are consistent with a similar metabolic pattern of decreased oxidative metabolism, recovering in parallel with function, and increased glucose uptake, which tends to normalize with time.

Conclusions

Postischemic canine myocardium displays prolonged depression of MVo2 despite a rapid normalization of MFB, indicating decreased oxygen extraction in the reperfused tissue. Abnormalities of substrate metabolism are also found in postischemic myocardium, in which the normal relations between substrate selection and plasma substrate concentrations are weak or absent. Palmitate kinetics are consistent with a similar degree of depression of fatty acid oxidation and overall oxidative metabolism. The recovery of oxidative metabolism in postischemic tissue parallels the recovery of regional function.

Acknowledgments

We thank Herbert W. Hansen, MS, for technical assistance. The preparation of radioisotopes by Dr. N. Satyamurthy and his cyclotron staff and the technical support of Ron Sumida and his staff in running the tomographs are gratefully acknowledged. We also thank Dr. Sanjiv Gambhir and Dr. Henry Huang for provision of computer programs used in the data analysis and for their helpful discussions, and Lee Griswold and Wendy Wilson for preparation of illustrations.

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Circulation. 1992;85:1842-1856
doi: 10.1161/01.CIR.85.5.1842

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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