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Sustained Nonoxidative Glucose Utilization and Depletion of Glycogen in Reperfused Canine Myocardium

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Ischemically injured reperfused myocardium is characterized by increased ^{18}F-fluorodeoxyglucose uptake as demonstrated by positron emission tomography. To elucidate the metabolic fate of exogenous glucose entering reperfused myocardium, D-[6-^{14}C] glucose and L-[U-^{13}C] lactate were used to determine glucose uptake, glucose oxidation and the contribution of exogenous glucose to lactate production. The pathologic model under investigation consisted of a 3 h balloon occlusion of the left anterior descending coronary artery followed by 24 h of reperfusion in canine myocardium. The extent and severity of myocardial injury after the ischemia and reperfusion were assessed by histochemical evaluation (triphenyltetrazolium chloride and periodic acid-Schiff stains). Thirteen intervention and four control dogs were studied.

The glucose uptake in the occluded/reperfused area was significantly enhanced compared with that in control dogs (0.40 ± 0.14 versus 0.15 ± 0.10 μmol/ml, respectively). In addition, a significantly greater portion of the glucose extracted immediately entered glycolysis in the intervention group (75%) than in the control dogs (33%). The activity of the nonoxidative glycolytic pathway was markedly increased in the ischemically injured reperfused area, as evidenced by the four times greater lactate release in this area compared with the control value. The dual carbon-labeled isotopes showed that 57% of the exogenous glucose entering glycolysis was being converted to lactate. Exogenous glucose contributed to >90% of the observed lactate production. This finding was confirmed by the histochemical finding of sustained glycogen depletion in the occlusion/reperfusion area. The average area of glycogen depletion (37%) significantly exceeded the average area of necrosis (17%).

These data demonstrate enhanced and sustained activity of the nonoxidative glycolytic pathway after a prolonged occlusion with reperfusion in canine myocardium. Because glycogen stores remain depleted, exogenous glucose becomes an important myocardial substrate under these pathologic conditions.

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Reperfusion to salvage ischemically injured myocardium has recently become the focus of experimental and clinical research (1–5). It is known that regional myocardial function remains impaired for a prolonged period after ischemia, even in reversibly injured myocardium (6). In addition, it has been shown that the delayed functional recovery is paralleled by a sustained decrease in the tissue concentration of high energy phosphate, indicating abnormalities in myocardial energy

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JACC Vol. 13, No. 3
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metabolism (7). Recent studies (8,9) addressing substrate metabolism in reperfused myocardium have demonstrated delayed recovery of fatty acid oxidation even after short periods of ischemia.

Increased glucose utilization has been observed during myocardial ischemia and hypoxia (10). More recent animal and clinical studies (11–13) using radiotracers have demonstrated a similar metabolic pattern in reperfused myocardium. In the canine model, sustained abnormalities in ^{11}C-palmitate kinetics in post-ischemic myocardium were matched by increased ^{18}F-fluorodeoxyglucose (^{18}F-deoxyglucose) uptake, suggesting increased use of exogenous glucose in the presence of impaired fatty acid metabolism after reperfusion (11).

Because ^{18}F-deoxyglucose traces only transmembraneous transport and phosphorylation of glucose, limited information about glucose metabolism can be derived from the use of this tracer. To define the metabolic fate of exogenous glucose taken up in ischemically injured reperfused canine myocardium, the relative contributions of oxidative and nonoxidative glucose utilization were assessed by a dual carbon-labeled isotope technique using D-[6-^{14}C] glucose and L-[U-^{13}C] lactate. Exogenous glucose oxidation based on ^{14}C-labeled carbon dioxide ($^{14}CO_2$) production and the isotopically measured lactate release were quantified. In addition, the contribution of exogenous glucose to lactate production was determined. We employed histochemical techniques to independently define the extent and severity of myocardial injury in the occlusion/reperfusion area and to correlate biochemical findings with the morphologic manifestation of ischemic injury in that area.

Methods

Study protocol and animal preparation. Nineteen adult mongrel dogs weighing 21.5 to 32.5 kg were studied during a 2 day protocol (Fig. 1). On day 1, after fasting overnight, the animals were anesthetized with sodium thiamylal (Surital) and ventilated with a mixture of oxygen and room air to maintain a partial pressure of oxygen in the arterial blood >100 mm Hg. Under sterile conditions, the left carotid artery was isolated by a cutdown procedure and a 7F catheter was advanced under fluoroscopic guidance into the ostium of the left anterior descending coronary artery for insertion of a 3F Fogarty balloon catheter. The balloon tip was placed distal to the first diagonal branch, which was identified by injection of contrast medium. The balloon was inflated with a mixture of saline solution and contrast medium, and occlusion of the left anterior descending artery was verified by repeat injections of contrast medium. Three hours after establishment of occlusion, the balloon catheter was slowly deflated, and both catheters were removed. The carotid artery was then ligated and the cutdown site closed. During ischemia and reperfusion, the electrocardiogram

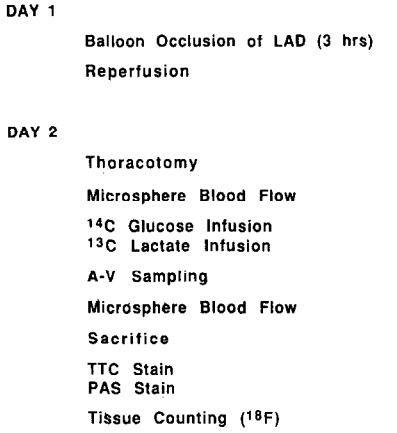

Figure 1. Study protocol of Day 1 and Day 2. LAD = left anterior descending coronary artery; TTC = triphenyltetrazolium chloride stain; PAS = periodic acid-Schiff stain.

(ECG) was continuously monitored. Before occlusion, an intravenous bolus of lidocaine (50 mg) was given, followed by constant infusion (2 mg/min). After reperfusion, the dogs received a single dose of procainamide (150 mg) intramuscularly. Fifteen dogs underwent coronary occlusion, to serve as controls, four dogs were subjected to the same study protocol, but no occlusion was performed.

On day 2, approximately 24 h (range 16 to 36 h) after reperfusion, all dogs were re-anesthetized, and a left thoracotomy was performed. The pericardium was widely incised and sutured to the chest wall to form a cradle in which the heart was suspended. To inject microspheres and sample arterialized blood, a polyvinyl cannula was inserted into the left atrium. To obtain blood samples from a vein draining the territory of the left anterior descending artery and the entire heart, two 23 gauge butterfly needles were inserted; one into a vein parallel to the left anterior descending artery, distal to the site of the balloon occlusion and one into the coronary sinus under the left atrial appendage. An additional catheter was placed into the femoral artery to monitor blood pressure and to withdraw blood for microsphere calibration.

Complete metabolic and flow data were obtained in all 4 control dogs but in only 13 of the 15 intervention dogs. This report is based on the data from these 17 animals (4 control and thirteen intervention).

D-[6-^{14}C] glucose and L-[U-^{13}C] lactate. After thoracotomy and instrumentation, D-[6-^{14}C] glucose (New England Nuclear; specific activity 56.1 mCi/mmol) was administered as a priming bolus of 24 μCi, followed by intravenous infusion at a constant rate of 15 μCi/h. To determine the myocardial lactate isotopic uptake, amount of lactate released and amount of glucose being converted to lactate, the stable (non-radioactive) isotope L-[U-^{13}C]lactate (Merck, Sharpe & Dohme L-[U-^{13}C] sodium lactate; 99% isotopic purity) was simultaneously infused. To achieve a

steady arterial level of L-[U-^{13}C] lactate (1.5% to 2.0% of the circulating chemical lactate), a priming dose of 30 mg of ^{13}C-lactate was introduced intravenously over 2 min, followed by an intravenous infusion of the isotope at a constant rate of 35 mg/h.

Equilibration of these tracers in arterial and myocardial venous blood is known to occur in 20 to 25 min after initiation of the infusion (14,15). At that time, blood samples were drawn simultaneously from the left atrium (for the arterial sample), the coronary sinus and the vein draining the territory of the left anterior descending artery to measure arteriovenous differences across the reperfused area as well as in the territory drained by the coronary sinus. Thirteen dogs (4 of 4 control and 9 of 13 intervention) received an infusion of D-[6-^{14}C] glucose and L-[U-^{13}C] lactate. Three sets of arteriovenous samples from four control and nine intervention dogs were drawn 10 min apart.

To determine the amount of lactate oxidation in this occlusion/reperfusion model, the remaining four intervention dogs received a priming bolus of 20 μCi of L-[1-^{14}C]-lactic acid (New England Nuclear Corp.; specific activity 55 mCi/mmol) followed by constant intravenous infusion at a rate of 25 μCi/h. After an equilibration period of 25 min (16), arterial and venous samples were withdrawn as outlined to determine concentrations and specific activities of lactate and glucose, as well as $^{14}CO_2$ content.

Chemical analysis. Weighed blood samples were analyzed for chemical concentrations of glucose, lactate and nonesterified fatty acids, as well as for $^{14}CO_2$ content and the specific activities of glucose and lactate (14,17). Samples from the dogs receiving ^{13}C-lactate were also analyzed for ^{13}C-lactate enrichment. Those samples to be analyzed for lactate, glucose, specific activities and ^{13}C-lactate were mixed immediately with a measured volume of cold 7% perchloric acid and centrifuged; the protein-free supernatant was then separated and stored at −4°C. Lactate and glucose concentrations were determined later by enzymatic spectrophotometric methods on this protein-free fluid (14,16). Free fatty acids were determined with a spectrophotometric method (18). Blood oxygen content was measured with use of an oxygen analyzer (Corning 920) (19).

D-[6-^{14}C] glucose analysis. Glucose, lactate and pyruvate were separated by ion exchange chromatography as previously described by Wisneski et al. (14). The protein-free fluid was neutralized and passed successively through cation and anion exchange columns to remove labeled ionic compounds. Portions of the eluates containing glucose in H_2O and lactate in 0.25 *M* sodium acetate were assayed by the described enzymatic methods. Other portions were mixed with Aquasol (New England Nuclear), and the ^{14}C content was measured in a scintillation counter. Results of scintillation counting were expressed as disintegrations per minute (dpm), and the specific activity was calculated as dpm/μmol. The coefficient of variation for specific activity of lactate was 2.5% in our laboratory (six analyses of one sample). The $^{14}CO_2$ was collected directly from blood by a previously established diffusion method having a 2.9% coefficient of variation (14,16).

L-[U-^{13}C] lactate analysis. L-[U-^{13}C] lactate content was determined with use of gas chromatography/mass spectrometry (16,17). Lactic acid was separated by ion exchange chromatography, converted to a volatile derivative (the trimethylsilyl ether of methyl lactate), and ion currents were recorded at m/e 161 and 164 and the results were compared with a standard curve (16). All isotopic and chemical analyses were performed in duplicate.

Calculations. This investigation used glucose labeled in the sixth carbon position. This carbon is released as $^{14}CO_2$ in the citric acid cycle. By measuring the venous-arterial (V-A) $^{14}CO_2$ difference, the amount of glucose being oxidized (μmol/ml blood) can be calculated as:

$$\frac{\text{(V-A) } ^{14}CO_2 \text{ dpm/ml blood}}{\text{arterial specific activity of glucose}},$$

in which dpm = disintegrations per minute.

When [6-^{14}C] glucose is used as a tracer, other substrates such as lactate become labeled secondarily. Therefore, before the oxidation rate of glucose is calculated, the V-A $^{14}CO_2$ value must be corrected for the oxidation of secondarily labeled substrates (14,16). As outlined, four of the intervention dogs received [1-^{14}C] lactate to determine the oxidation rate of lactate under these occlusion/reperfusion conditions. In these dogs, 78 ± 4% of the isotopically measured lactate uptake in the coronary sinus samples and 79 ± 6% of that in the left anterior descending venous samples were oxidized. These values were applied, together with the ^{13}C-lactate extraction ratio and the arterial specific activity of secondarily-labeled lactate, to correct the observed $^{14}CO_2$ production for the oxidation of secondarily-labeled lactate. The corrected $^{14}CO_2$ production was then used with the specific activity of glucose to calculate the oxidation of exogenous glucose.

The chemical extraction ratio (%) for a given substrate was calculated from the arterial and venous chemical concentrations as:

$$\frac{[A] - [V]}{[A]} \times 100,$$

in which [A] = arterial blood concentration and [V] = coronary sinus or left anterior descending venous blood concentration.

The isotope or [U-^{13}C] lactate extraction ratio (%) was calculated from the concentration of [U-^{13}C] lactate in the artery and vein as:

$$\frac{[A] \times \%\ ^{13}C_3 \text{ in artery} - [V] \times \%\ ^{13}C_3 \text{ in vein}}{[A] \times \%\ ^{13}C_3 \text{ in artery}} \times 100,$$

in which,

$$\%\ ^{13}C_3 = \frac{^{13}C_3\text{-lactate}}{\text{chemical lactate}} \times 100.$$

(% $^{13}C_3$ is obtained directly from gas chromatography/mass spectrometry).

Myocardial lactate uptake (μmol/ml blood) was determined by the isotope technique as:

$$[A] \times \frac{[U\text{-}^{13}C]\text{ lactate extraction ratio}}{100}.$$

The amount of lactate (μmol/ml blood) released or produced by the myocardium was calculated as:

$$[U\text{-}^{13}C]\text{ lactate uptake} - ([A] - [V]).$$

The contribution of exogenous glucose to the lactate released or produced was calculated from the observed and theoretical disintegrations per minute (dpm) of lactate/ml of blood in the vein and the specific activity of arterial glucose as:

$$\frac{\text{(Observed-theoretical) dpm lactate/ml blood in vein}}{\text{specific activity of arterial glucose}} \times 2.$$

(Note that the factor of 2 is used because one molecule of glucose yields two molecules of lactate.)

The actual or observed dpm of lactate/ml of venous blood were calculated as:

$$[V] \times \text{specific activity of venous lactate.}$$

The theoretical dpm due to lactate/ml of blood were determined as:

$$[A] - [U\text{-}^{13}C]\text{ lactate uptake (in μmol/ml blood)} \times \text{arterial specific activity of lactate.}$$

Histochemistry. At the end of each experiment, the left anterior descending artery was occluded at the site of the balloon inflation, which could easily be identified by perivascular edema. The dogs were killed with an intravenous injection of potassium chloride. Simultaneously, monastral blue was injected into the left atrium to outline the vascular territory of the left anterior descending artery. The heart was rapidly excised and cut into five cross sections, each 1 cm thick. Cross sections 2 and 4 were immediately immersed in Carnoy's fixative and subsequently stained with periodic acid-Schiff (PAS). Cross sections 1, 3 and 5 were incubated in triphenyltetrazolium chloride (TTC at 37°C for 20 min) for identification of tissue necrosis. The areas of glycogen depletion (PAS negative) and necrosis (TTC negative) were measured by planimetry from slides, with use of a Numonics planimeter and each measurement was expressed as a percent of the area of the cross section. The areas of necrosis and glycogen depletion were compared on the adjacent surfaces of cross sections 2 (PAS) and three (TTC).

Myocardial blood flow. Regional myocardial blood flow was determined with use of radioactivity labeled microspheres of tin, chromium and cerium (^{113}Sn, ^{51}Cr, ^{141}Ce, respectively) injected into the left atrium. Simultaneously, an arterial reference sample was withdrawn from the femoral artery (7.9 ml/min). Blood flow measurements were obtained before the first and between the second and third blood samples. Both measurements were averaged, and regional tissue activity concentrations determined by well counting were used to calculate blood flow in the areas of risk and in the nonischemic myocardium.

Statistical analysis. Data are expressed as a mean + SD. Analyses of variance and the paired *t* test were used to determine differences between data sets.

Results

Hemodynamic and blood flow measurements. At 24 h after reperfusion and immediately after the thoracotomy, the systolic blood pressure in all dogs averaged 124 ± 24 mm Hg with an average heart rate of 128 ± 11 beats/min. There were no significant changes in these variables during the blood sampling period. Myocardial blood flow was determined before the first and between the second and third blood samples. In the four control dogs, blood flow averaged 96 ± 14 ml/min per 100 g in the territory of the left anterior descending artery and 94 ± 18 ml/min per 100 g in the posterior wall (NS). In the intervention animals, blood flow averaged 87.2 ± 29.2 ml/min per 100 g in the posterior wall and 66.8 ± 35.4 ml/min per 100 g in the area of risk (77% of the flow in the nonischemic posterior area; $p < 0.001$). There were no significant differences between the first and second determinations of blood flow.

Myocardial extraction of substrates. The myocardial extractions determined by arteriovenous differences in concentrations of individual substrates are listed in Table 1. In the four control dogs, there were no significant differences in substrate extractions when the left anterior descending artery vein and coronary sinus sample sites were compared. Only 2% of glucose was extracted by the myocardium, whereas determination of nonesterified fatty acids revealed an arteriovenous extraction of 62%. The extraction of lactate and oxygen averaged 17% and 65%, respectively. In the nine intervention dogs, glucose extraction in the territory of the left anterior descending artery was 150% higher than that in the coronary sinus blood sample ($p < 0.05$), whereas extraction of fatty acids was 16% lower in the vein paralleling the left anterior descending artery ($p < 0.05$). Lactate extraction varied widely and averaged 0.02 ± 0.15 μmol/ml with net lactate production in seven of the nine dogs in the territory of the left anterior descending artery. Lactate extraction in the coronary sinus averaged 0.23 ± 0.17 μmol/ml, which was significantly higher than that found in the territory of the left anterior descending artery ($p < 0.001$). Oxygen extraction in the reperfused territory was 13% lower, than that in the coronary sinus sampling site ($p < 0.005$).

Table 1. Chemical Substrate Extraction in Control and Intervention Animals

	Arterial Level	LAD Vein Extraction	Coronary Sinus Extraction
Control (n = 4)			
Glucose (μmol/ml)	5.88 ± 0.85	0.15 ± 0.10 (2%)	0.10 ± 0.05 (2%)
NEFA (μmol/ml)	0.38 ± 0.06	0.22 ± 0.13 (58%)	0.25 ± 0.11 (66%)
Lactate (μmol/ml)	1.08 ± 0.60	0.20 ± 0.15 (19%)	0.16 ± 0.09 (15%)
Oxygen (μmol/ml)	8.66 ± 0.49	5.51 ± 0.36 (64%)	5.65 ± 0.67 (66%)
Intervention (n = 9)			
Glucose (μmol/ml)	4.38 ± 0.43	0.40 ± 0.14 (9%)	0.26 ± 0.13 (6%)*
NEFA (μmol/ml)	0.79 ± 0.25	0.32 ± 0.12 (40%)	0.38 ± 0.09 (48%)*
Lactate (μmol/ml)	1.06 ± 0.50	0.02 ± 0.15 (2%)	0.23 ± 0.17 (22%)*
Oxygen (μmol/ml)	8.81 ± 0.82	5.06 ± 1.16 (57%)	5.80 ± 0.94 (66%)*

*$p < 0.05$; paired t test comparing left anterior descending (LAD) vein and coronary sinus sample. NEFA = nonesterified fatty acids. Numbers in parenthesis represent chemical extraction fraction expressed in percent.

Table 2. Exogenous Glucose Utilization Measured by $^{14}C/^{13}C$ Analysis

	Control Animals (n = 4): LAD	Intervention Animals (n = 9) LAD	Intervention Animals (n = 9) CS
Glucose extraction (μmol/ml)	0.15 ± 0.10 (100%)	0.40 ± 0.14 (100%)	0.26 ± 0.13* (100%)
Exogenous glucose entering glycolysis (μmol/ml)	0.05 ± 0.02 (33%)	0.30 ± 0.05 (75%)	0.17 ± 0.06* (65%)
Exogenous glucose to lactate	0.01 ± 0.01 (6%)	0.17 ± 0.05 (43%)	0.08 ± 0.02* (31%)
Exogenous glucose oxidized	0.04 ± 0.05 (27%)	0.13 ± 0.08 (32%)	0.09 ± 0.07 (34%)
Lactate release (μmol/ml)	0.08 ± 0.03	0.37 ± 0.11	0.20 ± 0.11*

*$p < 0.05$ as compared with left anterior descending venous extraction (LAD) vein in intervention animals. CS = coronary sinus extraction. The percentages in parentheses reflect the amounts relative to glucose uptake.

Fate of extracted glucose. Tracer determinations of exogenous glucose oxidation and glucose conversion to lactate were related to overall extraction of glucose (Table 2). In the four control dogs, there were no significant differences in glucose utilization when the left anterior descending artery vein and coronary sinus samples were compared. Twenty-seven percent of exogenous glucose was being oxidized, whereas 6% were converted to lactate. Presumably the remainder (67%) entered a glucose storage pool, that is, glycogen.

The results from the intervention group revealed a different metabolic pattern. Exogenous glucose extraction was significantly increased compared with that of the control group. The amount of exogenous glucose entering glycolysis was also significantly increased, as evidenced by $^{14}CO_2$ production or ^{14}C-lactate in the venous effluent (75% in the vein paralleling the left anterior descending artery and 65% in the coronary sinus) (Table 2).

The amount of glucose converted to lactate averaged 0.17 ± 0.05 μmol/ml in the territory of the left anterior descending artery; this was significantly higher ($p < 0.001$) than that in the coronary sinus (0.08 ± 0.02 μmol/ml). However, the amount of glucose oxidized in both sample sites did not differ significantly (0.13 ± 0.08 versus 0.09 ± 0.07 μmol/ml). Lactate release derived from ^{13}C-lactate data was significantly higher ($p < 0.002$) in the reperfused segment (0.37 ± 0.11 μmol/ml) than in the coronary sinus site (0.20 ± 0.11 μmol/ml). The lactate release in the left anterior descending artery segment (0.37 ± 0.11 μmol/ml) was in close agreement with the amount of exogenous glucose converted to lactate (0.17 ± 0.05 μmol/ml), because one molecule of glucose is converted to two molecules of lactate. These metabolic data demonstrate that exogenous glucose accounts for most of the lactate release or production in this pathologic model.

Correlative findings. Neither the circulating glucose nor lactate arterial levels correlated significantly with the respective myocardial substrate extractions in this study. However, nonesterified fatty acid plasma levels correlated significantly with fatty acid extraction measured in the vein paralleling the left anterior descending artery ($r = 0.6$; $p < 0.05$) and in the coronary sinus ($r = 0.74$; $p < 0.02$; Fig. 2). Furthermore, fatty acid plasma levels were inversely related to the amount of glucose oxidized in the left anterior descending vein ($r = -0.69$; $p < 0.01$) and coronary sinus ($r = -0.61$; $p < 0.05$). There were no significant correlations between lactate or glucose blood levels and the relative amounts of glucose oxidized or that released as lactate. In the territory of the left anterior descending artery, the amount of glucose converted to lactate and exogenous glucose oxidized were both significantly related to glucose extraction ($r = 0.83$, $p < 0.002$ and $r = 0.79$, $p < 0.004$, respectively) (Fig. 2).

Histochemistry. Examples of the histochemical results are shown in Figure 3, which compares two adjacent cross-sectional stains from a control and an intervention dog. The stained tissue of the control dog shows homogeneous staining with periodic acid-Schiff (PAS) and triphenyltetrazolium chloride (TTC). For the intervention dog, the TTC stain shows a pale area of necrosis, whereas tissue with intact glycogen stores appears pink on the PAS stain and glycogen-depleted areas are not stained. In this dog the area of glycogen depletion was approximately 50% of the cross

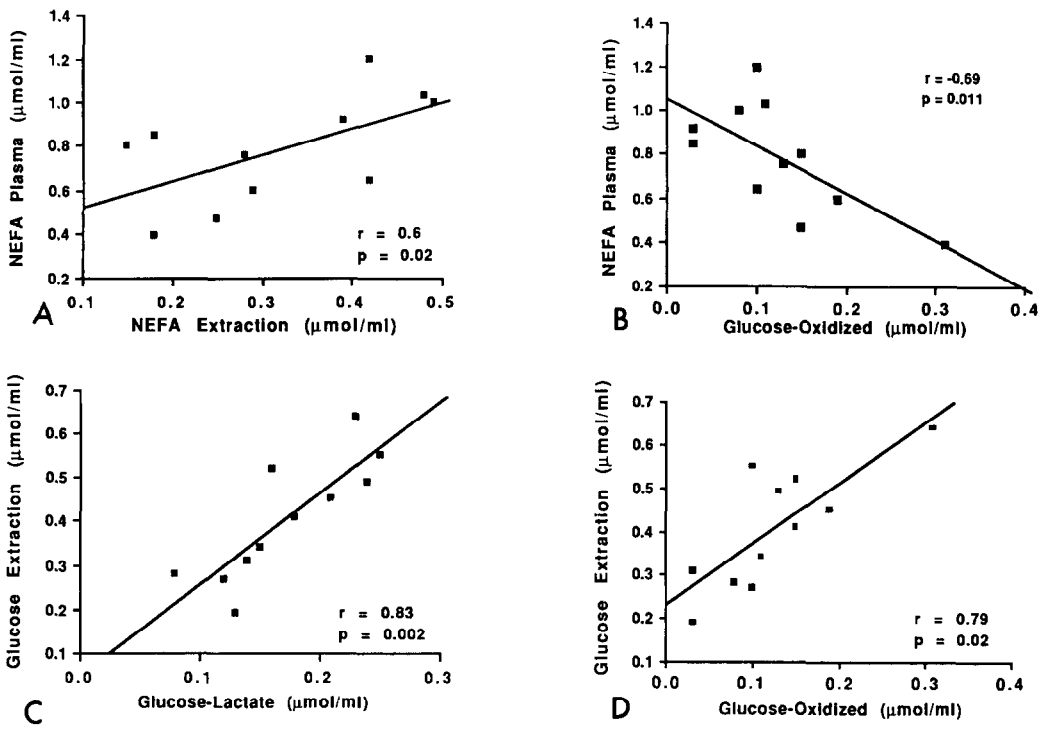

Figure 2. Substrate utilization in the reperfused myocardium. **A,** Plasma level of non-esterified fatty acids (NEFA) and regional extraction of nonesterified fatty acids in the left anterior descending coronary artery territory; **B,** Nonesterified fatty acid plasma levels and amount of ^{14}C glucose oxidized; **C,** Glucose extraction and amount converted to ^{14}C lactate; **D,** Glucose extraction and amount of exogenous glucose oxidized.

section and exceeded the nonstained area on the TTC stain (22%). In all intervention dogs, the areas of necrosis averaged 17.4 ± 10.5% of the mid-ventricular cross section, which was significantly smaller than the average glycogen-depleted area of 36.6 ± 19.2% ($p < 0.001$) (Fig. 4). In all four control dogs there was no evidence of necrosis or glycogen depletion.

Discussion

This study demonstrates for the first time that the activity of nonoxidative glucose pathway is enhanced at 24 h of reperfusion after a prolonged (3 h) ischemic injury. Our observation that lactate released by reperfused myocardium is predominantly derived (>90%) from nonoxidative metabolism of exogenous glucose was supported by the histochemical finding of sustained glycogen depletion in tissue surrounding the area of necrosis. Previous studies (11) have shown that the myocardial injury in this setting is reversible, that is, areas of enhanced glucose extraction measured by ^{18}F-deoxyglucose and positron emission tomography (PET) are associated with reversible wall motion abnormalities. Therefore, these data imply that exogenous glucose is very important for the functional recovery of the metabolically compromised tissue after occlusion and reperfusion.

Methodologic Considerations

Animal model. We chose a dog model that reproduces the clinical setting of acute myocardial infarction and reperfusion. In this model, we recently demonstrated (11) that PET measurements of increased ^{18}F-deoxyglucose uptake after 24 h of reperfusion predict reversible tissue injury as confirmed by subsequent functional recovery. A period of 24 h after reperfusion was chosen because previous serial studies in the same model (20) indicated that maximal ^{18}F-deoxyglucose uptake occurs at that time.

The disadvantage of the relatively long period of ischemia is the significant, but varying amount of necrosis that occurs in this model. However, independent of the duration of ischemia, the degree of ischemia in a vascular territory varies as a function of heterogeneous regional flow reduction and oxygen demands. Many previous investigations (21,22) indicated a "wave front" characteristic of myocardial necrosis during coronary occlusion with progression from subendocardium to subepicardium within relatively definite lateral borders. Unfortunately, there is, to our knowledge, no in vivo animal model producing homogeneous ischemic injury in an occluded vascular territory that could specifically

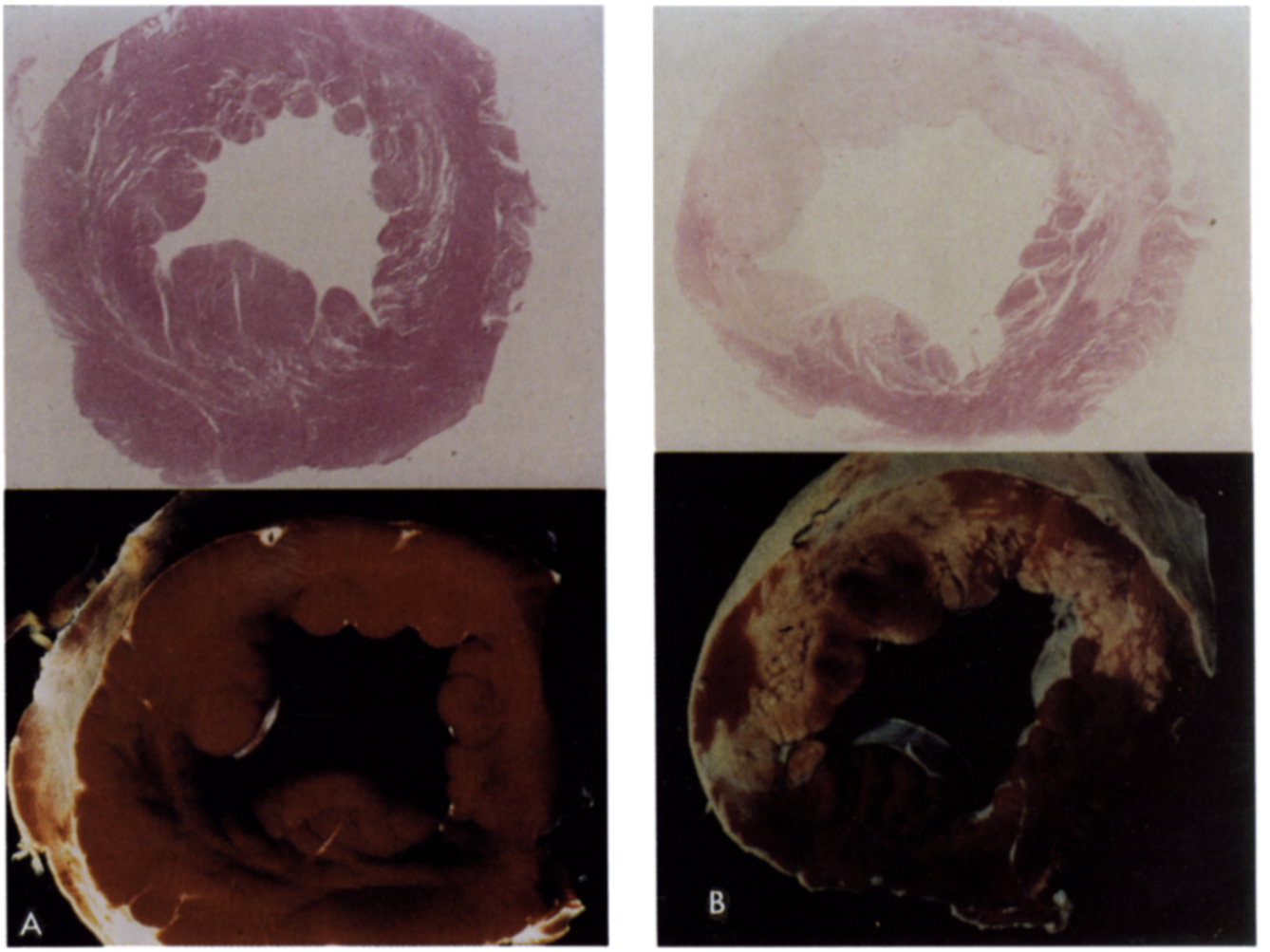

Figure 3. Two examples of triphenyltetrazolium chloride and periodic acid-Schiff stains. **A,** Control animal without intervention showed homogeneous staining after TTC (**below**) and PAS (**above**). **B,** Intervention animal with a large TTC negative area (**below**) that identifies areas of necrosis (22% of left ventricle cross section). The area of glycogen depletion (PAS negative) (**above**) is 50% and exceeds the area of necrosis in this section.

define the metabolic pattern in reversibly injured myocardium.

The heterogeneity of ischemic injury makes the interpretation of metabolic data derived from arteriovenous sampling more difficult. Because the accurate definition of the territory drained by a given coronary vein is limited, we chose to compare two venous sample sites. A vein paralleling the left anterior descending artery distal to the occlusion site was used for blood samples relating to the region of reperfused ischemic myocardium. To contrast these regional results with global myocardial metabolism, additional venous samples were obtained from the coronary sinus. This latter site does not represent a true control site because the coronary sinus drains blood from the entire heart and, therefore, contains mixed venous blood from reperfused injured and normal myocardium. However, the significant differences we observed between these two sample sites indicate a unique metabolic pattern in the reperfused segments. In addition, comparison with our data from dogs undergoing the same experimental protocol without coronary occlusion further confirms the regional metabolic abnormalities assessed in the intervention group.

Because of the difficulty in determining post mortem the exact extent of myocardium drained by a specific vein, correlation of data derived from arteriovenous sampling with data from tissue assays and microsphere blood flow determinations is limited. Therefore, we did not calculate regional substrate consumption, which depends on exact measurements of drained tissue volume and blood flow.

Histochemistry. Standard staining techniques for detecting necrosis (TTC) and glycogen tissue content (PAS) (23)

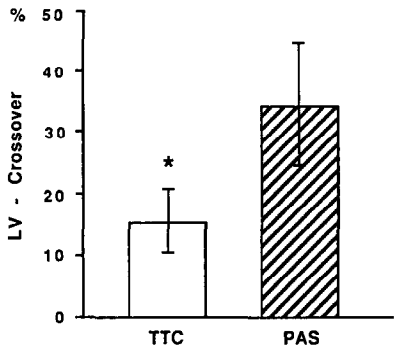

Figure 4. Histochemical stains. Summarized results of relative extent of necrosis as identified by triphenyltetrazolium chloride (TTC) and glycogen depletion by periodic acid-Schiff (PAS) negative in a mid-left ventricular (LV) cross section.

were employed to characterize the extent and severity of ischemic injury in the experimental animals. The histochemical results based on these two stains were calculated from cross-sectional surfaces directly facing each other and only microns apart so that comparison of respective nonstained areas was possible. Although both staining methods allow only qualitative evaluation of relative differences, these methods have been validated and proved practical in many experimental studies (23).

The histochemical results in this study verify the previously described heterogeneity of the ischemic injury; that is, necrotic, glycogen-depleted and histochemically normal tissue coexisting in the risk area. In most intervention dogs the necrotic zone was confined to the subendocardial layers, whereas glycogen depletion appeared to extend transmurally and significantly exceeded the area of necrosis. The course of glycogen depletion over time, has been fully characterized during ischemia (24), but little information is available about restoration of myocardial glycogen after reperfusion. The sustained depletion noted in our study contrasts with the reported (25) rapid recovery of glycogen stores after short periods of ischemia in heart or peripheral muscle.

Data Interpretation of Metabolic Results

Oxidative and nonoxidative glycolysis. The results of this study confirm previous PET studies in the same experimental model showing increased exogenous glucose extraction by the reperfused myocardium (11,20). After uptake by the myocyte, glucose can follow several metabolic pathways: oxidation through the citric acid cycle, storage as glycogen, utilization in the hexose monophosphate shunt pathway and conversion to lactate through the glycolytic pathway (26). Using [6-^{14}C]glucose and measuring the myocardial production of $^{14}CO_2$ enabled us to quantify the amount of exogenous glucose undergoing rapid oxidation, because carbon in the sixth position is released during oxidation in the citric acid cycle (26). The simultaneous infusion of [U-^{13}C]lactate and [6-^{14}C]glucose allowed us to calculate the amount of lactate being released and the conversion of exogenous glucose to lactate through glycolysis. Thus, in this study we were able to quantitate the metabolic activity in two of the glycolytic pathways: oxidation and nonoxidative glycolysis.

Myocardial oxidative metabolism in normal myocardium. In the control dogs, only a small amount of glucose was extracted by myocardium, confirming numerous previous studies demonstrating that nonesterified fatty acids are the primary substrate of myocardial oxidative metabolism in the normal heart. In addition, only about 30% of ^{14}C activity entering the cell as glucose was recovered in the effluent as $^{14}CO_2$ or ^{14}C-lactate in the control dogs. This finding suggests that the remaining 70% of labeled substrate entered a slow turnover pool that has not been equilibrated during the 25 to 35 min of [6-^{14}C] glucose infusion. In agreement with previous results obtained in the human heart using the same isotope technique (14), these observations indicate that the majority of glucose uptake in the fasting state is used to maintain tissue stores of glucose, such as glycogen. Turnover estimates of 5 to 6 h for the myocardial glycogen pool would support this hypothesis (27).

Exogenous glucose utilization in ischemic myocardium. In the intervention dogs, we observed a different pattern of exogenous glucose utilization. There were significant increases in glucose extraction and decreased extractions of fatty acids and oxygen compared with values in the control dogs (Tables 1 and 2). In contrast to the control dogs, in the intervention group 75% of the extracted glucose was accounted for by the ^{14}C-lactate and $^{14}CO_2$ in the venous effluent draining the reperfused segment. This finding suggests increased glycolytic flux with either reduced glycogen synthesis or a smaller glycogen pool having a higher turnover rate. Both hypotheses are consistent with the histochemical findings of sustained glycogen depletion in the reperfused myocardium. In addition, most of the lactate produced by the injured myocardium in this preparation (>90%) was derived from exogenous glucose. This hypothesis also agrees with the histochemical evidence of regional glycogen depletion in the risk area. Thus, this occlusion/reperfusion preparation differs from the experimental models of acute ischemia in which glycogen has been shown to be the major source of myocardial lactate production (24).

Prolonged nonoxidative glycolysis in reperfused myocardium. In the reperfused myocardium, 57% of the glucose entering glycolysis was converted to lactate. This amount was considerably more than that of glucose to lactate conversion in the control dogs (18%). The calculated lactate release was more than four times greater in the reperfused myocardium of the intervention animals than that in the control myocardium. These findings are consistent with

sustained and enhanced activity of the nonoxidative glucose pathway. Nonoxidative glycolysis has been observed primarily during acute ischemia and hypoxia and is thought to reflect compensatory nonoxidative high energy phosphate production in the presence of impaired oxidative metabolism (10,24,28). To our knowledge, this study demonstrates for the first time that nonoxidative glycolysis can be maintained for prolonged time periods after an ischemic insult. The significant correlation of glucose extraction, lactate production and glycogen depletion suggests the dependence of nonoxidative metabolism on the availability of exogenous glucose in this pathologic setting.

An additional source of nonoxidative glycolysis may be leukocytes that infiltrate ischemically injured myocardium (29). However, recent studies showed that ^{18}F-deoxyglucose distribution in reperfused myocardium differs from that of ^{111}Indium-labeled leukocytes, which indicate that increased ^{18}F-deoxyglucose uptake in reperfused myocardium predominantly traces myocyte metabolism (30).

Coexistence of oxidative and nonoxidative pathways in reperfused myocardium. In addition to contributing to the lactate production, about 40% of the exogenous glucose entering glycolysis was oxidized in the reperfused myocardium. The coexistence of both metabolic pathways for glucose reflects either normal cells intermixed with those affected by increased substrate demand *or* recovering cells that cannot utilize fatty acids but have a functioning citric cycle with preferential use of glucose and lactate as substrates. Previous studies using ^{11}C-palmitate in the same model indicated impaired long chain fatty acid metabolism in areas with increased glucose utilization, as evidenced by ^{18}F-deoxyglucose uptake (11). These findings would support the hypothesis of metabolically altered, recovering cells. It is possible that the enzymatic environment is altered in ischemically injured myocardium, with glucose becoming the preferred substrate for oxidative metabolism. Increased glucose oxidation has been described in postischemic skeletal muscle and attributed to a metabolic adaptation to repetitive ischemia (31).

On the other hand, the inverse relation of plasma nonesterified fatty acid levels with glucose oxidation noted in our experiments suggest an intact regulatory mechanism of oxidative substrate metabolism in reperfused myocardium (14). This characteristic of the reperfused tissue may reflect, primarily, the metabolism of an intermixed normal cell population, most likely in the subepicardium that can utilize both fatty acids and glucose.

Conclusions

This study demonstrates that enhanced nonoxidative glycolysis is still occurring after 24 h of reperfusion following a severe (3 h) ischemic insult to the myocardium. The observed increased exogenous glucose utilization confirms earlier findings of increased ^{18}F-deoxyglucose uptake identified in the same model by positron emission tomographic imaging. Both histochemical and metabolic data demonstrated heterogeneity of injury in the risk area. Metabolically and histochemically normal cells coexist with severely compromised and necrotic myocytes. The methods employed in this investigation do not provide enough spatial resolution to allow specific metabolic characterization on a cellular level. The relation between glucose extraction and lactate production in the reperfused territory indicates that sustained nonoxidative glycolysis is dependent on the exogenous glucose supply in the presence of depleted glycogen stores. Assuming a beneficial effect of nonoxidative glycolysis for tissue recovery, these findings demonstrate the importance of exogenous glucose for substrate metabolism in postischemic myocardium.

Therefore, our metabolic observations may have important future implications for the development of therapeutic strategies in patients with acute myocardial infarction. Recent clinical studies of this patient group with the use of PET revealed increased ^{18}F-deoxyglucose uptake in "infarcted" myocardium studies several days after the acute event (32,33). These clinical findings suggest a similar metabolic pattern in both the canine and human heart after an ischemic injury. Therefore, PET in combination with tracers such as ^{18}F-deoxyglucose, will allow metabolic characterization of tissue injury and may provide a noninvasive means of studying tissue recovery and the effects of therapeutic interventions on metabolism in ischemically injured human myocardium.

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