Persistent Changes in Myocardial Glucose Metabolism In Vivo During Reperfusion of a Limited-Duration Coronary Occlusion

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Background—Rapid reperfusion of an occluded coronary artery salvages regional mechanical function, but this benefit may not be realized for hours or days because of postischemic stunning. Recovery from stunning is incompletely understood but may involve adaptive changes in heart glucose metabolism.

Methods and Results—To examine whether reversible coronary occlusion produces sustained changes in regional glucose metabolism in vivo, we performed a 20-minute left coronary artery occlusion followed by 24 hours of open-artery reperfusion in intact rats. Coronary occlusion produced stunning of the anterolateral left ventricle that resolved over 24 hours. When examined at 24 hours, reperfused regions were fully contractile and viable by vital staining and microscopy but demonstrated 25% reduction in blood flow and 50% increased uptake of circulating glucose, as estimated by in vivo [13 N]NH₃ and [18 F]fluorodeoxyglucose (FDG) tracer uptake. Reperfused regions had largely inactive glycogen synthase, low rates of glycogen synthesis, and persistent 50% glycogen depletion but increased flux of plasma [1-13 C]glucose into myocardial [3-13 C]alanine, indicating preferential shunting of imported glucose away from storage and into glycolysis.

Conclusions—Sustained increases in regional glycolytic consumption of circulating glucose occur during reperfusion of a limited-duration coronary occlusion. This suggests a role for glycolytic ATP in the recovery from postischemic stunning in vivo. Furthermore, [13 N]NH₃/FDG regional mismatch may constitute a clinically accessible late metabolic signature of regional myocardial ischemia. (Circulation. 2000;101:917-922.)

Key Words: glucose ■ myocardium ■ glycogen ■ ischemia ■ metabolism

The goal of current therapy for acute myocardial infarction is to restore infarct-artery patency as rapidly and completely as possible. The rationale for this is the belief that reperfusion of the compromised region through an open artery is most likely to salvage mechanical function, the most important arbiter of prognosis, in the reperfused region. The natural history of recovery from a temporary coronary occlusion, however, is that the ischemically “stunned” myocardium resumes functioning only hours to days into reperfusion. Although the factors responsible for recovery of function during this time frame are not well understood, evidence suggests a role for increased utilization of exogenous glucose. For example, in the isolated perfused heart, glycolysis accelerates acutely during ischemia to allow anaerobic ATP formation.1,2 The provision of extra glycolytic substrate either during ischemia1,3 or during early reperfusion4 may improve short-term functional recovery; alternatively, the inhibition of glycolysis with 2-deoxyglucose5 or excess fatty acids6 delays or prevents it.

If increased flux of glucose through energy-generating pathways were important to the recovery of the ischemically stunned myocardium, one might expect to find evidence that this metabolic signature persists late into reperfusion, paralleling the recovery of function. Current evidence for this is ambiguous. In this report, we describe an intact rat model of reversible coronary artery occlusion-reperfusion suitable for examining glucose metabolism at time points late during the recovery from postischemic stunning. In this model, characteristic increases in regional myocardial glucose metabolism can be identified 24 hours after a limited-duration coronary occlusion in vivo. This appears to constitute a persistent adaptive response to postischemic stunning that may coincidentally provide a clinically accessible delayed metabolic signature of nonlethal myocardial ischemia.

Methods

Animal Preparation

Experiments were approved by the Animal Care Committee of the Yale School of Medicine. On day 1, male Sprague-Dawley rats...
(n=50; 250 to 300 g) were anesthetized with pentobarbital sodium (50 mg/kg IP), orotracheally intubated, and mechanically ventilated, with parameters set to maintain physiological arterial blood gases and hemodynamics. The heart was exposed via a small (1 cm) left anterolateral thoracotomy, the left coronary artery occluded 5 mm distal to its origin for 20 minutes with a 6-0 prolene suture snare, and the occlusion then released. This produces ischemia of an anterolateral segment of the left ventricle comprising >50% of left ventricular volume. The chest was closed, and rats were allowed to recover for 24 hours. The experimental protocol is shown in Figure 1.

Experimental Protocols
After 24 hours' reperfusion, rats were divided into 6 groups. Group 1 rats (n=6) were killed immediately, and their hearts were transversely sectioned and stained with triphenyltetrazolium chloride (TTC) to confirm the lack of necrosis resulting from the 20-minute day 1 coronary occlusion.

Group 2 rats (n=6) were killed immediately, and biopsy samples of the ischemic-reperfused anterior and control posterior segments of the left ventricle were removed for light and electron microscopy to assess cell morphology and glycogen stores.

Rats in groups 3, 4, and 5 were anesthetized and mechanically ventilated with room air. Polyethylene catheters were inserted into the right jugular vein and the left carotid artery for experimental infusions and blood sampling. Rats in group 3 (n=7) were used to compare myocardial blood flow and glucose uptake in ischemic-reperfused and control left ventricular regions. Each rat first received an intravenous trace bolus of 1.5 mCi [18F]fluorodeoxyglucose (18FDG). After a 30-minute period was allowed for myocardial uptake of 18FDG, a 1.5-mCi intravenous trace bolus of [13N]NH3 was given. Fifteen minutes after [13N]NH3 injection, the chest was opened by median sternotomy and a 6-0 prolene suture used to occlude the left coronary artery at the same level as the day 1 occlusion. With the left coronary thus temporarily occluded, a solution of methylene blue dye in water was injected into the left ventricular cavity of the heart beating in situ, to allow distinction of the blue-stained remote posterior left ventricle region from the unstained 24-hour reperfused anterior region on subsequent sectioning of the heart. Hearts were excised <30 seconds after coronary occlusion and processed as described below.

Group 4 rats (n=8) were given a 40-minute trace infusion of D-[3-13C]glucose at a rate of 0.5 mCi/min to label newly synthesized glycogen within ischemic-reperfused anterior and control posterior myocardial regions. After 40 minutes, hearts were excised and central portions of each region frozen in liquid nitrogen for measurement of tissue glycogen concentration, glycogen synthesis rates, and the activities of glycogen synthase (GS) and glycogen phosphor-ylase (GP) enzymes.

Group 5 rats (n=12) were used to assess the specific contribution of circulating glucose to glycolytic flux in each region. Rats were infused for 3 hours with a 20% solution of 99%-enriched D-[1-13C]glucose in saline at a rate of 2.0 mg·kg⁻¹·min⁻¹. This rate was chosen to raise the steady-state 13C enrichments of plasma glucose and its myocardial intermediary metabolite pools to levels sufficient for precise measurement without raising plasma glucose concentration. As originally demonstrated by Lewandowski and others, during continuous infusion of D-[1-13C]glucose, the myocardial pyruvate pool accumulates [3-13C]pyruvate in proportion to that fraction of total glycolytic substrate contributed by circulating glucose relative to other carbon sources (eg, 12C-glycogen). Myocardial concentrations of pyruvate are generally too low for its 13C enrichment to be measured in small samples, but pyruvate is in equilibrium through transaminase reactions with the larger alanine pool. Measurement of steady-state [3-13C]alanine enrichment in ischemic-reperfused and control regions therefore provides a comparison of exogenous glucose contribution with glycolytic flux in each region. After 3 hours, hearts were excised and myocardial regions frozen for analysis as described below. A sixth group of rats (n=8) were allowed to recover for 7 days after reperfusion to determine the final state of glycogen concentration and myocardial structural integrity.

Analytical Methods
Echocardiography
At 6, 12, and 24 hours into reperfusion, rats were lightly sedated with methoxyflurane vapor, and 2D echocardiography was performed with a 10-MHz Acuson probe positioned over the left lateral thoracotomy incision. Short-axis images of the left ventricle were obtained at the level of the papillary muscles, and the systolic thickening pattern of the reperfused anterolateral segment was compared qualitatively with an orthogonal segment of the posterior left ventricle.

Microscopy
Electron microscopic examination of biopsy material from viable, FDG-avid hibernating myocardium in humans demonstrates characteristic morphological changes, including increased glycogen content in many cells. To complement the measurement of total glycogen concentration in frozen myocardial samples and to determine the extent of cellular injury, biopsy samples taken from the central portions of the anterior and posterior left ventricle of group 2 rats were paraffin mounted and examined by light and electron microscopy. Electron micrographs were made at ×5600 magnification with a General Electric EM 5000 microscope.

Regional Myocardial Blood Flow and Glucose Uptake
In group 3 rats, reperfused anterior and control posterior (identified by blue-dye staining) sections of the left ventricle were excised, each section was separated into endocardial and epicardial halves, and these were further divided into samples of ~50 mg for radioactive counting. Samples were then stored for 2 hours to allow complete decay of 13N (t1/2=10 minutes) and recounted for residual 18F (t1/2=110 minutes). 13N counts per gram were
then obtained by subtracting the results of delayed from immediate counting.

Regional Glycogen Concentration and Glycogen Synthesis Rate
In group 4 rats, glycogen was isolated from frozen myocardium and digested with amyloglucosidase, and its concentration was expressed as micromoles of glucose per gram of wet weight.\(^{7,8}\) Purified glycogen samples were then counted for \(^{3}H\) and the results (dpm/g) divided by the average arterial plasma \([\text{D-}\]^{3-}H\text{glucose}\) specific radioactivity (dpm/\(\mu\)mol glucose) during each study to calculate regional glycogen synthesis rates (\(\mu\)mol glucose \(\cdot\) g\(^{-1}\) \(\cdot\) min\(^{-1}\)).\(^{7,8}\)

GS and GP Activities
GS and GP activities were measured in cell-free homogenates of frozen heart samples by modifications of the methods of Thomas\(^{11}\) and Tan\(^{12}\) respectively, as previously described.\(^{7,8}\) For GS, the activity of the physiologically active (glucose-6-phosphate independent; GS-i) form of the enzyme was defined as the rate (\(\mu\)mol/g/min) of incorporation of [U-\(^{14}\)C]uridine diphosphoglucose into glycogen at physiological (0.17 mmol/L) concentration of the enzyme’s activator, glucose-6-phosphate. Total GS activity was defined as that observed at saturating (7.2 mmol/L) glucose-6-phosphate. Similarly, physiological (GP-a) and total GP activities were measured as the rates of incorporation of [1-\(^{14}\)C]glucose-1-phosphate into glycogen in the absence and presence, respectively, of 5 mmol/L adenosine monophosphate. Activities are reported as the fraction of total activity present in the GS-i or GP-a forms.

Plasma and Myocardial Steady-State \(^{13}\)C-Enrichments
Analyses were performed as reported previously.\(^{8}\) Briefly, alanine was extracted from powdered, frozen myocardium by homogenization in cold 6% perchloric acid, then derivatized and analyzed by gas chromatography–mass spectrometry (GC-MS) as the trifluoroacetyl n-butyl ester. GC-MS analysis was performed with a gas chromatograph (model 5890, Hewlett-Packard Co) interfaced to a Hewlett-Packard 5971A mass detector operating in the positive chemical ionization mode with methane as reagent gas. Isotopic enrichment of alanine was determined from the ion intensities of mass-to-charge ratio (m/z) 342 to 348 and glutamate m\(+1\) tom+5 from m/z 356 to 363. Glucose was derivatized as the penta-acetate, and isotopic enrichment was determined from the ion intensities of m/z 331 to 334.

Data Analysis
Comparisons between anterior and posterior regions within each group were made by paired \(t\) tests. Data are presented as mean±SD.

Results

TTC Staining
Sections prepared from group 1 hearts exhibited uniform uptake of TTC, with no evidence of necrosis within the reperfused region.

Regional Myocardial Function
All rats examined with echocardiography demonstrated severe hypokinesis of the anterolateral segment of the left ventricle at 6 hours after reperfusion was begun. By 24 hours, normal concentric thickening, without any regional wall motion abnormalities, had resumed in every animal.

Microscopy
Light microscopy of the reperfused anterior myocardial region demonstrated only rare, scattered mononuclear cells. Electron microscopy revealed mitochondrial enlargement, moderate effacement of sarcomeres, and homogenous depletion of glycogen (Figure 2). By 1 week after reperfusion, these changes had largely resolved, leaving only rare, scattered foci of fibrosis.

Regional Myocardial Blood Flow
Results of radioactive counting of group 3 hearts for [\(^{13}\)N]NH\(_{3}\) are shown in Figure 3a. At 24 hours’ reperfusion, there was a consistent reduction in blood flow ([\(^{13}\)N]NH\(_{3}\) counts) within reperfused sections relative to the control posterior left ventricle. This reduction was greater in the endocardial (\(\sim\)33\%) than the epicardial (\(\sim\)25\%) layer of the reperfused region.

Regional Myocardial \(^{18}\)FDG Uptake
Reperfused anterior regions exhibited a corresponding increase in absolute \(^{18}\)FDG radioactivity relative to control posterior regions, ranging from \(\sim\)40\% in the endocardium to \(\sim\)15\% in the epicardium. In the posterior control region, \(^{18}\)FDG counts were distributed uniformly, with no difference between endocardial and epicardial layers (Figure 3b).
Correlation Between Blood Flow and 18 FDG Uptake

The observed increase in 18 FDG radioactivity within reperfused anterior regions correlated with the degree of blood flow reduction in each sample. When the relative excess 18 FDG radioactivity of each anterior sample was plotted against its relative reduction in [13 N]NH₃ radioactivity (Figure 4), an exponential relationship between the two was suggested such that glucose avidity was greatest in sections with the greatest reduction in reperfusion blood flow. Examination of the individual data in Figure 4 shows that for the 10 most ischemic samples (≥50% reduction in [13 N]NH₃ counts relative to control posterior regions), 18FDG radioactivity was on average 6.6±2.1-fold higher than in normally perfused posterior regions. 18FDG uptake was also increased in several reperfused samples with normal blood flow.

Glycogen Concentration and Glycogen Synthesis Rate

At 24 hours of reperfusion, glycogen concentration was lower in the ischemic-reperfused anterior region than in the control posterior region in every rat (10±5 versus 21±6 μmol/g; P<0.01). Despite this evidence of persistent glycogen depletion, spontaneous rates of glycogen synthesis were as low as those normally seen in fasted, glycogen-replete rats and not different from control regions of the same hearts (38±3 versus 38±4 nmol · g⁻¹ · min⁻¹). Glycogen content of the reperfused region returned to normal by 7 days (anterior 19±5 μmol/g, posterior 22±4 μmol/g; P=0.15).

GS and GP Activities

At 24 hours of reperfusion, 17±4% of GS was in the active, GS-i form in the anterior versus 15±4% in the posterior left ventricle (P=NS). This represents a low activation state for this enzyme, similar to that observed in fasting rats, and is consistent with the finding that incorporation of circulating 3 H-glucose into glycogen proceeded very slowly in both regions. Fractional GP-a activity was also similar between regions (35±4% versus 32±5%, P=NS), which indicates that the lower glycogen content of the ischemic-reperfused anterior region was not the result of its greater phosphorylase activity.

Myocardial GS is acutely but transiently activated after brief (3 to 5 minute) periods of ischemia in the rat. Therefore, we examined 6 additional rats at an early time point (30 minutes) after the 20-minute coronary occlusion. At 30 minutes, the reperfused anterior myocardium was significantly depleted of glycogen (11±3 versus 26±4 μmol/g in the posterior left ventricle), but only 12±4% of its GS was in the GS-i form versus 18±3% in the control posterior region (P=NS). Thus, despite significant glycogen depletion, GS activation (a consistent finding after short periods of ischemia) is not observed after a 20-minute coronary occlusion.

Regional Myocardial [3-13 C]Alanine Enrichment

Continuous infusion of d-[1-13 C]glucose achieved steady-state 13 C enrichment in plasma glucose at 36±3 atom percent excess (APE) above natural abundance. Simultaneous steady-state [3-13 C]alanine enrichment was consistently higher
(P=0.007) in the ischemic-reperfused anterior regions (8.5±2.1 APE) than in control posterior regions (7.2±2.5 APE), which indicates ~18% greater proportional contribution of circulating glucose to glycolytic flux in the ischemic-reperfused region.

Discussion

The results of this study demonstrate that in the intact rat, a 20-minute left coronary artery occlusion produces regional mechanical stunning that resolves over 24 hours of subsequent conscious, closed-chest reperfusion. At 24 hours, the reperfused region had regained normal function and was without necrosis by TTC staining and microscopy. However, it exhibited 4 characteristics that distinguished it from control regions: its blood flow remained reduced; its uptake of FDG from the circulation was increased, both per unit blood flow and in absolute terms; it remained glycogen depleted; and its relative utilization of circulating glucose as a glycolytic substrate increased. These findings imply that even fairly late into the recovery from postischemic stunning, the reperfused region remained more reliant on circulating glucose as an energy substrate than remote regions of the same heart.

Regional Glucose Metabolism 24 Hours After Reversible Coronary Occlusion

Increased regional glucose uptake has been observed late after coronary occlusion in a number of species. However, in both pigs and patients with coronary artery disease, such glucose-avid regions are observed to have increased glycogen content. The factors that determine whether glucose imported into the postischemic myocardium is energetically metabolized, as opposed to merely being stored as glycogen, have not been clear.

Myocardial glycogen normally turns over only slowly but is rapidly consumed during ischemia. GS is activated both during the reperfusion period after a brief coronary occlusion and during prolonged low-flow ischemia, in both cases catalyzing accelerated glycogen resynthesis. In contrast, in the present study, myocardial regions reperfused after a 20-minute coronary occlusion failed to activate GS either acutely or later during reperfusion and remained glycogen depleted. The combination of increased glucose uptake (indicated by greater FDG radioactivity) and persistently low GS-i activity is consistent with shunting of imported glucose away from glycogen repletion and toward energy-generating pathways in the recovering myocardium. The finding that proportional labeling of glycolytic end product ([3-13]C]alanine) by circulating [13C]-glucose was greater in reperfused than control regions confirms this hypothesis. In part, this finding could reflect reduced contribution to glycolysis of unlabeled glucose derived from glycogen. Indeed, our data do not distinguish whether overall glycolytic flux increased in reperfused myocardium or only that portion of glycolysis supported by circulating glucose. Regardless, evidence suggests the latter is the key determinant of functional recovery.

Whether increased energetic glucose utilization during reperfusion is relevant to the functional recovery from postischemic stunning is a critical question. Certainly, it seems unlikely that glucose becomes the dominant myocardial ATP source during reperfusion. In the isolated rat heart reperfused after 30 to 45 minutes of global ischemia, Lopaschuk et al demonstrated that palmitate oxidation recovers within 1 hour and supplies the same fraction of total ATP (~90%) as in aerobic control hearts. Similarly, in the extracorporeally perfused pig heart, fatty acid oxidation returns to normal even supernormal levels within 1 hour of reperfusion after a 45- to 60-minute ischemic period. Myeas et al observed moderate (48%) impairment in regional fatty acid oxidation and increased (273%) regional glucose oxidation during reperfusion after a 1-hour coronary occlusion in canines, but even under those circumstances, fatty acid oxidation continued to account for ~75% of total ATP synthesis.

Glucose-derived ATP may nevertheless be qualitatively important. Evidence suggests myocardial energy metabolism is compartmentalized such that ATP formed in the cytosol by glycolysis is used preferentially to support homeostatic processes (eg, ion transport), whereas ATP formed oxidatively in mitochondria supports mechanical work. Glycolysis may in fact be functionally coupled to sarcoplasmic reticulum calcium transport, a process whose impairment may contribute to postischemic stunning. Thus, increased glycolytic flux during reperfusion, even if not a large fraction of total ATP production, may contribute to maintaining cellular viability. Several investigators, including Johnston and Lewandowski, Liedtke et al, and Lopaschuk et al, have reported that recovery of mechanical function in vitro is improved by pharmacological stimulation of pyruvate dehydrogenase flux and therefore pyruvate oxidation. Additional studies will be needed to distinguish whether the increased FDG uptake observed 24 hours into reperfusion reflects acceleration of glycolysis alone or a concomitant increase in pyruvate oxidation.

Regional Blood Flow 24 Hours After Reversible Coronary Occlusion

Reduction in regional myocardial blood flow late after reperfusion has been reported in a number of species after coronary occlusions of > 15 minutes’ duration. Although data in rats are limited, perhaps owing to the technical challenge of measuring regional blood flow in this species, the magnitude of flow reduction we observed 24 hours after reperfusion was begun (25% to 30%) was similar to that reported 3 or 4 hours into reperfusion after a 15-minute coronary occlusion in canines. This “no-reflow” phenomenon has been suggested to account, at least in part, for delayed recovery of mechanical function after ischemia. In the present study, significant no-reflow clearly persisted beyond the point at which regional function had fully recovered.

Methodological Considerations

The model used in the present study allows metabolic processes to be examined in a relatively homogeneous region of ischemically stunned but viable myocardium in the territory of a coronary artery and compared with control regions of the same heart. Furthermore, examinations can be made at late time points, in living animals, with blood flow and metabolism tracers ([13N]NH, and [18FDG] directly relevant to

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cardiac PET imaging in humans. However, the present study has limitations. Perhaps most importantly, \(^{18}\)FDG may underestimate myocardial uptake of glucose when the heart is ischemic\(^{29}\) or stimulated with insulin.\(^{30}\) The relevance of these observations to studies like ours that are conducted under physiological conditions in intact animals is not yet clear. Indeed, the concordance between our observation of increased regional uptake of \(^{18}\)FDG and \([1-13\,\text{C}]\)glucose would suggest that \(^{18}\)FDG does track a true increase in postischemic regional glycolytic metabolism. Nonetheless, the absolute \(^{18}\)FDG radioactive counts of anterior and posterior myocardial regions (Figure 3) may not precisely mirror absolute glucose uptake into these regions.

**Clinical Implications**

Patients frequently come to medical attention because of recent chest pain. Knowing whether this symptom represents myocardial ischemia and, if so, the size of the region affected are important diagnostic challenges. The regional \(^{18}\)FDG/\([13\,\text{N}]\text{NH}_3\) mismatch we observed 24 hours after reversible coronary occlusion in rats constitutes a delayed metabolic signature of regional ischemia that would potentially be identifiable in patients long after the resolution of traditional clinical, electrocardiographic, and functional manifestations of ischemia. Finally, the observation that increased glycolytic utilization of circulating glucose accompanies the late mechanical recovery of postischemic stunned myocardium provides additional theoretical support for clinical trials of therapies designed to further augment myocardial glycolytic flux after reperfusion.

**Acknowledgments**

This study was supported by a merit review grant from the Department of Veterans Affairs and by National Institutes of Health grants RO1 DK-40936 and PO1 DK-45735. Parts of this work were performed during the tenure of Dr McNulty as a Clinician-Scientist Awardee (AHA 91004370) of the American Heart Association.

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_Circulation_. 2000;101:917-922
doi: 10.1161/01.CIR.101.8.917
_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

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