Glucose uptake and glycogen levels are increased in pig heart after repetitive ischemia

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McFalls, Edward O., Bilal Murad, Jeih-San Liow, Mary C. Gannon, Howard C. Haspel, Alex Lange, David Marx, Joseph Sikora, and Herbert B. Ward. Glucose uptake and glycogen levels are increased in pig heart after repetitive ischemia. Am J Physiol Heart Circ Physiol 282: H205–H211, 2002.—Repetitive myocardial ischemia increases glucose uptake, but the effect on glycogen is unclear. Thirteen swine instrumented with a hydraulic occluder on the circumflex (Cx) artery underwent 10-min occlusions twice per day for 4 days. After 24 h postfinal ischemia and in the fasted state, echocardiogram and positron emission tomography imaging for blood flow ([13N] ammonia) and 2-[18F]fluoro-2-deoxy-D-glucose (FDG) uptake were obtained. Tissue was then collected for ATP, creatine phosphate (CP), glycogen, and glucose transporter-4 content, and hexokinase activity. After reperfusion, regional function and CP-to-ATP ratios in the Cx and remote regions were similar. Despite the absence of stunning, the Cx region demonstrated higher glycogen levels (33 ± 11 vs. 24 ± 11 μmol/g; P < 0.05), and this increase correlated well with the increase in FDG uptake (r² = 0.78; P < 0.01). Hexokinase activity was also increased relative to remote regions (0.62 ± 0.29 vs. 0.37 ± 0.19 IU/g; P < 0.05), with no difference in GLUT-4 content. In summary, 24 h after repetitive ischemia, glucose uptake and glycogen levels are increased at a time that functional and biochemical markers of stunning have recovered. The significant correlation between glycogen content and FDG accumulation in the postischemic region suggests that increased rates of glucose transport and/or phosphorylation are linked to increased glycogen levels in hearts subjected to repetitive bouts of ischemia.

A single episode of prolonged regional myocardial ischemia with reperfusion results in a sustained decrease in function and increase in glucose metabolism. The increase in glucose uptake 24 h after stunning follows nonoxidative pathways and is associated with incomplete recovery of glycogen (25, 34). Along with other indexes of metabolic abnormalities, however, normalization occurs within 1–3 wk in parallel with wall thickening (15, 23, 35). Unlike one episode of ischemia, repetitive ischemia with reperfusion might be expected to cause dissociation between the recovery of function and glucose metabolism. After multiple episodes of ischemia, for instance, regional function normalizes earlier as a result of “preconditioning against stunning” (37), whereas glucose uptake might remain elevated as a result of increased expression of the glucose transporters (GLUT) and/or hexokinase (8, 38). Such a sustained increase in the rate of glucose uptake could, in turn, be an important determinant of glycogen storage, as observed in myocardium from patients subjected to chronic ischemia (6) and skeletal muscle from rats exposed to chronic exercise training (28).

The primary objective of this study was to determine whether a glycogen overshoot occurs in repetitively ischemic-reperfused myocardium and, if increased, whether the levels correlate with a relative increase in glucose transport and/or phosphorylation. A secondary objective was to determine whether these changes are present at a time that other indexes of stunning have recovered (i.e., wall thickening and creatine phosphate (CP)-to-ATP ratios). Positron emission tomography (PET) was used to estimate changes in myocardial glucose transport and phosphorylation with the glucose analog 2-[18F]fluoro-2-deoxy-D-glucose (FDG) during fasting conditions.

MATERIALS AND METHODS

This study was performed under the guidance of the animal care committee at the Veterans Affairs Medical Center and conformed to the National Institutes of Health Guide For the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Animal Instrumentation

Domestic pigs (~30 kg) were sedated with xylazine (2 mg/kg im) and telazol (4 mg/kg im), intubated, ventilated, and anesthetized with isoflurane (1%). With the use of sterile techniques, the external jugular vein and internal carotid

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artery were cannulated with 7-Fr sterile catheters and subcutaneously tunneled through the neck. A left thoracotomy was performed in the fifth intercostal space, and the proximal circumflex artery was dissected free and instrumented with a Doppler flow probe (2.5 mm) and hydraulic occluder. Incisions were closed in layers and sterile dressings were applied. Postoperative prophylactic cephazolin (1 g im) was given twice per day for 2 days.

**Experimental Protocol**

Animals were allowed to recover for 72 h after surgery. They were then subjected to the 4-day protocol of repetitive ischemia. Before each ischemic period, the animals were brought from the animal cage into the adjacent laboratory suite and sedated with xylazine (2 mg/kg im) and telazol (4 mg/kg im). They underwent 10 min of coronary artery occlusion with complete reperfusion. The Doppler coronary flow signal and the presence of electrocardiogram changes confirmed ischemia. Occlusion of the coronary artery was performed twice per day with 4 h of reperfusion between each ischemic period.

Before the final episode of ischemia on the final day, animals were sedated, intubated, and anesthetized with isoflurane (1%). A PET scan was performed during the last period of ischemia, allowing spatial localization of the circumflex region at risk. Animals were then allowed to recover. Twenty-four hours later, the pigs were sedated, reintubated, and anesthetized with isoflurane (1%) for the terminal echocardiogram and PET studies.

**Regional Wall Thickening**

Two-dimensional echocardiograms were obtained (model 2500, Hewlett-Packard) from the right parasternal and apical views. Regional wall thickening was measured at the midparasternal short-axis view, and analyses were performed in the posterior (circumflex) and anterior (remote) walls. Wall thickening was computed as the difference between end-systolic and end-diastolic wall thicknesses and expressed as a percentage of end-diastolic thickness (Hewlett-Packard software program). End diastole and end systole were defined as the onset of the QRS and the frame with the smallest chamber size, respectively.

**Positron Emission Tomography Studies**

After the animals were positioned on the PET imaging table, images were acquired with an ECAT 953B/31 (CTI/Siemens; Knoxville, TN). Myocardial blood flows were obtained with $^{13}$N-ammonia (15 mCi) infused intravenously over 20 s during acquisition of dynamic scans. The scanning protocol consisted of one 30-s, twelve 10-s, two 30-s, three 60-s, and one 900-s frame. During the final PET study, FDG (6 mCi) was infused for 50 min after the blood flow study and dynamic scans were acquired over the next 40 min. The FDG scanning protocol included twelve 10-s, six 30-s, four 60-s, three 120-s, three 300-s, and one 600-s frame. Arterial blood samples were obtained during the FDG scan, and plasma substrate levels were later analyzed by enzymatic techniques.

**Data Analysis of PET Images**

For analysis of the studies, ~10 regions of interest (ROIs) were obtained from both the ischemic (circumflex) and non-ischemic (remote) territories and saved for later analysis. A circular ROI was obtained from the largest portion of the LV cavity to serve as the arterial input. Time-activity curves for each ROI were obtained from the dynamic $^{13}$N-ammonia and FDG scans.

**Myocardial blood flow.** For estimation of regional myocardial blood flow, we applied a three-compartment model (27). Myocardial blood flows were obtained by a nonlinear least-square fitting to the model equation using the input function and 18 tissue samples acquired in the first 6 min of the dynamic scan.

**FDG uptake.** Patlak plots were generated from the time-activity curves. Values were averaged for the circumflex and remote regions. The Patlak plot defines a constant ($K$), which incorporates the forward ($k_1$) and reverse ($k_2$) rate constants from plasma to tissue as well as the phosphorylation ($k_3$) constant. The formula is expressed as $K = (k_1 \times k_2)/(k_2 + k_3)$. The dephosphorylation constant ($k_4$) is assumed to be zero. The metabolic rates of glucose uptake were determined from arterial plasma glucose samples an assumed lumped constant of 0.67 (29).

**Postmortem Analysis**

After the final PET scan, animals were returned to the adjacent surgical suite for tissue extraction. To avoid hypotension during exposure of the heart, anesthesia was supplemented with α-chloralose (150 mg/kg iv) while the isoflurane was weaned. A midline sternotomy was then performed. During stable hemodynamics and within 30 min of the final PET study, transmural biopsies were obtained from the circumflex and remote regions using a modified variable high-speed dental drill. The drill bit was capable of acquiring 100 mg of full-thickness specimens, which were transferred to liquid nitrogen-cooled 2-methyl-butane within 1 s. They were then stored at −70°C (24). Discrimination was not made between endocardial and epicardial tissue and therefore subsequent analysis was expressed as transmural tissue levels.

**ATP and CP.** The tissue was extracted within 24 h in 7.1% perchloric acid, homogenized, and centrifuged. The supernatant was neutralized (pH 7.2) with 2 N KOH, 0.4 M imidazole, and 0.4 M KCl and centrifuged to remove potassium perchlorate. Samples were stored at −70°C. ATP and CP were assayed spectrophotometrically in a two-step coupled enzymatic system with hexokinase and glucose-6-phosphate in which the reduction of nicotinamide adenine dinucleotide phosphate (NADP) at $E_{440}$ was followed.

### Table 1. Hemodynamics, myocardial function, and blood flow 24 h postrepetitive ischemia

<table>
<thead>
<tr>
<th>Systemic Hemodynamics</th>
<th>Regional Wall Thickening, %</th>
<th>Myocardial Blood Flow, ml/min/m²·100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>Mean BP, mmHg</td>
<td>Circumflex Remote</td>
</tr>
<tr>
<td>105 ± 17</td>
<td>78 ± 11</td>
<td>28 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.02 ± 0.22</td>
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<tr>
<td></td>
<td></td>
<td>1.04 ± 0.29</td>
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</tbody>
</table>

Values are means ± SD; $n = 13$ pigs. HR, heart rate; BP, blood pressure. Hemodynamics were recorded during the echocardiogram and were similar to measurements taken during the positron emission tomography study.

### Table 2. ATP and CP at 24 h postrepetitive ischemia

<table>
<thead>
<tr>
<th></th>
<th>ATP, μmol/g wet wt</th>
<th>CP, μmol/g wet wt</th>
<th>CP/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumflex</td>
<td>4.40 ± 1.36</td>
<td>6.69 ± 1.51</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Remote</td>
<td>3.84 ± 0.71</td>
<td>6.48 ± 1.14</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n = 13$ pigs. CP, creatine phosphate.
Table 3. Plasma substrates and myocardial glucose uptake 24 h postrepetitive ischemia

<table>
<thead>
<tr>
<th>Plasma Substrates</th>
<th>Glucose, μmol/ml</th>
<th>Lactate, μmol/ml</th>
<th>Fatty acids, μmol/ml</th>
<th>Insulin, μU/ml</th>
<th>Circumflex</th>
<th>Remote</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.4 ± 0.5</td>
<td>1.0 ± 0.3</td>
<td>580 ± 100</td>
<td>6.1 ± 2.5</td>
<td>0.32 ± 0.05*</td>
<td>0.26 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 13 pigs. *P < 0.05 vs. remote region.

**GLUT content.** Samples were given a number code and analyzed for GLUT content without knowledge of the sample origin. Membrane isolation, protein assays, and immunoblotting of GLUTs were performed as previously described (14). Antisera to the carboxyl-termini of rat GLUT-1 and GLUT-4 elicited to the 13 and 18 COOH-terminal amino acid residues were employed. Frozen tissue (30–50 mg) was homogenized (4 × 30 s), sonicated, and centrifuged (200,000 g at 4°C) for 22 min. Membranes were subjected to sodium dodecyl sulfate-11% polyacrylamide gel electrophoresis and the resolved proteins were electrothermically transferred to nitrocellulose. 11% polyacrylamide gel electrophoresis and the resolved proteins were electrophoretically transferred to nitrocellulose. The blots were blocked with 5% nonfat dried milk and probed with antibodies to the GLUTs (1:1,000 dilution), and bound IgG detected horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000 dilution) as required. Enhanced chemiluminescence detection was performed with X-ray film. For detection of 125I on dried blots, a Bio-Rad (Hercules, CA) luminescence detection was performed with X-ray film. Rabbit IgG (1:10,000 dilution) as required. Enhanced chemiluminescence detection was performed with X-ray film.

**Hexokinase activity.** Frozen tissue was homogenized and hexokinase activity was estimated by determining the rate of NADP production measured over 20 min at 340 nm and 30°C.

**Immunohistochemistry.** Transmural sections (5 μm) from frozen samples were cut on a cryostat microtome, mounted on Superfrost Plus slides, air dried for 2 min, and stored at −80°C. Sections were fixed in methanol at −20°C for 20 min, rehydrated with Tris-buffered saline (TBS), and blocked with 1% bovine serum albumin (fraction V) and 0.5% Tween 20. For colocalization of GLUT-4 with nuclei, slides were incubated with a polyclonal anti-hexokinase antibody (NeoMarkers; Fremont, CA). Sections were used at a dilution of 1:50 and 1:200, respectively, and bound IgG detected horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1,000 dilution) as required. Enhanced chemiluminescence detection was performed with X-ray film. For detection of 125I on dried blots, a Bio-Rad (Hercules, CA) luminescence detection was performed with X-ray film. Rabbit IgG (1:10,000 dilution) as required. Enhanced chemiluminescence detection was performed with X-ray film.

**Statistical Analysis**

Results are expressed as means ± SD. Differences between the postischemic circumflex and remote regions were tested at the P < 0.05 level of significance with paired Student’s t-test.

Fig. 1. In one animal, myocardial blood flow (MBF; [13N]ammonia) is shown during the final circumflex artery occlusion and 24 h later together with 2-[18F]fluoro-2-deoxy-D-glucose (FDG) uptake. The transaxial images demonstrate that FDG uptake during fasted conditions is higher in the postischemic region 24 h after repetitive ischemia-reperfusion.
Glycogen Levels

Glycogen in the postischemic circumflex region was \( \sim 25\% \) higher than in the remote region, with no regional differences detected in activities of either GS-I or phosphorylase (Table 4). When normalized to the remote region, the degree of increased glycogen within the postischemic circumflex region correlated well with the relative increase in FDG uptake within the same regions (\( r^2 = 0.78; P < 0.001 \)). This suggests that the increased rates of glucose transport and/or phosphorylation are important determinants of the relative accumulation of glycogen.

GLUT-4 and Hexokinase Activity

The content of GLUT-1 and GLUT-4 in the heart tissue samples was compared with that of membranes from the pig and rat brains and adipose tissues. The pig brain contained easily measurable quantities of \( \sim 50\-kDa \) GLUT-1. However, the content of GLUT-1 in the pig heart samples was \( \sim 10\% \) of brain tissue and near the limit of detection with our method. GLUT-4 of \( \sim 50\-kDa \) was easily detected in all the pig heart membrane samples and was about twofold more abundant than in adipose membranes (Fig. 2). There was no difference in total membrane associated GLUT-4 content between the postischemic circumflex and remote regions. Hexokinase activity was significantly higher in the postischemic circumflex region compared with the remote territory (Fig. 3). Although quantitative differences in the degree of GLUT-4 translocation were not determined, qualitative estimates of regional difference in the degree of translocation by immunohistochemistry were not apparent (Fig. 4). Immunohistochemical staining of hexokinase showed that the activated form from the postischemic circumflex region was dispersed within the cytoplasm in the vicinity of the mitochondria, whereas the inactive form from the remote region was clumped in perinuclear areas (Fig. 5).

DISCUSSION

Several new observations are reported in this study. First, in a model of repetitive regional myocardial ischemia, abnormalities in glucose metabolism during fasting conditions persist at a time that mechanical and bioenergetic markers of stunning have recovered. Second, the degree of glycogen “overshoot” in this model correlates with the relative degree of increased FDG accumulation, suggesting that increased rates of

<table>
<thead>
<tr>
<th>Glycogen, ( \mu \text{mol/g} )</th>
<th>Synthase Activity</th>
<th>Phosphorylase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumflex</td>
<td>33 ± 11*</td>
<td>1.33 ± 0.32</td>
</tr>
<tr>
<td>Remote</td>
<td>24 ± 11</td>
<td>1.30 ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 13 \) pigs. GS-I, glycogen synthase I; GPa, glycogen phosphorylase type a. *\( P < 0.05 \) vs. remote region.
glucose transport and/or phosphorylation are linked to increased glycogen levels in hearts subjected to repetitive bouts of ischemia. Third, the augmented glucose uptake 24 h after repetitive ischemia is associated with increased hexokinase activity.

**Glucose Uptake and Reperfused Myocardium**

Many animal studies utilizing a variety of techniques have characterized changes in myocardial substrate utilization after ischemia-reperfusion. Although glycolysis may play an important role in supporting some cellular functions during early reperfusion (9, 16), it is clear that the predominant substrate for overall energy expenditure remains fatty acids (20, 31). Within severely stunned myocardium, a sustained increase in glucose uptake has been observed at least 24 h after ischemia and signifies an increase in non-oxidative pathways (34). It is interesting that the increase in glucose uptake occurs at a time of incomplete recovery of glycogen, suggesting that reperfused myocardium shunts glucose away from glycogen synthesis and toward alternate energy-generating pathways (25). Glycogen turnover is a dynamic process in the heart and a small change in the rate of synthesis or degradation could impart a larger change in total glycogen accumulation (11). If glycolysis were slowed or unchanged after reperfusion, increased rates of glucose transport and/or phosphorylation would be important determinants of overall glycogen levels.

**Glucose Transport and Phosphorylation**

Multiple episodes of ischemia-reperfusion could have a sustained effect on glucose transport and/or phosphorylation. Prolonged moderate ischemia provides a strong stimulus for translocation of GLUTs from cytoplasmic stores to active sites on the sarcolemma (41). The signal involved with this process acts independently of phosphatidylinositol-3 kinase or insulin-mediated mechanisms (36) and is likely dependent on cell signaling enzymes such as mitogen-activated protein kinases (MAPK), p38 MAPK, and MAPK-activated protein kinase-2 (40). Conversely, it might also involve activation of other kinases in response to ischemia, such as AMP-activated protein kinase (32). Whether intracellular translocation of GLUTs is a prerequisite for a sustained increased in glucose uptake post ischemia-reperfusion is not clear. In the present study, the degree of GLUT-4 translocation did not appear by immunohistochemistry to differ between the postischemic circumflex and remote regions 24 h after the final episode of ischemia. Although this was not a quantitative assessment of the degree of translocation, the findings support data from other models of reperfused myocardium, in which a sustained increase in glucose uptake was observed at a time that translocation of GLUTs was not evident (26). We also found no difference in GLUT-4 translocation in this protocol of repetitive ischemia-reperfusion, which is consistent with another swine model of chronic ischemia involving a 4-day coronary artery stenosis (18). In the present study, we analyzed GLUT-1 content in all of the samples, with specific probes that showed a robust signal for rat and pig brain. The total content of GLUT-1 was <10% of the standards in all samples and, although clearly detectable, was beyond the limits of adequate quantitation. Previous studies (17) from rat cardiac myocytes have

![Fig. 4. Dual histochemical staining of GLUT-4 (green) and nuclei (yellow) with confocal microscopy (×200)](http://ajpheart.physiology.org/)

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![Fig. 5. Dual histochemical staining of hexokinase (red) and mitochondria (green) with confocal microscopy (×200)](http://ajpheart.physiology.org/)

Hexokinase activity is increased 24 h after repetitive ischemia, suggesting that reperfused myocardium shunts glucose away from glycogen synthesis and toward alternate energy-generating pathways. Prolonged moderate ischemia provides a strong stimulus for translocation of GLUTs from cytoplasmic stores to active sites on the sarcolemma. The signal involved with this process acts independently of phosphatidylinositol-3 kinase or insulin-mediated mechanisms and is likely dependent on cell signaling enzymes such as mitogen-activated protein kinases (MAPK), p38 MAPK, and MAPK-activated protein kinase-2. Conversely, it might also involve activation of other kinases in response to ischemia, such as AMP-activated protein kinase. Whether intracellular translocation of GLUTs is a prerequisite for a sustained increased in glucose uptake post ischemia-reperfusion is not clear. In the present study, the degree of GLUT-4 translocation did not appear by immunohistochemistry to differ between the postischemic circumflex and remote regions 24 h after the final episode of ischemia. Although this was not a quantitative assessment of the degree of translocation, the findings support data from other models of reperfused myocardium, in which a sustained increase in glucose uptake was observed at a time that translocation of GLUTs was not evident. We also found no difference in GLUT-4 translocation in this protocol of repetitive ischemia-reperfusion, which is consistent with another swine model of chronic ischemia involving a 4-day coronary artery stenosis. In the present study, we analyzed GLUT-1 content in all of the samples, with specific probes that showed a robust signal for rat and pig brain. The total content of GLUT-1 was <10% of the standards in all samples and, although clearly detectable, was beyond the limits of adequate quantitation. Previous studies from rat cardiac myocytes have
shown that GLUT-1 content is ~25% of that of GLUT-4 after a 5-h fast. After a 24-h fast, however, as was instituted in the present study, GLUT-1 was reduced by ~40%, whereas GLUT-4 content remained unaffected (17). Therefore, the explanation for low levels of GLUT-1 relative to GLUT-4 in the present study may reflect the intense fasting conditions. Ischemia has been shown to induce an upregulation in GLUT-1 mRNA expression within the heart (7), and it is possible that the metabolic state during the present study abolished significant differences in the protein after the repetitive ischemia protocol.

Of interest, hexokinase activity was increased 24 h after the final episode of ischemia. Although this observation is new, it is not surprising, considering that hexokinase activity was shown (28) as both activated and expressed within skeletal muscle from rats undergoing vigorous exercise training. Although transport of glucose within myocardium plays a predominant role in the determination of overall glucose utilization, increased rates of phosphorylation may be important during various metabolic conditions (21). The immunohistochemical localization of hexokinase in its activated state was dispersed throughout the cytoplasm in the vicinity of the mitochondria. This is compatible with previous studies (33) that have provided evidence for compartmentalization of the active form of hexokinase within the cytoplasm compared with the inactive state, which is clumped in perinuclear locations. From the present study, it is difficult to determine the physiological significance of these sustained abnormalities in glycogen and hexokinase. However, the findings support the notion that a sustained metabolic imprint may be observed well after other indexes of stunning have normalized.

Effects of Repetitive Ischemia-Reperfusion

Although the length of time required for recovery of the glucose abnormalities is dependent upon the severity of the period of ischemia, metabolic markers of reperfusion injury return to normal in parallel with function within weeks of a severe episode of ischemia (3, 15, 23, 35). In models of repetitive ischemia-reperfusion, regional function normalizes earlier than after a single episode of ischemia as a result of “preconditioning against stunning” (37). As such, one would expect that functional recovery within models of repetitive stunning might precede recovery of other metabolic markers of reperfusion injury, such as the sustained increase in glucose uptake. In the present study, reperfused myocardium was studied within 24 h of the final ischemic episode. In pigs subjected to multiple episodes of ischemia-reperfusion, that period of time has been shown to be within the late window of preconditioning against stunning. During this time period, the elaboration of nitric oxide (NO) via increased expression of inducible NO synthase may account for early recovery of function after stunning (2). Although NO has been shown to alter substrate utilization by decreasing glucose uptake (30), activated p38 MAPK and MAPK-activated protein kinase-2 has been observed at this time (5) and might be expected to increase glucose transport (40). Whether overall fatty utilization is altered differently after repetitive ischemia-reperfusion compared with a single episode of ischemia is unclear (12).

Limitations

In these studies, regional wall thickening was measured by echocardiography rather than by piezoelectric crystals, because implantation of crystals could create an inflammatory reaction on the epicardial surface and confound the interpretation of the final FDG study. A two-dimensional echocardiogram has been deployed in PET-related studies (1, 4, 22) using a variety of chronically ischemic animal models. Although multiple estimates of regional function and glucose uptake during evolution of the model would have been informative, the primary focus was to correlate tissue levels of glycogen and hexokinase at the conclusion of the protocol, along with in vivo estimates of glucose uptake.

Analogs of glucose such as deoxyglucose are transported and phosphorylated at different rates than glucose. Approximations have been made in the present study, which have been previously validated. However, it has been well described (13, 19) that the factor that corrects for differences in rates of transport between glucose and deoxyglucose may vary dependent upon substrate and insulin levels. To minimize this problem, we used fasted conditions, in which levels of insulin and glucose were low.

In conclusion, in this model of repetitive ischemia-reperfusion in swine, glucose uptake and glycogen storage are increased 24 h after the final ischemic period, at a time that functional and bioenergetic markers of stunning have recovered. The significant correlation between glycogen content and FDG accumulation in the postischemic region suggests that increased rates of glucose transport and/or phosphorylation are linked to increased glycogen levels in hearts subjected to repetitive bouts of ischemia.

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