Profound Underestimation of Glucose Uptake by \([^{18}F]2\text{-Deoxy-2-fluoroglucose in Reperfused Rat Heart Muscle}

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**Background**—\([^{18}F]2\text{-deoxy-2-fluoroglucose (FDG) is widely used as a tracer for glucose uptake in ischemic heart muscle. We tested the effects of low-flow ischemia and reperfusion on the ratio of tracer/tracee (lumped constant, LC).**

**Methods and Results**—Isolated working rat hearts were perfused with Krebs-Henseleit buffer containing only glucose 5 mmol/L (group 1) or glucose 5 mmol/L plus oleate 0.4 mmol/L (group 2, fed; group 3, fasted). Dynamic glucose uptake was measured simultaneously with \([2-^{3}H]\text{glucose and with FDG. After 20 minutes, coronary flow was reduced by 75% for 30 minutes before it was returned to control conditions for the final 20 minutes. Hexokinase activity in the cytosolic and mitochondrial fractions and tissue metabolites were determined. Rates of glucose uptake were highest when glucose was the only substrate. Glucose uptake, FDG uptake, and the LC increased during ischemia only in group 3. There was no change of these parameters during ischemia in groups 1 and 2. FDG uptake decreased significantly with reperfusion in groups 2 and 3, and there was a striking fall in the LC (from >1.0 to <0.2, \(P<.001\)). The fall in the LC was associated with a significant increase in intracellular free glucose. Neither ischemia nor reperfusion affected the kinetic properties of hexokinase.

**Conclusions**—FDG profoundly underestimates glucose uptake during reperfusion in the presence of fatty acids. In the fasted state, however, FDG overestimates glucose uptake during ischemia. The results indicate limitations in the use of FDG to quantify myocardial glucose uptake in human heart. (*Circulation*. 1998;97:2454-2462.)

**Key Words:** ischemia ■ radioisotopes ■ nuclear medicine ■ enzymes ■ glucose ■ fatty acids

To date, the gold standard for the detection of viability in dysfunctional myocardium of patients suffering from coronary artery disease is positron emission tomography using FDG as a tracer for glucose uptake (PET viability studies).\(^1\) The method is based on the principle that in reversibly ischemic myocardium, uptake of FDG is increased relative to myocardial blood flow. This discrepancy between the accumulation of FDG and a flow tracer (eg, \([^{13}N]H_3\)i) is thought to reflect increased glucose uptake by the myocardium.

However, FDG is a glucose tracer analogue rather than a simple glucose tracer. Like glucose, FDG is transported and phosphorylated, but unlike glucose, FDG is not further metabolized to products that are ultimately released by the myocardium. The kinetic differences of transport and phosphorylation between FDG and glucose are reflected in a ratio called the “lumped constant” (LC).\(^2\) This ratio allows for the quantification of glucose uptake from the accumulation of FDG. A fixed value for the LC of 0.67 has been derived from an animal model\(^3\) and has since been used to quantify glucose uptake in human heart.\(^4\) However, the correlation between \([5-^{3}H]\text{glucose and [U-}^{14}\text{C]deoxyglucose as markers of glycolysis in reperfused myocardium is very poor.}^7\)

Recently, we\(^8\) and others\(^10\) found that the LC is a quotient, which changes as a result of changes in the metabolic environment. We established in rat heart that the addition of insulin or competing substrates leads to a decrease in the LC.\(^8,9\) Two different mechanisms have to be considered for the explanation of these changes. The first mechanism involves the translocation of hexokinase from the cytosolic to the mitochondrial cell compartment, with a concomitant decrease in the affinity of hexokinase for FDG.\(^9\) The second mechanism is based on Michaelis-Menten kinetics and the fact that FDG is transported in favor of glucose but glucose is phosphorylated in favor of FDG.\(^12\) Thus, depending on the rate-limiting step for glucose uptake, the LC will range from high values (transport rate limiting) to low values (phosphorylation rate limiting).

In the brain, ischemia has been shown to cause an increase in the LC.\(^13-15\) The influence of ischemia on the LC in heart muscle has thus far been investigated only under in vitro conditions with glucose as the only substrate.\(^16,17\) Marshall et
al found no change in the LC during low-flow ischemia in a rabbit septum, and we did not observe any change in the LC with reperfusion of the globally ischemic isolated rat heart. The observations of Liedtke et al prompted us to examine the influence on the LC of physiological cosubstrates to glucose during ischemia and reperfusion. Knowledge about the LC under these conditions would be important, for two reasons: first, to quantify glucose uptake in underperfused or reperfused myocardium in vivo, and second, to possibly provide an explanation for conflicting reports underperfused or reperfused myocardium in vivo, and second, for two reasons: first, to quantify glucose uptake in underperfused or reperfused myocardium in vivo, and second, to possibly provide an explanation for conflicting reports of clinical PET studies on FDG retention by dysfunctional myocardium.

We therefore investigated the effects of low-flow ischemia and reperfusion on both myocardial glucose and FDG uptake of the isolated working rat heart. We tested the effects under conditions in which glucose was the only substrate and both glucose and oleate were present at physiological concentrations. We also determined hexokinase activity and tissue metabolites of glucose metabolism to obtain evidence in support of one or the other proposed mechanism in the setting of ischemia and reperfusion. We were able to confirm the earlier results that the LC is unaffected by ischemia and reperfusion as long as glucose was the only substrate. However, under the far more physiological condition in which oleate is present as a second substrate, we observed a profound underestimation of glucose uptake by FDG during reperfusion.

**Methods**

**Animals**

Male Sprague-Dawley rats (275 to 300 g) were obtained from Harlan (Indianapolis, Ind). Animals were either fed ad libitum or fasted overnight (16 to 20 hours) with free access to water. The use of animals and the experimental protocol were approved by the Animal Welfare Committee of the University of Texas–Houston Health Science Center.

**Materials**

Chemicals were obtained from Fisher Scientific or Sigma Chemical Co. Enzymes and cofactors for metabolite assays were obtained from Boehringer Mannheim or Sigma. Regular human insulin (Humulin R) was obtained from Eli Lilly and Co.

**Radioisotopes**

The positron-emitting glucose tracer analogue FDG (specific activity >3000 Ci/mmol) was prepared by the method of Hamacher et al at the University of Texas–Houston Health Science Center Cyclotron Facility. High-performance liquid chromatography–purified [2-3H]glucose, [1,2-3H]2-deoxyglucose, [U-14C]sucrose, and [U-14C]glucose were obtained from Amersham Corp. The purity of the H-labeled tracers was ascertained by measuring the intrinsic 3H2O content.

**Equipment**

PET = positron emission tomography

V_{max} = maximum reaction velocity

Selected Abbreviations and Acronyms

- FDG = [18F]2-deoxy-2-fluoroglucose
- G6P = glucose 6-phosphate
- K_m = Michaelis constant
- LC = lumped constant
- PET = positron emission tomography

**Working Heart Preparation**

The preparation is a modification of the model described by Neely et al and has been described in detail earlier. Hearts were then perfused as working hearts at 37°C with recirculating Krebs-Henseleit buffer (200 mL containing either glucose 5 mmol/L or glucose 5 mmol/L plus sodium oleate 0.4 mmol/L) bound to 1% BSA, Cohn fraction V, and fatty acid free (Intergen Co) and equilibrated with 95% O_2/5% CO_2. Total perfuse [Ca^2+] was 2.5 mmol/L. All experiments were carried out with a preload of 15 cm H_2O. The afterload was varied between 10 and 35 cm H_2O according to the perfusion protocol. The hearts were beating spontaneously at an average rate of 200 bpm. Aortic flow and coronary flow were measured every 5 minutes. Heart rate as well as systolic and diastolic aortic pressures were measured continuously with a 3F Millar transducer (Millar Instruments) and a MacLab physiological recording system (ADInstruments). Cardiac performance was expressed as cardiac power (the product of cardiac output and mean aortic pressure) in terms of milliwatts as described earlier. The perfusion chamber was placed between a pair of coincidence detectors for the detection of positron annihilation (see below).

**Perfusion Protocol**

All hearts were perfused for 70 minutes. After 5 minutes, the radioactive tracers FDG (363±38 μCi/mL) and [2-3H]glucose (10 μCi) were added to the recirculating perfusate (200 mL). At 20 minutes, the afterload was lowered from 100 to 35 cm H_2O for the following 30 minutes. This procedure to induce ischemia has been described in detail earlier. The afterload was readjusted to 100 cm H_2O for the final 20 minutes of the perfusion protocol. Hearts in group 1 (n=4) came from fed animals and were perfused with Krebs-Henseleit buffer containing glucose (5 mmol/L) as the only substrate. In group 2 (n=6), the perfusate contained both glucose (5 mmol/L) and oleate (0.4 mmol/L) as substrates. In group 3 (n=6), hearts came from fasted animals, and the perfusate contained both glucose (5 mmol/L) and oleate (0.4 mmol/L) as substrates. We used fasted animals because glucose metabolism after ischemia differs between the fed and the fasted state.

For the final 5 minutes of the experiments, the hearts in groups 1 and 2 were perfused with nonradioactive, nonrecirculating perfusate to wash out any tracer from the extracellular fluid space. To determine the extracellular fluid space (group 3), [U-14C]sucrose (5 μCi) was added after 15 minutes of reperfusion, and recirculating perfusion was continued for the final 5 minutes. At the end of the perfusions, all hearts were freeze-clamped with aluminum tongs cooled to the temperature of liquid nitrogen.

In separate sets of perfusions, hearts were perfused according to protocol 2 or 3, but perfusions were ended either after 20 minutes (before ischemia; group 2, n=5; group 3, n=4), after 50 minutes (end of ischemia; group 2, n=4; group 3, n=5), or after 70 minutes (end of reperfusion; group 2, n=4). Hearts in group 2 were perfused without any radioactive tracers and arrested in ice-cold isolation medium (180 mmol/L KCl, 10 mmol/L Tris, and 0.5 mmol/L MgCl_2, pH 7.4) for the determination of hexokinase and citrate synthase activity. The hearts perfused for protocol 3 were freeze-clamped for metabolite extraction.

**Measurement of Radioactivity**

**FDG**

Tissue accumulation of the positron-emitting FDG was counted on a second-to-second basis by a pair of coincidence detectors placed on opposite sides of the heart. Positron annihilation between the detectors was measured with a fast/slow coincidence system connected to a personal computer for data acquisition. FDG radioactivity in the perfusate was continuously counted by β-counting of a portion of the arterial side of the recirculating perfusate. All counts were decay-corrected to the time FDG was added. The system was calibrated with a heart-shaped model (bar phantom) containing a known amount of radioactivity. A calibration factor (cps/μCi) was obtained from the decay curve of the bar phantom, which was used to calculate glucose uptake rates (μmol·min⁻¹·g dry wt⁻¹) from the slopes of the time-activity curves.
H and 14C

Dual-label counting of these isotopes was performed on a Packard 1900 TR liquid scintillation analyzer by the method of spectral index analysis as described by the manufacturer (Packard Instruments).

Glucose uptake was determined by the rate of 1H2O production from [2-3H]glucose.25 Release of H2O into the perfusate was analyzed in 5-minute intervals. H2O was separated from [2-3H]glucose in the perfusate by anion exchange chromatography on AG-1X8 resin (BioRad Laboratories).26 The amount of H2O in the perfusate was plotted against time, and the slopes of the desired intervals were used to calculate glucose uptake rates, which were expressed as μmol · min⁻¹ · g dry wt⁻¹. Expression of glucose uptake, measured by FDG or [2-3H]glucose, as rates of uptake ([1,2-3H]2-deoxyglucose and [U-14C]glucose were used for the hexokinase assay as described below. [U-14C]sucrose was used to determine the extracellular fluid space.

Calculation of the LC

The LC was calculated for every experiment before ischemia, during ischemia, and during reperfusion by dividing the uptake rate as determined by FDG accumulation by the uptake rate as determined from H2O release from [2-3H]glucose.

Tissue Analysis

The frozen tissue, ground under liquid nitrogen, was extracted with 6% perchloric acid. The tissue extracts were neutralized and immediately assayed for G6P and lactate by standard enzymatic methods. Glycogen was assayed by the method of Walaas and Walaas27 with amyloglucosidase. The amount of unphosphorylated intracellular glucose (intracellular free glucose) was determined by measuring the glucose content in the tissue and subtracting the portion in the extracellular fluid space (ECFS) was determined with [U-14C]sucrose as described below. [U-14C]sucrose was used to determine the extracellular fluid space.

Perfusate Samples

Samples of the coronary effluent (1 mL) were withdrawn every 5 minutes. Samples were stored on ice until they were assayed for glucose and lactate with a glucose/lactate analyzer (2300 STAT, YSI Inc). Myocardial lactate release was calculated from the appearance of lactate in the perfusate.25 The samples were analyzed for the specific activity of [2-3H]glucose and for 3H2O content.

Subcellular Fractionation

The cooled hearts were homogenized in isolation medium (180 mmol/L KCl, 10 mmol/L Tris, and 0.5 mmol/L MgCl2, pH 7.4) as described earlier.28 We modified the method of Chemnitius et al29 to isolate subsarcolemmal and interfibrillar mitochondria. Percoll (Pharmacia LKB) step gradients were prepared with isolation medium in polyallomer tubes (Du Pont Corp). Heart muscle homogenates (600 μL) were suspended in isolation medium and Percoll to give a 2-mL fraction containing 65% (vol/vol) Percoll at the bottom of the tube. Lighter layers containing 50% (2 mL), 30% (3 mL), and 5% (1 mL) of Percoll in isolation medium, respectively, were successively layered on top. Gradients were spun for 60 minutes at 20,000 rpm (50,000g maximal gravitational force) in a Sorvall RC-5B centrifuge with a SS-34 rotor. Eight fractions (1 mL each) were collected through a small hole melted in the bottom of the centrifugation tube. Subsarcolemmal mitochondria were recovered in gradient fraction 4 (buoyant density, 1.068 g/mL) and interfibrillar mitochondria in fraction 7 (buoyant density, 1.040 g/mL).28 The cytosol was recovered in fractions 1 and 2.

Enzyme Assays

Citrate Synthase

Citrate synthase (E.C. 4.1.3.7) was determined by the method of Srere.30 Assays were performed at 25°C. Total and free citrate synthase activity of homogenate and gradient fractions were determined after preincubation with or without 2.5% (vol/vol) Triton X-100. The free citrate synthase activity is considered an indicator for structurally damaged mitochondria. Latent citrate synthase activity was calculated as the difference of total and free activity and represents structurally intact mitochondria.31

Hexokinase

Hexokinase was determined by a modification of the method of Gots and Bessman.31 The method is based on the conversion of [U-14C]glucose to [U-14C]G6P or [1,2-3H]2-deoxyglucose to [1,2-3H]2-deoxyglucose 6-phosphate and the separation of the two compounds by batch chromatography with Dowex 2-X8 resin (BioRad Laboratories). All assays were performed in duplicate at room temperature.

The Vmax of hexokinase was determined in all eight fractions and in the homogenate. Kh was determined in the cytosolic and the mitochondrial fractions. For the determination of the Kh values of hexokinase associated with mitochondria, equal volumes of fractions 4 (subsarcolemmal mitochondria) and 7 (interfibrillar mitochondria) were combined. The sample with the cytosolic hexokinase was obtained by passing 600 μL of fraction 1 (which was free of latent citrate synthase) through a Sephadex G25 column (5 mL) to remove any endogenous glucose. The sample was eluted from the column with 4 volumes of isolation medium. Analysis of enzyme kinetics was performed according to Eadie and Hofstee.32

Statistical Analysis

All data are presented as mean±SD. Statistical comparison was made by paired or unpaired Student’s t test or by single-factor ANOVA with post hoc comparison by Newman-Keuls test as appropriate.33 Differences were considered statistically significant when P<.05.

Results

Cardiac Performance

Before ischemia, cardiac power was 9.69±2.84 mW in group 1, 8.39±2.43 mW in group 2, and 9.52±2.67 mW in group 3. Coronary flow was 20.1±3.98 mL/min in group 1, 22.6±5.67 mL/min in group 2, and 20.5±1.26 mL/min in group 3. Flows are considerably higher than coronary flow in vivo, which is due to the absence of erythrocytes on the one hand and a lower viscosity of the perfusate compared with blood on the other hand. The absence of ischemia under these conditions has been previously documented.32 Low-flow ischemia resulted in a reduction of coronary flow to 25.2%, 26.0%, and 26.3% of flow before ischemia and of cardiac power to 24.4%, 26.5%, and 31.8% of power before ischemia, respectively. With reperfusion, there was immediate and full recovery to preischemic values in all three groups. There were no significant differences among the groups during the three perfusion periods. See bottom panels of Figures 1 and 2 for representative tracings of cardiac power.

Glucose Uptake

The upper panel of Figure 1 shows the tissue time-activity curve for FDG accumulation and the release of 3H2O from
[2-13C]glucose of a heart perfused with glucose as the only substrate (group 1). Both FDG accumulation and release of 3H2O were linear before, during, and after ischemia. Glucose uptake did not change with ischemia or reperfusion. FDG uptake decreased slightly with ischemia and remained the same during reperfusion. Hence, the ratio between the two uptake rates decreased slightly with ischemia, but there was no change during reperfusion.

The upper panel of Figure 2 shows the tissue time-activity curve for FDG accumulation and 3H2O release from [2-13C]glucose of a heart perfused with Krebs-Henseleit buffer containing glucose (5 mmol/L) as sole substrate. At t=20, afterload was reduced from 100 to 35 cm H2O. At t=50, afterload was raised to 100 cm H2O. At t=65, tracer-containing perfusate was changed to perfusate containing glucose (5 mmol/L) but no tracers, and radioactivity was washed out rapidly from ventricular chambers. Radioactivity in perfusate (input function) was constant throughout experiment. Numerical values at tracing present ratios of FDG to glucose uptake rates (LC) obtained before, during, and after low-flow ischemia. Cardiac power of experiment is shown at bottom. See perfusion protocol for details.

Tissue Metabolites and Lactate Production
Figure 3 shows intracellular free glucose, G6P, glycogen, and lactate content in hearts from fasted animals perfused with glucose and oleate before ischemia, at the end of ischemia, and at the end of reperfusion. The intracellular free glucose...
content was slightly increased at the end of ischemia \((P=\text{NS})\). At the same time, G6P and glycogen were decreased and lactate was increased, suggesting increased anaerobic glycolytic activity. At the end of reperfusion, G6P, glycogen, and lactate had returned to preischemic values, whereas the intracellular free glucose content was significantly elevated, suggesting that glucose transport exceeded phosphorylation during reperfusion.

We calculated the amount of myocardial lactate release in the following three perfusion periods: before ischemia, during ischemia, and during reperfusion. Lactate production before ischemia was not different among groups. Lactate production doubled during ischemia when glucose was the only substrate (from \(2.12\pm 0.62\) to \(4.86\pm 2.22\) \(\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}\)) and tripled when oleate was present as second substrate (from \(2.41\pm 1.78\) to \(7.33\pm 2.89\) [fed] and from \(1.86\pm 1.76\) to \(6.63\pm 2.12\) [fasted] \(\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}\), \(P<.01\)). During reperfusion, lactate release returned to preischemic values.

**Hexokinase and Citrate Synthase**

In the subcellular fractionation procedure, we obtained 8 fractions with densities between 1.095 and 1.027 g/mL. Both hexokinase and citrate synthase activities were quantitatively recovered from the gradient (85.0\% to 93.6\% recovery of hexokinase, 89.1\% to 91.1\% recovery of citrate synthase).

The distribution of hexokinase and citrate synthase on the gradient is shown in Figure 4 for a representative experiment.

**Figure 3.** Tissue content of intracellular free glucose, G6P, glycogen, and lactate in hearts from fasted animals perfused with glucose (5 mmol/L) and oleate (0.4 mmol/L) before ischemia \((n=5)\), at end of ischemia \((n=5)\), and at end of reperfusion \((n=6)\). Values are mean\(\pm\)SD. *\(P<.05\) vs before ischemia; +\(P<.05\) vs end of ischemia.

### Influence of 30 Minutes of Low-Flow Ischemia and 20 Minutes of Reperfusion on Glucose Uptake Measured by Both \(^{3}H_{2}O\) Release From \([2-^{3}H]\)glucose and FDG Accumulation and on the LC

<table>
<thead>
<tr>
<th>Group</th>
<th>Perfusion Period</th>
<th>(^{3}H_{2}O) Slope, (\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1})</th>
<th>FDG Slope, (\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1})</th>
<th>LC (FDG/(^{3}H_{2}O))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 ((n=4))</td>
<td>Before ischemia</td>
<td>5.23(\pm)1.26</td>
<td>6.28(\pm)1.73</td>
<td>1.27(\pm)0.47</td>
</tr>
<tr>
<td></td>
<td>Low-flow ischemia</td>
<td>4.17(\pm)0.98</td>
<td>3.81(\pm)1.22*</td>
<td>0.98(\pm)0.43</td>
</tr>
<tr>
<td></td>
<td>Reperfusion</td>
<td>4.22(\pm)1.69</td>
<td>2.76(\pm)0.52*</td>
<td>0.72(\pm)0.24</td>
</tr>
<tr>
<td>Group 2 ((n=6))</td>
<td>Before ischemia</td>
<td>2.83(\pm)1.26</td>
<td>2.27(\pm)1.14</td>
<td>0.86(\pm)0.50</td>
</tr>
<tr>
<td></td>
<td>Low-flow ischemia</td>
<td>3.08(\pm)0.85</td>
<td>3.55(\pm)1.38</td>
<td>1.16(\pm)0.38</td>
</tr>
<tr>
<td></td>
<td>Reperfusion</td>
<td>2.26(\pm)0.74†</td>
<td>0.27(\pm)0.57†*</td>
<td>0.10(\pm)0.25†*</td>
</tr>
<tr>
<td>Group 3 ((n=6))</td>
<td>Before ischemia</td>
<td>1.79(\pm)0.72</td>
<td>2.02(\pm)0.99</td>
<td>1.12(\pm)0.35</td>
</tr>
<tr>
<td></td>
<td>Low-flow ischemia</td>
<td>2.87(\pm)0.66‡</td>
<td>4.34(\pm)1.31*</td>
<td>1.51(\pm)0.27*</td>
</tr>
<tr>
<td></td>
<td>Reperfusion</td>
<td>1.83(\pm)0.88‡</td>
<td>0.47(\pm)0.61*</td>
<td>0.20(\pm)0.25*</td>
</tr>
</tbody>
</table>

Isolated working rat hearts were perfused with Krebs-Henseleit buffer containing glucose 5 mmol/L (group 1) or glucose 5 mmol/L plus oleate 0.4 mmol/L (group 2, fed; group 3, fasted). After 20 minutes of perfusion, the afterload was lowered for 30 minutes from 100 to 35 cm H\(_{2}\)O. Total perfusion time was 70 minutes. Values are mean\(\pm\)SD. *\(P<.01\) vs ischemia; †\(P<.01\) vs low-flow ischemia; ‡\(P<.05\) vs FDG slope.
Mitochondria.29 Fractions 1 and 2 are subsarcolemmal (fraction 4, 1.068 g/mL) and interfibrillar and 7, which have been classified as the fractions containing the latent citrate synthase is located primarily in fractions 4 in the first two fractions of the gradient. This activity represents completely solubilized enzyme. The free activity in fractions 3 to 8 represents particle-associated activity.29 Figure 4A shows that free citrate synthase is located primarily in the cytosol and 43.5 ± 20.2 μmol/L when associated with mitochondria. The $K_m$ of hexokinase for 2-deoxyglucose was 237 ± 186 μmol/L in the cytosol and 327 ± 143 μmol/L in the mitochondrial fractions.

The $V_{max}$ for glucose was 7.34 ± 3.94 μmol/L in the cytosol and 17.2 ± 7.14 μmol/L in the mitochondrial fraction, which is also reflected in the distribution of hexokinase on the gradient. $V_{max}$ for 2-deoxyglucose was not significantly different from $V_{max}$ for glucose.

**Discussion**

We performed this study to document any effects of low-flow ischemia and reperfusion on the LC in heart muscle and found that in hearts perfused with glucose as the only substrate, neither ischemia nor reperfusion had a significant effect. However, when oleate was present in physiological concentrations as a competing substrate to glucose, FDG accumulation was suppressed during reperfusion and there was a profound fall in the LC. During ischemia, the LC increased and FDG overestimated glucose uptake only in the fasted state. The results are in agreement with the observations in reperfused swine heart and have direct implications for the quantitative assessment of glucose uptake in human heart, because fatty acids are the main substrate for respiration of the heart in vivo. The striking suppression of FDG uptake without a comparable suppression of true glucose uptake may be the cause for serious errors in the assessment of rates of glucose uptake in dysfunctional heart muscle in vivo. Furthermore, the differential effect of the nutritional state on the LC may also bear clinical implications. A critique of the experimental model and its clinical relevance is therefore in order.

**Experimental Model and Clinical Relevance**

The isolated working rat heart model permits simultaneous measurement of FDG and glucose uptake ([2-18F]glucose) and provides a means to control the metabolic environment at physiological contractile performance. In this model, we subjected hearts to 30 minutes of low-flow ischemia as described before24 by reducing the afterload from 100 to 35 cm H$_2$O. This reduction in coronary perfusion pressure reduces coronary flow by ~75%. We were guided by the observation that in acute myocardial infarction, collateral flow defines the area at risk and limits the size of the infarct. Restoration of antegrade blood flow creates a situation of reperfusion after low-flow ischemia. We measured a significant reduction in glycogen, together with increases in tissue lactate content and lactate release, suggesting increased anaerobic glycolysis during ischemia.39

For the present experiments, we chose two different perfusate compositions. One composition with glucose as the only substrate, resembling the situation in the brain, where glucose is the only substrate that can pass the blood-brain...
barrier. Unlike in the brain, where ischemia (low-flow, total, or hypoxia) causes an increase in the LC, the LC in rat heart remained unchanged or showed a tendency toward a reduction of the values. These results are consistent with an earlier study in which we found no effect of total ischemia on the LC during reperfusion, when glucose was the only substrate. The other composition consisted of physiological concentrations of glucose (5 mmol/L) and oleate (0.4 mmol/L), the two main substrates of the heart in vivo. Under these conditions, we investigated hearts from fed or fasted animals for two reasons: first, because glucose metabolism after ischemia differs between the fed and the fasted states, and second, because patients undergoing PET studies with FDG may be in either of the two nutritional states at the time of the study. Our results suggest that the nutritional state influences glucose uptake during ischemia (fasting resulted in increased glucose uptake during ischemia). It is important to note that the LC increased during ischemia only in the fasted state. This phenomenon results in the overestimation of true glucose uptake. Increased rates of FDG uptake in the fasted state have been reported in chronically ischemic (hibernating) compared with normal myocardium in vivo. The increased rates of FDG uptake in the ischemic myocardium could not be reproduced when the same patients were investigated with a euglycemic hyperinsulinemic clamp. This phenomenon was interpreted as reduced insulin sensitivity in the ischemic myocardium, which would abolish detectable differences in glucose uptake between the normal and ischemic areas on insulin stimulation. Although our results may not be representative for chronically ischemic myocardium, they suggest a second potential explanation for the clinical findings: a change in the LC. When glucose uptake in the ischemic region is assessed in the fasted state, FDG will overestimate true glucose uptake. This is not likely to occur when the study is performed in the fed state (hyperinsulinemic-euglycemic clamp), in which the LC is unchanged during ischemia. Thus, it may be conceivable that the increased glucose uptake as assessed by FDG in hibernating myocardium may be due to an artifact inherent to an increase in the LC in the ischemic regions, because all cited studies were performed in the fasted state and a fixed value of 0.67 was used for the LC. Obviously, this effect does not eliminate the ability of PET to detect ischemic myocardium, but the quantitative determination of glucose uptake in those regions has to be questioned and can only be reliable when changes in the LC are taken into account. The role of mathematical modeling of the LC has to be considered in this context.

The profound decrease of the LC during reperfusion was not affected by the nutritional state. The almost complete suppression of FDG uptake during reperfusion may raise concerns regarding the determination of glucose uptake in patients with coronary artery disease, although the direct clinical relevance of our results may be low, because it is extremely rare to use PET in a situation similar to our experimental protocol. However, it appears to be necessary to assess the effect of reperfusion on the LC in a model of chronic ischemia and reperfusion.

Possible Mechanisms
We were not able to detect any influence of ischemia or reperfusion on the kinetics or the intracellular distribution of the enzyme hexokinase. Hence, it seems unlikely that translocation and changes in the kinetics of hexokinase, as suggested for the effects of insulin on the LC, are responsible for the large decrease of the LC during reperfusion, although the studies were performed in vitro and may not reflect the kinetic behavior of hexokinase in the intact organ or in vivo.

Another explanation is based on kinetic observations. The glucose transporters prefer FDG transport over glucose transport, whereas hexokinase prefers phosphorylation of glucose over phosphorylation of FDG. Depending on the rate-limiting step for glucose uptake, the LC will range from high values (transport rate limiting) to low values (phosphorylation rate limiting). Because in the latter case transport exceeds phosphorylation, intracellular free glucose will accumulate.

We measured a significant increase of intracellular free glucose during reperfusion that accompanied the decrease in the LC supporting the principle explained above (LC fell from 1.51 ± 0.27 during ischemia to 0.20 ± 0.25 during reperfusion). We obtained less experimental evidence in support of this hypothesis when the increase in the LC during ischemia was not accompanied by decreased levels of intracellular free glucose at the end of ischemia. The interpretation of these results remains difficult because the dynamic assessment of intracellular free glucose is not possible.

During ischemia, glucose uptake in hearts from fasted animals increased and glycogen decreased (Figure 3), presumably providing a substrate for anaerobic glycolysis. The increase in lactate release during ischemia provides further evidence for the stimulation of anaerobic glycolysis. It is reasonable to assume that hexokinase is activated under these conditions because G6P, a strong inhibitor of hexokinase, is decreased at the end of ischemia. This increase in hexokinase activity appears to be matched by transport, because intracellular free glucose was not decreased at the end of ischemia. Young et al recently reported translocation of GLUT 1 and GLUT 4 to the cell membrane during low-flow ischemia in dog heart. Translocation of glucose transporters may also be responsible for the increase in glucose uptake measured during ischemia in the present study, but a change in the intrinsic activity of the transporters present at the cell membrane has to be considered.

During reperfusion, glycolytic activity is reduced because glucose uptake returns to preischemic values, glycogen is resynthesized, and the elevated levels of G6P are imposing stronger inhibition on hexokinase. It may be speculated that the increased energy demand during reperfusion, which is due to the increased afterload, may be met by reestablishment of oleate oxidation. It has been demonstrated that free fatty acid oxidation returns to preischemic values within minutes of reperfusion after 40 minutes of low-flow ischemia in extra-corporally perfused pig hearts.
When glucose is the only substrate for the heart (group 1), there is no alternative substrate that can inhibit glycolysis during reperfusion. Presumably, the phosphorylation rate remains high during ischemia and reperfusion, which may explain why the LC does not change. An explanation for the fact that glucose uptake in this group did not increase during ischemia may be the reduced energy demand due to the reduced afterload.

Limitations of the Study

The study is limited by the relatively short duration of the experiments. The effects of chronic ischemia and reperfusion cannot be investigated with this in vitro model of low-flow ischemia. To verify that the suppression of FDG uptake during the 20-minute reperfusion period after ischemia represents steady-state conditions, we performed experiments with reperfusion times of up to 1 hour. FDG continued to be suppressed for the entire reperfusion periods (data not presented). Protocols reflecting clinical circumstances more closely will have to be investigated.

In conclusion, we have extended our earlier observations on the dynamic nature of the LC to the ischemic and reperfused myocardium. Although the increased LC during ischemia in the fasted state is of direct clinical importance, the relevance of the decreased LC during acute reperfusion remains to be established.

Acknowledgments

This study was supported by a grant from the US Public Health Service (RO1-HL-43133). Dr Doenst was a recipient of a research fellowship from the German Research Foundation (Deutsche Forschungsgemeinschaft). We thank Prof Dr F. Beyersdorf for encouragement, Drs Gary W. Goodwin and James E. Holden for helpful discussions, Quying Han and Patrick H. Guthrie for technical assistance, and the staff of the Positron Diagnostic and Research Center at the University of Texas-Houston Health Science Center for the preparation of FDG.

References


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Circulation. 1998;97:2454-2462
doi: 10.1161/01.CIR.97.24.2454

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