Estimating glucose metabolism using glucose analogs and two tracer kinetic models in isolated rabbit heart

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The purpose of this investigation was to 1) evaluate the relative accuracy of the Sokoloff and Patlak tracer kinetic models in estimating glucose metabolic rate (GMR) in the presence and absence of insulin; 2) evaluate the effect of nutritional state on the lumped constant (LC); and 3) compare the kinetics of 2-fluoro-2-deoxy-[14C]glucose (FDG) and 2-deoxy-d-[3H]glucose (DG) membrane transport and phosphorylation. The experimental preparation was the isolated, red blood cell-albumin-perfused rabbit heart. Our results showed that both tracer kinetic models provided GMR estimates that correlated well with the Fick method (for FDG, R = 0.84 and 0.91 for the Sokoloff and Patlak models, respectively); nutritional state did not affect the LC; and FDG and DG have different transport and/or phosphorylation parameters. We also observed that 1) the addition of a fourth compartment to the Sokoloff model reduced the mean squared error between measured and modeled data by a factor of 7.4; 2) a longer time (21.8 min) was required to obtain a linear phase of the Patlak plot than is allowed in clinical studies; and 3) accurate GMR estimates were obtained only by using different LCs reflecting insulin’s presence or absence. Our results indicate potential sources of error in the use of FDG and positron emission tomography to quantify GMR in patients.

fluorodeoxyglucose; deoxyglucose; positron emission tomography

ALTHOUGH POSITRON EMISSION tomography (PET) and 2-[18F]fluoro-2-deoxy-d-glucose are in general clinical use (43), the question remains as to how well this approach quantifies myocardial glucose metabolism. Accurate quantification of glucose metabolism with PET and FDG requires an appropriate tracer kinetic model. Evaluation of the accuracy of a tracer kinetic model in patients is difficult because of limitations in spatial and temporal resolution with PET (5), the need to be invasive to independently measure glucose consumption with the Fick principle, and the potential for uncontrolled factors affecting glucose and/or FDG uptake and metabolism. In this investigation, we evaluated, in a well-controlled in vitro environment, two tracer kinetic models that have been used with PET and FDG in patients (13, 14, 19, 24) and compared the results with the Fick-determined glucose metabolic rate (GMR).

The first of these tracer kinetic models was originally developed by Sokoloff et al. (41) for analysis of glucose utilization in the brain using 2-deoxy-d-[14C]glucose and autoradiography. This approach employs a three-compartment model to assess tracer transport from blood into the cell and phosphorylation in the cytosol. Patlak and co-workers (3, 33, 34) developed a second model, also for use in the brain, which uses an unrestricted number of reversible compartments to represent tracer transport and one irreversible phosphorylation compartment. In contrast to the Sokoloff model in which it is necessary to estimate transport and phosphorylation parameters from kinetic analysis of the entire tissue FDG uptake curve, the Patlak technique estimates GMR from the “steady-state” portion of FDG myocardial and blood curves using a graphical approach. Although there are potential errors related to the difficulty in attaining a true tracer steady state in vivo (23), the Patlak graphical analysis has been used preferentially in recent clinical studies evaluating glucose metabolism with FDG and PET (14, 19, 24). In the study by Gambhir et al. (13), both tracer kinetic models were employed, but the results were not validated against the Fick principle. The relative accuracy of these two tracer kinetic models in the heart has not been evaluated and compared against an independent measure of glucose consumption.

To quantify GMR, both the Sokoloff and Patlak models depend on a term known as the lumped constant to correct for differences in the kinetics of FDG and glucose membrane transport and phosphorylation. Two recent reports indicate that the value of the lumped constant changes in the presence vs. the absence of insulin (16, 31). However, neither of these studies quantified glucose metabolism using a tracer kinetic model: both investigations employed the crystalloid-perfused working rat heart, which has a high coronary flow rate, making it difficult to simultaneously perform kinetic analysis of FDG accumulation curves to estimate glucose consumption and measure arteriovenous differences of both tracer and glucose to assess the lumped constant. There have been no reports demonstrating that the use of different, condition-
specific lumped constants results in improved accuracy of GMR estimates using either tracer kinetic model.

The first goal of this investigation was to evaluate the relative accuracy of the Sokoloff and Patlak methodologies in estimating myocardial GMR in the presence and absence of insulin using different measured lumped constant values. Second, feeding vs. fasting has been reported to mask the effect of insulin on glucose metabolism in isolated hearts (39, 40). We therefore compared results obtained in the presence of insulin with those observed in its absence in hearts from fed vs. fasted rabbits to determine if the nutritional state of the animal independently affected lumped constant values. Third, because the kinetics of FDG vs. DG membrane transport and phosphorylation have not been compared in the heart, we assessed possible differences in computed transport and phosphorylation parameters between these two glucose analogs.

The experimental preparation was the isolated, isovolumetric, retrograde red blood cell (RBC) plus albumin perfused rabbit heart (26, 27), which has more physiological perfusion rates than crystalloid-perfused hearts. Lumped constants were determined with and without insulin in the perfusate in hearts from fed and fasted rabbits using a model-independent approach. The GMRs estimated using the Sokoloff and Patlak tracer kinetic models and average lumped constant values determined to be appropriate for each experimental condition were compared with results obtained with the Fick principle. Our results indicate that 1) the original Sokoloff model needed to be modified to include a fourth compartment to account for tracer delivery and distribution in the heart; 2) the modified, four-compartment model derived from Sokoloff and the Patlak graphical approach yielded comparably accurate estimates of myocardial GMR in the presence and absence of insulin provided the appropriate lumped constant relevant to each condition was used; 3) the nutritional state of the animal had no effect on the lumped constant; and 4) FDG and DG have different lumped constant and combined rate constant values, reflecting the fact that fluorine substitution in 2-deoxy-D-glucose has an effect on membrane transport and/or phosphorylation.

METHODS

Experimental Preparation

Preparation of isovolumic, retrograde RBC-albumin-perfused rabbit hearts was similar to that reported previously (25–27). All procedures were done in accordance with institutional guidelines for animal research. Male New Zealand White rabbits (3.5–4.5 kg) were given 4,000 U heparin sodium (Upjohn, Kalamazoo, MI) and 250 mg pentobarbital sodium (Abbott, North Chicago, IL) via an ear vein. The heart was immediately excised through a median sternotomy, arrested in ice-cold buffer, and rapidly attached to a cannula to allow retrograde perfusion. An apical drain was inserted into the left ventricle (LV). The atrioventricular node was crushed to allow controlled stimulation, and a fluid-filled latex balloon connected to a Gould-Statham P23ID pressure transducer (Gould, Oxnard, CA) was inserted into the LV. The balloon was inflated to maintain diastolic pressure at 8–10 mmHg. A coronary venous sampling catheter and needle thermistor (Bailey Instrument, Saddlebrook, NJ) were inserted into the right ventricle. The venae cavae and pulmonary artery were ligated so all coronary venous drainage flowed out of the sampling catheter. Stimulating electrodes connected to a Grass SD44 stimulator were placed against the left and right ventricles, and 4-V, 4-ms stimuli were delivered at a rate of 180 min⁻¹. Temperature was maintained between 36 and 38°C with a water-jacketed heating coil and heart chamber. Coronary flow was held constant with a peristaltic pump (Radin Instrument, Woburn, MA). The RBC-albumin perfusate was not recirculated. The rate of coronary blood flow was measured as the volume of blood discharged per minute from the coronary venous sampling catheter. Control plasma flow rate was ~1.2 ml·min⁻¹·g LV wet wt⁻¹. The perfusion line included an in-line 20-µm blood transfusion filter (Stacorp, Columbia, TN) to remove RBC aggregates.

Hearts were perfused with a modified Tyrode solution containing oxygenated bovine RBCs and 22 g/l bovine serum albumin (fraction V, fatty-acid free; Sigma Chemical, St. Louis, MO). The bovine serum albumin was dialyzed overnight at 4°C against buffer and filtered through a 0.8-µm Millipore filter. Bovine RBCs were separated from whole blood by centrifugation in 250 ml polyethylene bottles at 2,800 g for 20 min at 4°C and then were washed, resuspended with oxygenated ice-cold buffer, and spun again; this separation procedure was repeated four times. The specific electrolyte concentrations of the buffer solution were (in mmol/l) 110 NaCl, 2.5 CaCl₂, 6 KCl, 1 MgCl₂, 0.435 NaH₂PO₄, and 28 NaHCO₃. The pH and oxygen tension were measured on the RBC-albumin perfusate using an IRMA Blood Gas Analyzer (Diametrics Medical, St. Paul, MN). The mean ± SD pH value was 7.38 ± 0.04, and the PO₂ value was 303 ± 72 mmHg. The concentration of RBCs in the perfusate buffer was adjusted to a hematocrit of 0.17–0.25. The flask containing the RBC-albumin perfusate was gassed with a mixture of 98% O₂–2% CO₂ during the experiment.

Hearts from rabbits that were fed ad libitum or fasted overnight (~18 h) were studied in the presence or absence of insulin in the perfusate. There were three experimental groups: 1) hearts from fed rabbits perfused with insulin (5 mU/ml); 2) hearts from fed rabbits perfused without insulin; and 3) hearts from fasted rabbits perfused without insulin. A high insulin concentration was used to ensure a maximum insulin effect, since Taegtmeyer et al. (42) observed that insulin binds to an unpredictable extent to glassware and tubing.

Plasma glucose concentration in hearts perfused with insulin was 5.3 ± 0.5 mmol/l (range, 4.5–6.5 mmol/l). Work by Ng et al. (31) has shown that reducing perfusate glucose concentration in the presence of insulin affects lumped constant values. To study the effect of reducing perfusate glucose concentration in the absence of insulin, we varied perfusate glucose concentration from 2.8 to 6.2 mmol/l (mean, 4.9 ± 1.3 mmol/l) in hearts from fed rabbits and 1.9 to 6.4 mmol/l (mean, 3.1 ± 1.5 mmol/l) in hearts from fasted rabbits perfused without insulin. To study the effect of flow on the lumped constant, plasma flow was varied from ~0.5 to 1.5 ml·min⁻¹·g LV wet wt⁻¹ in all three experimental groups.

Radiopharmaceuticals and Synthesis of 131I-Labeled Albumin

Radioisotopes were purchased from the following sources: 2-fluoro-2-deoxy-D-[U-14C]glucose (FDG) and 2-deoxy-D-[6-3H]glucose (DG) from American Radiolabeled Chemicals (St. Louis, MO), and 131I from Du Pont-NEN Research Products. Bovine serum albumin was labeled with 131I utilizing the IODO-GEN-based protein iodination technique (29).
Experimental Protocol

After the heart was prepared, an equilibration period of at least 15 min preceded all experimental interventions. A heart was acceptable for study if it developed at least 60 mmHg pressure (peak systolic — diastolic). After equilibration, myocardial perfusion was gradually changed to the experimental flow rate and subsequently maintained constant throughout the remainder of the experiment. Constant infusion of FDG and DG (−12 µCi of each isotope/l) was initiated 5–10 min after equilibration at the experimental flow rate and continued for 60 min. In some experiments, only FDG was infused. Isotope infusion was initiated as a step function by employing two parallel perfusion circuits (one with and one without isotope) and two, in-line, three-way stopcocks placed just above the aortic cannula. Rapid venous sampling (∼7–15 s/sample, depending on the flow rate) from the right ventricular cannula into microcentrifuge vials commenced with radioisotope introduction and continued uninterrupted for 2–3 min. The interval between samples was subsequently lengthened (from 15 to 240 s) until ∼55–60 venous samples were taken in each experiment. Samples from the perfusate flask were taken at 4-min intervals (“arterial samples”). All samples were immediately chilled and centrifuged in an Eppendorf microcentrifuge. Glucose and lactate concentrations in the supernatant were assayed in duplicate using a YSI Biochemistry Analyzer (Model 2700; Yellow Springs Instrument, Yellow Springs, OH).

At the end of an experiment, the LV was separated from the heart, blotted dry, and weighed (wet weight). The LV was sliced into ∼10 pieces, dried for 24 h at 90°C, and reweighed (dry weight).

Tissue FDG and DG Content

Tissue content of FDG or DG was computed as the summed product of plasma flow, the arteriovenous concentration difference in plasma 14C or 3H activity, and sampling interval at each sampling time

$$A_t(t_j) = \sum_{j=1}^{k} F[C_A - C_V(t_j)]\Delta t_j$$

where $A_t(t_j)$ is the tissue FDG or DG content, $F$ is plasma flow, $C_A$ is the arterial FDG or DG activity, $C_V(t_j)$ is the venous FDG or DG activity, and $\Delta t_j$ is the sampling interval.

Lumped Constant

Lumped constants were computed as the steady-state extraction fraction ratio of FDG to glucose or DG to glucose

$$\left[\frac{|C_A - C_V(t)|}{|C_A - C_V|}\right]_{\text{analog}}$$

$$\left[\frac{|C_A - C_V(t)|}{|C_A - C_V|}\right]_{\text{phys}}$$

where $C_V(t)$ is venous tracer concentration as a function of time and $C_A$ is constant venous glucose concentration. In all experiments, the final steady-state extraction fraction value for FDG and DG used to compute the lumped constant was reached −20–25 min after initiation of isotope infusion. This method of determining the lumped constant is model independent and has been used before by Sokoloff et al. (41) in the brain and by Ng et al. (31) in the heart.

Patlak Graphical Analysis

As described by Patlak et al. (34), FDG-DG phosphorylation rate was estimated by inspection of the asymptotic behavior of the plot of tissue tracer content vs. integrated plasma tracer content after both were normalized by arterial plasma activity, i.e., $A_t(t)/C_A$ vs. $\int_0^t C_A \, dt/C_A$, where $A_t(t)$ is tissue tracer content as a function of time. The linear portion of the plot can be expressed by the equation

$$\frac{A_t(t)}{C_A} = \frac{K_i}{C_A} \int_0^t C_A \, dt + V_i$$

where $K_i$ and $V_i$ are the slope and ordinate intercept of the linear asymptote, respectively. $K_i$ is the influx constant and provides a measure of the rate of FDG-DG phosphorylation. $V_i$ is equal to or less than the reversible tissue FDG-DG distribution volume. To accurately estimate the FDG-DG phosphorylation rate from the Patlak graph, tracer must have equilibrated between plasma and reversible tissue regions before the linear phase of the graph used to estimate the $K_i$ (see Discussion). Under the experimental conditions employed here (i.e., constant isotope infusion), once tracer equilibration has developed, it should continue through the end of the experiment. Therefore, $K_i$ and $V_i$ were estimated as the slope and ordinate intercept of the straight line fitted to the final “steady-state” portion of the graph starting at a point when the curve first appeared linear (usually ∼20 min after isotope injection) through the end of the experiment (60 min). The lowest correlation coefficient was $>0.99$.

Four-Compartment Model

Figure 1 shows the four-compartment model that was used to describe the kinetics of DG and FDG phosphorylation in the isolated rabbit heart. The principles of this model are derived from Sokoloff et al. (41). However, the form of the model and fitting procedures are different from that originally described by Sokoloff. The specific mathematical approach is outlined in the APPENDIX. Briefly, the fractional utilization (FU) of FDG-DG per unit flow was estimated as the asymptotic rate of isotope accumulation in the fourth compartment. This number multiplied by flow provides a combined rate constant, $K_{FU}$, that estimates the fractional phosphorylation rate of FDG-DG.

GMR

Patlak graphical analysis and four-compartment model. $K_i$ and $K_{FU}$ are combined rate constants that provide a measure of the rate of FDG-DG phosphorylation. To convert these estimates of FDG-DG phosphorylation rate to GMR, the following relationship was used

$$GMR = \frac{C_A \times K}{LC}$$
where \( K \) is either \( K_i \) or \( K_{FUR} \), \( C_A \) is the arterial glucose concentration, and \( LC \) is the lumped constant. Lumped constant values for each experimental group were computed by averaging the results from the corresponding individual experiments.

Fick GMR. GMR was computed using the Fick equation, arterial and venous chemical glucose concentrations, and plasma flow

\[
GMR = F(C_A - C_V)
\]

The GMR is expressed as micromoles per minute per gram LV wet weight.

Statistical Methods

Data are expressed as means ± SD. Statistical significance was analyzed using both parametric and nonparametric methods. Paired observation tests (paired t-test and Wilcoxon signed rank) were used to compare FDG vs. DG within each experimental group; differences between experimental groups were analyzed using an unpaired t-test and Mann-Whitney U-test. In all cases, the results using parametric and nonparametric methods were in agreement. Reported P values were obtained using the nonparametric approach, since these were consistently higher than those obtained with the parametric method. A P value < 0.05 was considered significant.

RESULTS

RBC Metabolism and FDG-DG Uptake

Lactate was present in arterial plasma samples due to RBC metabolism occurring during both storage and the experiment. Lactate concentration (mmol/l) averaged 0.11 ± 0.06 in hearts perfused with insulin, 0.13 ± 0.05 in hearts from fed rabbits perfused without insulin, and 0.11 ± 0.04 in hearts from fasted rabbits without insulin in the perfusate (no statistically significant difference between groups; smallest P value > 0.15). These lactate concentrations are more than two orders of magnitude below that shown to alter lumped constant values (16).

We evaluated FDG-DG uptake in bovine RBCs at 37°C by 1) incubating RBCs (0.20 hematocrit) with FDG and DG for 15 min and acquiring samples at 5-min intervals and 2) pumping RBC-albumin perfusate containing FDG and DG through the perfusion apparatus (no heart attached) for up to 70 min at speeds ranging from 2 to 20 ml/min with "venous" (exit drain) samples acquired at 5- to 10-min intervals. Collected samples were immediately chilled, centrifuged, and counted. No significant FDG and DG uptake into RBCs was observed. Because FDG uptake in RBCs has been observed in vivo during PET studies (35), the absence of demonstrable FDG or DG uptake in our experiments presumably relates to species differences and to the fact that our RBCs were harvested and stored at 6°C before in vitro use.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>GMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed with insulin</td>
<td>Fick 0.50±0.12</td>
</tr>
<tr>
<td>Fed with insulin</td>
<td>[3H]DG 0.49±0.17*</td>
</tr>
<tr>
<td>Fed with insulin</td>
<td>[14C]FDG 0.26±0.08*</td>
</tr>
<tr>
<td>Fasted without insulin</td>
<td>[3H]DG 0.15±0.05</td>
</tr>
<tr>
<td>Fasted without insulin</td>
<td>[14C]FDG 0.15±0.05</td>
</tr>
</tbody>
</table>

Table 1. Glucose metabolic rates as assessed using the Fick principle, Patlak graphical analysis, and four-compartment model.

Values, expressed as µmol·min⁻¹·g LV wet wt⁻¹, are means ± SD. P values for the Fick method are given in the text. [3H]DG, 2-deoxy-D-[3H]glucose; [14C]FDG, 2-fluoro-2-deoxy-D-[14C]glucose. P values for the Patlak and compartment glucose metabolic rate (GMR) are *P < 0.05 vs. fed without insulin and fasted; †P < 0.01 vs. fed with insulin and fasted; and ‡P < 0.001 vs. fed with insulin and fed without insulin.

0.001) and in hearts from fasted rabbits perfused without insulin (0.15 ± 0.05; n = 15; P < 0.001; see Table 1). Comparing the two groups perfused without insulin, the GMR was statistically higher in hearts from fed rabbits than from fasted rabbits (P < 0.002).

Lumped Constant

Lumped constant values were compared between the three groups to assess the effect of feeding, fasting, and insulin on computed transport and phosphorylation parameters for both glucose analogs. The results are illustrated in Fig. 2. For both FDG and DG, the lumped constant values in hearts from fed rabbits perfused with insulin (FDG, 0.45 ± 0.09; DG, 0.32 ± 0.08) were significantly less than in hearts from fasted rabbits perfused without insulin (FDG, 1.05 ± 0.27; P < 0.001; and DG, 0.96 ± 0.32, P < 0.001) and in hearts from fasted rabbits perfused without insulin (FDG, 0.92 ± 0.16, P < 0.001; and DG, 0.82 ± 0.17, P < 0.003). There was
no significant difference between the fed and fasted groups perfused without insulin. These results indicate that the presence vs. the absence of insulin has a significant effect on the value of the lumped constant for both FDG and DG while the nutritional state of the animal does not.

In each group, plasma flow ranged from ~0.5 to 1.5 ml·min⁻¹·g LV wet wt⁻¹. In the two groups perfused without insulin, plasma glucose concentration ranged from 1.9 to 6.4 mmol/l. Neither plasma flow rate nor perfusate glucose concentration in the absence of insulin had a significant effect on lumped constant values (data not shown).

Patlak Graphical Analysis

A graphical representation of a typical Patlak analysis of an experiment from a fed rabbit heart perfused with insulin is shown in Fig. 3. Visual inspection of these curves indicates that a linear phase of the Patlak graph for both glucose analogs begins ~1,200 s (20 min) after isotope introduction. For all experiments, the time taken to develop a linear phase was identical for both isotopes (average, 21.8 min; range, 15–26 min) and did not vary between experimental groups. These results provide an estimate of the time required to achieve plasma-tissue free FDG-DG equilibration under the constant isotope infusion conditions used here.

The \( K_i \) and reversible distribution volumes (\( V_i \)) determined from Patlak graphical analysis of FDG-DG tissue accumulation curves for each of the three groups are listed in Table 2. For both FDG and DG, \( K_i \) did not vary significantly between the fed with insulin, fed without insulin, and fasted without insulin groups, indicating that the presence or absence of insulin and the nutritional state of the animal did not affect \( K_i \) values for either isotope. Similarly, flow did not affect \( K_i \) values for either isotope, and the perfusate glucose concentration did not affect \( K_i \) values in the two groups perfused without insulin (data not shown).

\( V_i \) provides a lower-limit estimate of the reversible distribution volume of nonphosphorylated FDG and DG (3, 33, 34). Possibly because of variable results, neither insulin in the perfusate nor the nutritional state of the animal had a significant effect on \( V_i \) values (Table 2). Plasma flow rate and perfusate glucose concentration also had no effect on \( V_i \) values (data not shown). Similarly, there were no significant differences in the \( V_i \) values for FDG vs. DG in any of the groups. The reversible distribution volume of free FDG-DG, as estimated by \( V_i \), exceeded the free water space in the isolated rabbit heart (0.79 ± 0.03 ml/g, computed as 1 – dry wt/wet wt) in all three groups. These results indicate that the reversible distribution volume of nonphosphorylated FDG-DG is greater than the tissue water space and is unaffected by insulin in the perfusate or the nutritional state of the animal.

With the use of Eq. 4 and the averaged lumped constant for each group, the mean GMRs for the three experimental groups estimated from the Patlak graphical analysis agreed well with those determined from the Fick equation (Table 1) for both FDG and DG. As observed with the Fick method, there were significant differences in mean GMRs estimated from the Patlak plot between the fed with insulin, fed without insulin, and fasted without insulin groups for both FDG and DG.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>( K_i ), ml·min⁻¹·g⁻¹</th>
<th>( V_i ), ml/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed with insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^{3}H)DG</td>
<td>0.0419 ± 0.0144</td>
<td>1.21 ± 0.68</td>
</tr>
<tr>
<td>(^{14}C)FDG</td>
<td>0.0586 ± 0.0154</td>
<td>1.18 ± 0.61</td>
</tr>
<tr>
<td>Fed without insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^{3}H)DG</td>
<td>0.0460 ± 0.0144</td>
<td>1.21 ± 0.68</td>
</tr>
<tr>
<td>(^{14}C)FDG</td>
<td>0.0544 ± 0.0307</td>
<td>1.03 ± 0.62</td>
</tr>
<tr>
<td>Fasted without insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^{3}H)DG</td>
<td>0.0419 ± 0.0213</td>
<td>0.94 ± 0.55</td>
</tr>
<tr>
<td>(^{14}C)FDG</td>
<td>0.0544 ± 0.0307</td>
<td>1.03 ± 0.62</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD. \( K_i \), slope of linear asymptote; \( V_i \), y-intercept of linear asymptote. Each value represents the mean of 7–18 experiments.

Four-Compartment Model

Figure 4 illustrates the results of fitting a three-compartment and a four-compartment model to an experimentally derived curve for DG. The four-compartment model and the measured data are concordant. In contrast, the three-compartment model underestimates the experimental curve between 200 and 500 s and overestimates the entire terminal portion of the curve. Because the terminal phase of the curve is crucial to estimating the asymptotic rate of filling of the fourth compartment, overestimation of the later part of the curve corresponds to an increased estimate of GMR. These results were observed consistently for both FDG and DG in all experiments.
Quantitatively, using the four- vs. the three-compartment model reduced the mean squared error between the measured and modeled arteriovenous difference by an average of 6.8 for FDG (range, 1.5–32) and 8.4 for DG (range, 1.4–39). Estimates of the combined rate constant could be estimated with greater certainty than the individual rate constants. Consistent with this observation is the fact that widely divergent values for the first three individual rate constants in hearts from fasted rabbits perfused without insulin were not accompanied by comparable scatter in $K_{\text{FUR}}$ values.

Similar to $K_i$, $K_{\text{FUR}}$ values did not vary significantly between the three experimental groups. Similar to the results with the Patlak graphical analysis, mean GMR values estimated using Eq. 4 and average lumped constants for each of the three experimental groups agreed well with the results using the Fick equation (Table 1).

FDG vs. DG. Table 4 illustrates the comparison of lumped constants and combined rate constants for FDG vs. DG in hearts perfused with both isotopes. In each group, the lumped constant values for FDG were greater than those for DG. Similarly, $K_i$ and $K_{\text{FUR}}$ values for FDG were higher than those for DG in all three experimental groups. Taken together, the results for the lumped constant and combined rate constants for FDG vs. DG indicate that these two glucose analogs have different transport and/or phosphorylation parameters and suggest that these two glucose analogs should not be used interchangeably.

### Table 3. Four-compartment model individual and combined rate constants for FDG and DG

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Rate Constants, min⁻¹</th>
<th>$K_{\text{FUR}}$, ml·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{21}$</td>
<td>$k_{22}$</td>
</tr>
<tr>
<td>Fed with insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[^{3}\text{H}]\text{DG}$</td>
<td>1.3 ± 0.2</td>
<td>2.1 ± 1.6</td>
</tr>
<tr>
<td>$[^{14}\text{C}]\text{FDG}$</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>Fed without insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[^{3}\text{H}]\text{DG}$</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 1.8</td>
</tr>
<tr>
<td>$[^{14}\text{C}]\text{FDG}$</td>
<td>1.5 ± 0.2</td>
<td>2.4 ± 1.5</td>
</tr>
<tr>
<td>Fasted without insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[^{3}\text{H}]\text{DG}$</td>
<td>3.3 ± 5.2*</td>
<td>25.7 ± 63.7*</td>
</tr>
<tr>
<td>$[^{14}\text{C}]\text{FDG}$</td>
<td>2.2 ± 3.4*</td>
<td>12.0 ± 41.6*</td>
</tr>
</tbody>
</table>

Values are the means ± SD. $K_{\text{FUR}}$, combined rate constant for fractional utilization. *The large scatter in these three rate constant values is due to the inclusion of an experiment in which there was a discrepancy between the recorded start time for the isotope infusion and the appearance of radioactivity in the venous samples.

![Fig. 4. Data points from one experimental curve for DG in a fed rabbit heart plotted as the normalized arteriovenous (A–V) difference vs. time together with the best fits of the 3-compartment and the 4-compartment models. Data are measured in triplicate, and • represents the mean value at each time point. Error bars are SD.](image-url)
Comparison of GMR Estimates Using Patlak Graphical and Four-Compartment Analyses

The correlation between GMR estimated by the Patlak graphical approach, the four-compartment model, and the Fick method for both FDG and DG is illustrated in Fig. 5. The three experimental groups are combined, and each data point represents an individual experiment. GMR was computed using Eq. 4, the appropriate average lumped constant value determined for each experimental group, and the individual $K_i$ values or $K_{FUR}$ values computed for each experiment. Because FDG and DG were shown to have different lumped constants, a total of six lumped constant values were used (3 each for FDG and DG). The equations for the linear regression and correlation coefficient are shown in Fig. 5, A–D. For both tracer kinetic models and both isotopes, the slopes of the linear regression were not significantly different from one (smallest $P > 0.2$), and the ordinate intercepts were not significantly different from zero (smallest $P > 0.5$). These results indicate that both tracer kinetic models provided comparably accurate GMR estimates using both glucose analogs.

In clinical studies evaluating myocardial glucose metabolism with PET and FDG, a single lumped constant value of 0.67 has been used to compute GMR (13, 14, 19, 24). To compare our results with those obtained using an invariant lumped constant, we computed GMRs using Eq. 4, the single lumped constant value of 0.67, and the individual combined rate constants determined in this study using both the Sokoloff and Patlak tracer kinetic models. The correlation between each model-estimated and Fick-determined GMR using this single lumped constant value for FDG is illustrated in Fig. 6. The format of this illustration is the same as in

Fig. 6. Fick GMR vs. Patlak (A) or compartment (B) GMR. Compartment and Patlak GMRs were calculated using a single lumped constant value of 0.67. Equation for the linear regression is included in A and B.
constant values when evaluating glucose metabolism. Consequently, slower GMRs are underestimated. These results clearly demonstrate that accurate GMR estimates can only be obtained using different lumped constant values when evaluating glucose metabolism in the presence vs. the absence of insulin.

DISCUSSION

In this investigation, we have evaluated the accuracy of the Sokoloff compartment model and the Patlak graphical analysis in estimating myocardial GMR in the presence and absence of insulin in hearts from fed and fasted rabbits using both FDG and DG as tracers. To fit experimental heart curves, the original Sokoloff model had to be altered to include a fourth compartment to account for tracer delivery and distribution. We found that accurate GMR estimates were produced by both tracer kinetic models only by using the appropriate lumped constant for each experimental condition. In contrast to insulin, the nutritional state of the animal did not appear to have any effect on the lumped constant. FDG and DG were also observed to have different transport and/or phosphorylation parameters.

Compartment Model

A three-compartment model has been used to quantify GMR with PET and FDG (13, 17, 36). In our analysis of glucose metabolism in the isolated heart, a fourth compartment was required to obtain satisfactory fits to the data. The simplest explanation for the need to use an additional compartment is that tissue FDG and DG accumulation curves were computed using in vitro arteriovenous differences instead of in vivo residue detection with PET. Compared with the present data, in vivo myocardial and blood FDG curves acquired with PET are degraded due to limitations in spatial and temporal resolution and statistical uncertainties. As a result, parameter estimation is less accurate, reducing the number of compartments that can be fit to the data.

Two previous studies used the three-compartment model to quantify GMR in an isolated heart preparation (20, 21). In contrast to our investigation, these studies evaluated FDG accumulation kinetics from externally detected tissue residue curves using coincidence detection. Before myocardial FDG-6-phosphate accumulation, perfusate FDG content represents a significant fraction of detected activity when measured by external detection. In contrast, tissue FDG and DG accumulation determined from arteriovenous differences excludes plasma tracer activity from computed residue curves. Therefore, the early dynamics of FDG and DG exchange between plasma and tissue are probably better assessed from arterial and venous concentrations, which might have contributed to the need to use an additional compartment in this study.

One of the fundamental assumptions of the original Sokoloff model is that it is possible to estimate capillary FDG, DG, and glucose concentrations from arterial measurements (41). This assumption is based on the concept that brain uptake of these substances is completely barrier limited and independent of the rate of blood flow. However, because of the structural and functional dissimilarities of the two organs, it is not clear if this assumption is valid in the heart. One important difference is that glucose enters the brain by facilitated diffusion through the blood-brain barrier, whereas entry into the heart is via diffusion through pores in the myocardial capillary wall. Because of this difference, FDG-DG might diffuse more rapidly into the heart than into the brain. If diffusion into the heart is rapid, then myocardial uptake of FDG-DG is not completely barrier limited, invalidating Sokoloff's original assumption for use in the heart.

To test the validity of this assumption in the heart, we assessed the rapidity of FDG diffusion by measuring its peak instantaneous extraction relative to an intravascular reference tracer (1). The peak instantaneous extractions of FDG and DG are expected to be similar in the heart, differing only by their respective diffusion coefficients (22). Using 131I-labeled albumin as the intravascular tracer, we measured the peak instantaneous extraction of FDG in eight additional hearts using published techniques (1, 9, 25, 27; see Fig. 7). An average of 72% of the FDG diffused out of the capillary during its initial transit through the heart. This value is approximately two times that reported for glucose in brain at comparable blood flow rates and plasma glucose concentrations (9, 44). Because of the rapid extravascular diffusion in the heart, initial distribution of FDG (and DG) is not completely barrier limited, indicating that initial capillary FDG (and DG) concentration cannot be estimated from arterial measurements. In the present study, the addition of a fourth compartment and the inclusion of flow in the model (see Appendix) allowed us to avoid this assumption.

Patlak Graphical Analysis

The Patlak graphical approach provides an alternative method to that of Sokoloff for measuring GMR from
FDG-DG tissue and blood curves (3, 33, 34). Compared with the Sokoloff three-compartment model, the Patlak model is more general with an unrestricted number of reversible compartments and one irreversible compartment. The advantages of the Patlak approach are that it does not depend on an explicit compartment model, and it is easier to use than the Sokoloff model. However, one of the fundamental requirements for successful use of the Patlak graphical analysis is that there is a relatively rapid equilibration of FDG between plasma and reversible tissue regions so that a unidirectional flux of tracer into the irreversible compartment dominates the curve for a measurable time period during the experiment.

The fact that the Patlak graphical analysis provided GMR estimates that correlated well with results obtained with the Fick principle suggests that the requirement for rapid tracer equilibration was fulfilled in the isolated rabbit heart. These results were obtained during constant isotope infusion. In contrast, isotopes are usually delivered as a bolus in patients such that blood tracer activity constantly declines after introduction. In the first clinical application of the Patlak graphical analysis to the heart, Gambhir et al. (13) recommended that scans acquired as early as 12 min after FDG injection be used to quantify GMR. However, the accuracy of the GMR estimates obtained with the Patlak technique in that study was not validated against results obtained with the Fick principle. In the present study, >20 min were required to achieve tracer equilibration during constant isotope infusion (Fig. 3). Because of the differences in tracer introduction and delivery, neither the time at which tracer equilibration occurs in vivo nor the errors caused by tracer nonequilibration are known. These considerations indicate that careful evaluation and validation of the Patlak graphical analysis under appropriate experimental and clinical conditions should be completed before final acceptance of this approach.

The ordinate intercept of the linear portion of the Patlak plot is equal to or less than the sum of the plasma volume and reversible tissue distribution volume of free FDG-DG (3, 33, 34). In our studies, this combined volume averaged >1.0 ml/g LV wet wt (Table 2). Because total tissue water space in the RBC-albumin-perfused rabbit heart is 0.79 ml/g, the average value for the summed FDG-DG plasma and tissue distribution volume exceeds tissue water space. Given that membrane transport prefers FDG-DG to glucose while phosphorylation by hexokinase favors glucose over FDG-DG (8), it is predictable that the concentration of FDG-DG in the cytoplasm should be greater than that for glucose. However, these relative affinities are insufficient to account for the fact that the distribution volumes for free FDG and DG were greater than total tissue water space. For this latter observation to be valid, the myocyte must concentrate FDG-DG relative to the plasma. In order for the cell to concentrate FDG-DG, membrane transport has to be asymmetric with the rate of inward transport exceeding outward transport. If correct, this unexpectedly high distribution volume for unphosphorylated FDG-DG could have an effect on the successful use of the Sokoloff compartment model to estimate GMR.

Effect of Insulin on the Lumped Constant

The lumped constant is a combined factor that converts FDG-DG phosphorylation rate into the net rate of glucose utilization (41). It is composed of six terms in the following order: $\lambda V_m^* K_m^* / V_m K_m^*$, where $\lambda$ is the ratio of distribution volumes for FDG-DG to glucose, $V_m^* K_m^*$ and $V_m K_m^*$ are the maximum velocities and Michaelis constants of hexokinase for FDG-DG and glucose (symbols with * refer to FDG-DG; those without refer to glucose, respectively), and $\phi$ is the fraction of phosphorylated glucose that proceeds through glycolysis. Because the value for $\phi$ is close to one (11) and is not known to be affected by insulin, the terms most likely to be altered by insulin are $\lambda$ and $V_m^* K_m^* / V_m K_m^*$. In the brain, the ratio of distribution volumes for FDG-DG to glucose ($\lambda$) has been shown to be a function of tissue glucose concentration (10, 15). Because insulin alters tissue glucose concentration in the heart, it is possible that insulin changes the value for the lumped constant through its effect on $\lambda$.

In contrast to the brain, insulin is known to affect the activity of hexokinase in the heart (2). Russell et al. (38) investigated the possibility that insulin’s effect on the lumped constant was due to a change in the relative affinity of hexokinase for DG vs. glucose ($K_m^*$). These investigators reported that insulin increased the fraction of hexokinase bound to mitochondria and that bound hexokinase had a decreased affinity for DG relative to glucose. Based on this observation, they proposed that the reduction in the lumped constant in hearts perfused with insulin was due to an insulin-related increase in $K_m^*$ of mitochondrial-bound hexokinase for DG. At present, there is insufficient information to elucidate the mechanism whereby insulin alters the value of the lumped constant in the heart. Additional research is needed to assess the effect of insulin on both $\lambda$ and $V_m^* K_m^*/V_m K_m^*$ before this mechanism can be more precisely understood.

Nutritional State

An increase in GMR in the presence of insulin has been observed in isolated hearts from fasted but not fed animals (32, 39, 40). One explanation for this finding is that hearts from fed animals maintain a “metabolic memory” due to a residual insulin effect acquired during in vivo feeding (39). Direct comparison of the GMR results obtained in the present study with those previously published is difficult because of differences in experimental design. However, the observation that the nutritional state of the animal had no effect on the lumped constant is inconsistent with the concept of a metabolic memory, i.e., the presence of a residual insulin effect should have lowered the lumped constant in hearts from fed compared with fasted rabbits perfused without insulin. A possible explanation for this discrepancy is that a different experimental prepara-
tion (crystalloid-perfused working rat heart) was employed in the previous studies than was employed here.

FDG vs. DG

The present data indicate that FDG and DG have different lumped constant and combined rate constant values in the isolated RBC-albumin-perfused rabbit heart. In all cases, the values for FDG were higher than those for DG, reflecting a preference of either membrane transport or phosphorylation for FDG over DG. Although these two glucose analogs have not been compared in the heart, Reivich et al. (37) compared \(^{13}F\)FDG and \(^{13}C\)DG in the brain and did not observe any difference in lumped constant values. In their study, lumped constants were measured in different experimental groups (humans) with PET and then compared, whereas, in our study, FDG and DG were infused simultaneously, and lumped constant values for the two isotopes were determined from results obtained in a single heart. One possible explanation for the discrepant results between heart and brain is in the experimental design. Experimental errors and biological variability might obscure differences in FDG vs. DG lumped constants when different experimental subsets are compared; these may become more apparent when paired observations made in a single experiment are compared. It is also possible that transport and phosphorylation for human brain and rabbit myocardium might have different relative affinities for FDG vs. DG.

Clinical Implications

The results of the present study provide evidence that different lumped constant values are required to obtain accurate estimates of GMR with PET and FDG in the presence vs. the absence of insulin. It is also possible that factors other than insulin could affect the value of the lumped constant. High concentrations of lactate and \(\beta\)-hydroxybutyrate have been shown to affect the value of the lumped constant for FDG (16). In two recent studies, free fatty acids were observed to affect the value of the lumped constant (4, 12). Furthermore, the effects on the lumped constant of myocardial pathology such as acute low-flow ischemia and “chronic” stunning and/or hibernation have not been reported. Unfortunately, it is not clear what lumped constant value should be used under many of the conditions that are routinely encountered in the clinical applications of PET and FDG to study glucose metabolism. However, our value of 0.45 in the presence of insulin, combined with a previously reported value of 0.33 (31), suggests that more accurate quantification of GMR with FDG and PET in normal myocardium during euglycemic hyperinsulinemic clamp might be achieved using a lumped constant value of 0.4.

The ability of both the Sokoloff and Patlak tracer kinetic models to provide accurate GMR estimates suggests that the assumptions required to obtain convergence of model-predicted and experimentally measured curves were fulfilled under the experimental conditions evaluated in the isolated RBC-albumin-perfused rabbit heart. Although the present in vitro environment is dissimilar to that encountered in vivo (since tissue residue curves were determined using arteriovenous sampling and isotopes were delivered as a constant infusion), the current results might be relevant to in vivo data acquired with PET. Because the factors determining the kinetics of initial FDG delivery and distribution are probably similar in vivo and in vitro, our need to use an additional compartment to fit experimental heart curves could indicate that more accurate GMR estimates would be obtained in vivo using a four- rather than a three-compartment model and high time resolution PET (6, 30). It is also possible that errors produced by employing PET images acquired before FDG equilibration after bolus introduction might account for the poor separation of viable vs. nonviable dysfunctional myocardium noted in recent clinical studies using the Patlak graphical analysis to quantify GMR (14, 19). However, until further studies are completed comparing model-estimated and Fick-determined GMR using high time resolution tomographs and/or arteriovenous sampling during tracer nonsteady states, it cannot be predicted that the present success in obtaining accurate quantitative estimates of glucose metabolism with both the Sokoloff and Patlak models will be reproduced in vivo with PET.

APPENDIX

Mathematical Description of the Multicompartment Model

In our experiments, the measured parameters were the arterial and venous blood concentrations as a function of time, which are denoted as \(C_A(t)\) and \(C_V(t)\), respectively. The tissue activity content per unit flow as a function of time, denoted as \(R(t)\), was computed as

\[
R(t) = \int_0^t [C_A(t) - C_V(t)] dt
\]  

Figure 1 shows the four-compartment model used to model the kinetics of FDG and DG phosphorylation. The differential equations governing the time courses of the compartment activity contents per unit flow as a function of time, which are denoted as \(Q_2(t)\), \(Q_3(t)\), and \(Q_4(t)\), are

\[
\frac{dQ_2(t)}{dt} = k_{23}Q_3(t) - k_{12}C_A(t) - k_{32}Q_2(t) + k_{22}Q_3(t)
\]  

\[
\frac{dQ_3(t)}{dt} = k_{32}Q_2(t) - (k_{23} + k_{43})Q_3(t)
\]  

\[
\frac{dQ_4(t)}{dt} = k_{43}Q_3(t)
\]

where \(k_{ij}\) is the transfer rate to compartment \(i\) from compartment \(j\). The modeled tissue uptake \(R(t)\) is

\[
\hat{R}(t) = Q_2(t) + Q_3(t) + Q_4(t)
\]
It can be shown that the solution to Eqs. A2–A5 for $\hat{R}(t)$ can be written in the form

$$\hat{R}(t) = \int_0^t h(\tau) C_A(t - \tau) d\tau$$  \hspace{1cm} (A6)

Thus the stimulated tissue uptake $\hat{R}(t)$ is just the convolution of the measured arterial blood input with a function $h(t)$ that depends on the set of compartment model parameters ($k_{21}$, $k_{12}$, $k_{32}$, $k_{23}$, and $k_{43}$). The function $h(t)$ is called the impulse response for the compartment model.

We used the software package RFIT (7, 18, 28) to estimate a set of compartment model parameters ($k_{21}$, $k_{12}$, $k_{32}$, $k_{23}$, and $k_{43}$) that locally minimizes the mean squared error between the time derivatives of the measured tissue uptake $R(t)$ described by Eq. A1 and a simulated tissue uptake $\hat{R}(t)$ described by Eq. A6, given the time derivative of the measured blood input $C_A(t)$. Taking the time derivative of both sides of Eqs. A1 and A6, we obtain the equations

$$\frac{dR(t)}{dt} = C_A(t) - C_V(t)$$ \hspace{1cm} (A7)

$$\frac{d\hat{R}(t)}{dt} = \int_0^t h(\tau) \frac{dC_A(t - \tau)}{dt} d\tau$$ \hspace{1cm} (A8)

Thus the compartment model impulse response $h(t)$, which relates the blood curve $C_A(t)$ to the simulated tissue curve $R(t)$ via Eq. A6, also relates the blood derivative curve $dC_A(t)/dt$ to the simulated tissue derivative curve $dR(t)/dt$ via Eq. A8 because of the linearity of convolution and differentiation.

Providing RFIT with the blood and tissue derivative curves $dC_A(t)/dt$ and $dR(t)/dt$ is preferable in this case because our samples of the arteriovenous difference (Eq. A7) have uncorrelated errors, in keeping with the use of an unweighted least squares curve fit. For a constant infusion

$$C_A(t) = \begin{cases} 0 & t < 0 \\ C_{A_0} & t \geq 0 \end{cases}$$ \hspace{1cm} (A9)

where $C_{A_0}$ is constant arterial tracer concentration during constant infusion, and the blood derivative curve is the Dirac delta function

$$\frac{dC_A(t)}{dt} = C_{A_0} \delta(t)$$ \hspace{1cm} (A10)

Having estimated a set of compartment model parameters ($k_{21}$, $k_{12}$, $k_{32}$, $k_{23}$, and $k_{43}$), we are interested in using the model to calculate the fraction of FDG or DG that is phosphorylated. This corresponds to the amount of isotope that is trapped in compartment 4, given a unit amount of isotope in the arterial blood input. It can be shown that this fractional utilization, which we denote by $FU$, is given by

$$FU = \frac{k_{21}}{1 + \frac{k_{12}}{k_{32}} \left( \frac{k_{23}}{1 + k_{43}} \right)}$$ \hspace{1cm} (A11)

As the equations have been developed, $k_{31}$ is dimensionless and $FU$ multiplied by flow provides an estimate of that fraction of the total delivered FDG or DG that is taken up by the heart. This term ($FU \times \text{flow}$) has the dimensions of milliliters per minute per gram LV wet weight and is denoted in this report as $K_{FUR}$. This study was supported by National Heart, Lung, and Blood Institute Grants PO1 HL-25840 and PO1 HL-48068 and by the Director, Office of Energy Research, Office of Biological and Environmental Research (OBER), Medical Applications and Biophysical Research Division of the United States Department of Energy under OBER contract DE-AC03–76SF00098. Address for reprint requests: R. C. Marshall, Lawrence Berkeley National Laboratory, Bldg. 55–121, 1 Cyclootron Rd., Berkeley, CA 94720. Received 9 February 1998; accepted in final form 30 April 1998.

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