

Myocardial Glucose Uptake Measured with Fluorodeoxyglucose: A Proposed Method to Account for Variable Lumped Constants

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Quantitative assessment of myocardial glucose uptake by the glucose tracer analog 2-deoxy-2-[¹⁸F]fluoro-D-glucose (FDG) depends on a correction factor (lumped constant [LC]), which may vary. We propose that this variability is caused by different affinities of FDG and glucose for membrane transport and phosphorylation and can be predicted from the time course of FDG retention. We therefore measured the LC under steady-state metabolic conditions and compared the results with values predicted from the tracer retention alone. **Methods:** We measured rates of myocardial glucose uptake by tracer ([²⁻³H]glucose) and tracer analog methods (FDG) in isolated working Sprague-Dawley rat hearts perfused with Krebs buffer and glucose, or glucose plus insulin or β -hydroxybutyrate. In separate experiments, we established the theoretical upper and lower limits for the LC (R_t and R_p), which are determined by the relative rates of FDG and glucose membrane transport (R_t , 1.73 ± 0.22) and the relative rates of FDG and glucose phosphorylation (R_p , 0.15 ± 0.04). **Results:** The LC was decreased in the presence of insulin or β -hydroxybutyrate or both (from 1.14 ± 0.3 to 0.58 ± 0.16 [insulin], to 0.75 ± 0.17 [β -hydroxybutyrate] or to 0.53 ± 0.17 [both], $P < 0.05$). The time-activity curves of FDG retention reflected these changes. Combining the upper and lower limits for the LC with the ratio between unidirectional and steady-state FDG uptake rates allowed the prediction of individual LCs, which agreed well with the actually measured values ($r = 0.96$, $P < 0.001$). **Conclusion:** The LC is not a constant but is a predictable quotient. As a result of the fixed relation between tracer and tracee for both membrane transport and phosphorylation, the quotient can be determined from the FDG time-activity curve and true rates of myocardial glucose uptake can be measured.

Key Words: PET; enzyme kinetics; hormones; membrane transport; hexokinase

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Determination of myocardial glucose uptake (MGU) with 2-deoxy-2-[¹⁸F]fluoro-D-glucose (FDG) and PET is

based on the tracer model developed for 2-deoxy-[¹⁴C]D-glucose and quantitative autoradiography in the brain (1). The method was adapted for the heart, because myocardial uptake of FDG and glucose occurs through a series of similar steps. These steps include facilitated transport of hexoses into the myocyte by transporter proteins and phosphorylation by the enzyme hexokinase. Because the phosphorylated tracer analog is neither dephosphorylated nor a substrate for the glycolytic pathway, the accumulation of FDG is thought to trace glucose uptake (2).

The FDG method has provided valuable qualitative information about reversibility of left ventricular dysfunction in patients with ischemic heart disease (3–5). However, without quantitation, it is impossible to assess any mechanisms. Quantitative assessment of MGU by FDG is difficult because the correction factor that equates net myocardial FDG uptake to net MGU, the lumped constant (LC), is not a true constant (6–9). In theory, the main mechanism underlying its variability can be ascribed to the variable distribution of the metabolic control strength among the serial steps regulating uptake of hexoses (8,10). The concept of control strength has its origin in analyses of the regulation of metabolism (11) and quantifies the influence of transport and phosphorylation on myocardial uptake of hexoses (12). The assignment of control among these steps is modulated by determinants of MGU, for example, insulin and ketone bodies (12), which also generate changes of the LC (6,8,9).

Both membrane transport and the hexokinase reaction obey Michaelis-Menten kinetics, which means that the reaction velocity is dependent on substrate concentration and is saturable. Incorporation of these kinetics into the tracer model describing myocardial uptake of FDG and glucose implies that unmeasurable transfer coefficients of the tracee can be substituted by measurable transfer coefficients of the tracer such that the magnitude of the LC is predictable by the kinetics of FDG uptake into the heart (9).

The aim of this study was to determine whether the magnitude of the LC could be assessed from the time course of myocardial FDG uptake data. We examined different metabolic states under steady-state conditions. We found

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that changes in the metabolic control strength of membrane transport and phosphorylation, and thus the corresponding changes of the magnitude of the LC, were reflected in the kinetics of FDG retention. The use of measurable parameters of FDG kinetics offers a solution to the problem of the variable LC required for the correct measurement of MGU.

MATERIALS AND METHODS

Theoretical Background

The LC was originally defined by Sokoloff et al. (1) as the ratio between the net clearances (fractional uptake rates, mL/min/g) of FDG (K^*) and glucose (K), independent of any model:

$$LC = K^*/K. \quad Eq. 1$$

The LC consists of several components to correct for all of the kinetic differences between FDG and glucose in the metabolic pathways of transport and phosphorylation. To clarify the influence of each of these components on the magnitude of the correction factor, other exact descriptions of the LC have been derived. Sokoloff et al. (1) related the LC to a specific combination of kinetic rate constants for transport of deoxyglucose and glucose across the blood-brain barrier and Michaelis-Menten constants for the activity of hexokinase:

$$LC = \lambda V_{max}^* K_m / \Phi V_{max} K_m^*, \quad Eq. 2$$

where λ is the ratio of distribution volumes (the steady-state tissue-to-plasma concentration ratio), V_{max} is the maximum reaction velocity of hexokinase, Φ is the fraction of phosphorylated glucose that is further metabolized and K_m is the Michaelis constant for hexokinase. Asterisks refer to the tracer analog. In this model, the LC was considered constant, because each of the six elements included was thought to be constant.

Kuwabara et al. (13) extended the model to predict a variable LC related to the ratio between net (K^*) and unidirectional transport (K^\dagger) of FDG:

$$LC = R_p + [(R_t - R_p)K^*/K^\dagger], \quad Eq. 3$$

where R_t is the ratio between rates of membrane transport of FDG and glucose, and R_p is the ratio between the rates of phosphorylation of FDG and glucose. Equation 3 is more useful than Equation 2 because it defines the LC in terms of the measurable parameters K^* and K^\dagger and the invariant ratios R_t and R_p (10,13,14). The K^*/K^\dagger ratio quantitatively describes the relative influence of the transport and phosphorylation processes on the overall rate of hexose uptake (12,15,16). It has a theoretical range of 0–1. When the rate of phosphorylation approaches that of transport, net uptake is determined by the transport rate and the K^*/K^\dagger ratio approaches its maximum value of 1. As phosphorylation declines (for a given transport rate), the net uptake rate and the K^*/K^\dagger ratio decrease, approaching 0 when phosphorylation is rate limiting. An intermediate value of the transport limitation ratio corresponds to the situation where both transport and phosphorylation determine the overall rate of uptake.

The mathematical derivation of the LC in terms of the components included in Equation 3 therefore predicts that the LC can be determined when R_t and R_p are known (9). The variability of the LC is dependent on the metabolic control strength of membrane transport and of hexokinase, which is determined by the metabolic environment of the organ. The theoretical magnitudes of the LC range from a lower value that equals R_p , when phosphorylation is

rate limiting, to an upper value of R_t , when transport is rate limiting. As a result of independent changes at each of the serial steps, changes of the LC are associated with fluctuations of the intracellular free glucose and FDG.

Experimental Strategy

The experimental plan addressed four specific aims: (a) to compare the time course of FDG retention and glucose uptake during different steady-state metabolic conditions, (b) to test whether the LC is inversely related to the intracellular content of free glucose, (c) to establish values for the parameters R_t and R_p and (d) to test whether LCs that are calculated by Equation 3 and K^*/K^\dagger ratios that are individually determined agree with measured LCs.

Materials

Chemicals of the highest grade came from Fisher Scientific (Pittsburgh, PA) or Sigma (St. Louis, MO). Enzymes and cofactors were from Boehringer Mannheim (Indianapolis, IN) or Sigma (St. Louis, MO). Regular human insulin (Novolin) was from Novo-Nordisk (Ballerup, Denmark). FDG (specific activity >185 TBq/mmol [>5000 Ci/mmol], 56 kBq/mL [1.5 μ Ci/mL] per perfusion) was prepared by the method of Hamacher et al. (17). [2^3 H]glucose (1830 Bq/mL [0.05 μ Ci/mL] per perfusion) and [14 C]sucrose (550 Bq/mL [0.015 μ Ci/mL] per perfusion) were from Amersham Corporation (Chicago, IL).

Working Heart Preparation

We used male Sprague-Dawley rats (275–350 g) from Harlan Laboratory (Indianapolis, IN) fed ad libitum on Purina Chow (Purina, St. Louis, MO) with free access to water. The investigations conform with the *Guide for Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). The preparation has been described in detail earlier (2,18). After a brief period of retrograde perfusion (<5 min) with oxygenated buffer containing 5 mmol/L glucose to wash out any blood from the heart and to perform the left atrial cannulation, hearts were switched to the working preparation. The perfuse (37°C) consisted of Krebs-Henseleit bicarbonate saline (200 mL) equilibrated with 95% O₂/5% CO₂, containing the substrates as indicated in the protocols, and was recirculated. Perfuse [Ca²⁺] was 2.5 mmol/L. All perfusions were performed at standard workload conditions (left atrial pressure, 10 cm H₂O; aortic afterload, 100 cm H₂O). Heart rate and aortic pressures were continuously measured with a 3F Millar transducer (Millar Instruments, Houston, TX) and a MacLab physiological recording system (ADIInstruments, Milford, MA). Aortic flow and coronary flow were measured every 5 min. Cardiac power (mW) was calculated as mean aortic pressure (mm Hg) \times cardiac output (mL/min) \times 2.22 \times 10⁻³. At the end of the perfusion, hearts were freeze-clamped between aluminum blocks cooled to the temperature of liquid nitrogen and stored at –70°C until tissue extraction. In separate experiments, the recirculating tracer infusion period was followed by perfusion with nonradioactive perfusate for an additional 40 min (washout studies).

Experimental Groups

We studied five groups of hearts perfused with (a) 2.5 mmol/L glucose (n = 5), (b) 5 mmol/L glucose (n = 10), (c) 5 mmol/L glucose and 40 μ U/mL insulin (n = 10), (d) 5 mmol/L glucose and 4 mmol/L D,L- β -hydroxybutyrate (n = 8) or (e) 5 mmol/L glucose, 4 mmol/L D L- β -hydroxybutyrate and 40 μ U/mL insulin (n = 6).

Radioactive Tracer Protocols and Measurement of Radioactivity

We assessed glucose transport and phosphorylation with [2^3H]glucose (19). FDG was introduced into the perfusate reservoir as a bolus (150 μL , 11 MBq [300 μCi] or 1.11×10^{10} Hz) after an equilibration period of 15 min and was subsequently present at a stable concentration (see Fig. 1, input function). Radioactivity of FDG in the perfusate and in the myocardium were recorded continuously. Metabolic rate calculation included a correction for the amount of perfusate removed (18). $^3\text{H}_2\text{O}$ was measured in perfusate samples (1 mL) withdrawn at 5-min intervals. [^{14}C]sucrose was added for the final 10 min of the perfusion.

Radioactivity of ^{18}F in the tissue was counted by a pair of bismuth germanate detectors placed on opposite sides of the heart. The signal was sent to a fast-slow coincidence system (Canberra, Meriden, CT) for counting positron annihilation (2). Coincidence counting rate was recorded on a second-to-second basis, and counts were sent to a personal computer for further analysis. FDG radioactivity in the recirculating perfusate (input function) was recorded continuously by a positron-counting device connected to the arterial side of the perfusion apparatus (2). All counts were decay corrected to the time of FDG addition. The conversion of tissue coincidence counting rates into microcurie per milliliter was performed with a bar phantom similar in shape and size to a rat heart containing a known amount of ^{18}F radioactivity. The same bar phantom was used for initial calibration. In all experiments, counts were acquired at a rate that did not exceed the linear range of the coincidence detection system (i.e., <8000 Hz). A well counter (Gamma Products, Inc., Palos Hills, IL) was used to determine the radioactivity of FDG in the perfusate ($\mu\text{Ci}/\text{mL}$).

Radioactivity of ^3H and ^{14}C was counted on a Packard 1900TR liquid scintillation counter (Packard Instruments, Meriden, CT) by the method of spectral index analysis as described by the manufacturer. Rates of glucose uptake were determined from the rate of $^3\text{H}_2\text{O}$ production from [2^3H]glucose (19). $^3\text{H}_2\text{O}$ is released during rapid equilibrium at the stage of the enzyme phosphoglucose isomerase. Therefore, the release of $^3\text{H}_2\text{O}$ is a measure of net glucose utilization. We separated $^3\text{H}_2\text{O}$ from [2^3H]glucose by

anion exchange chromatography (19) on AG-1 \times 8 resin (Bio-Rad Laboratories, Hercules, CA).

To determine the intracellular concentration of nonphosphorylated glucose, the extracellular fluid space (ECFS) was measured with [^{14}C]sucrose (0.055 MBq [1.5 $\mu\text{Ci}/100 \text{ mL}$] perfusate), as described previously (2). Approximately 100 mg of the freeze-clamped tissue, ground to a fine powder under liquid nitrogen, were solubilized directly in glass scintillation vials by the addition of 1.5 mL Soluene-350 (Packard Instruments, Chicago, IL) followed by incubation at 50°C–55°C. To reduce quenching, 100 μl 30% hydrogen peroxide was used to decolorize the sample before the addition of 10 mL scintillation mixture (Ultima Gold; Packard Instruments, Chicago, IL). The ECFS was calculated as the ratio of [^{14}C]sucrose (dpm) found in 100 mg tissue over the [^{14}C]sucrose (dpm) found in 100 μL perfusate.

Perfusate Assays, Tissue Extraction and Metabolite Determination

Glucose was determined immediately with a glucose analyzer (2300 STAT; YSI, Yellow Springs, OH). The glucose concentration ($\mu\text{mol}/\text{mL}$ perfusate) was used to determine the specific activity of [2^3H]glucose.

Approximately 200 mg frozen tissue powder were extracted with 6% perchloric acid (1:4, wt/vol). The tissue sample was centrifuged at 7000g for 10 min in a refrigerated centrifuge. The supernatant fraction was neutralized with 3 mol/L KOH, 0.25 mol/L 2-[N-morpholino] ethanesulfonic acid and 0.25 mol/L 3-[N-morpholino] propanesulfonic acid. Glucose and glucose-6-phosphate were determined by enzymatic methods. The intracellular free glucose concentration ($[\text{glucose}]_{\text{ic}}$) was calculated using the formula:

$$[\text{glucose}]_{\text{ic}} = ([\text{glucose}]_{\text{tissue}} - [\text{glucose}]_{\text{perfusate}} \times \text{ECFS}) / ([1 - 1/(W/D) - \text{ECFS}] \times 0.75), \quad \text{Eq. 4}$$

where W/D is the wet-to-dry weight ratio. The units for $[\text{glucose}]_{\text{perfusate}}$ are micromole per milliliter, and the units for $[\text{glucose}]_{\text{ic}}$ and for $[\text{glucose}]_{\text{tissue}}$ are micromole per gram wet weight.

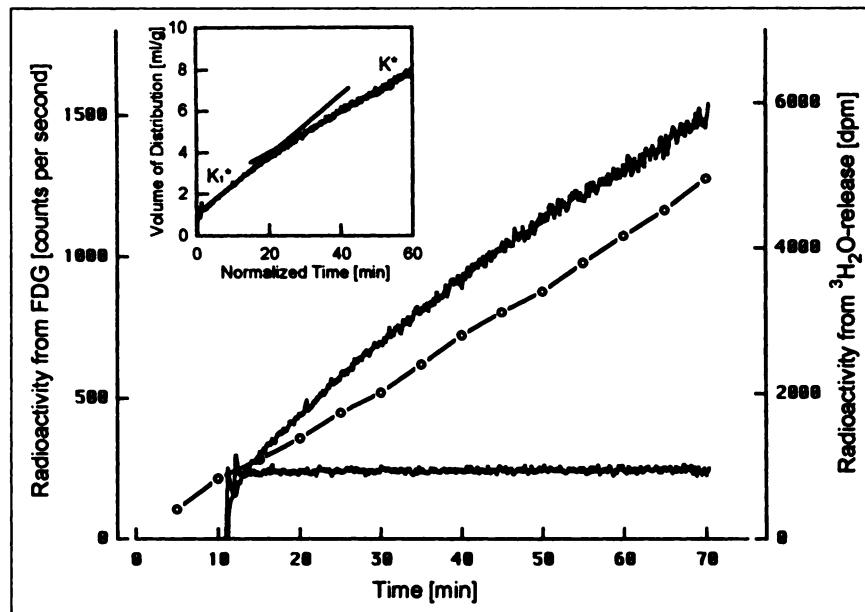


FIGURE 1. Representative time-activity curve of heart perfused with 5 mmol/L glucose. Line with open circles represents $^3\text{H}_2\text{O}$ production from [2^3H]glucose. Solid lines represent tracing curves of FDG: Upper line represents tissue time-activity curve, and lower line represents input function, which remains constant throughout experiment. Inset depicts apparent volume of distribution as function of normalized blood concentration-time integral, which is application of graphical analysis to FDG tissue time-activity curve and input function ($K^*/K_i^* = 0.95$).

The factor of 0.75 was introduced because only 75% of the intracellular water is available for sugar distribution (20). A small portion of the pulverized tissue was oven dried for dry weight determination. Values are reported as micromole per gram dry weight by multiplying by the W/D weight ratio. Glycogen content was determined by enzymatic digestion with amyloglucosidase and is reported as glycosyl units (21).

Determination of R_t

The ratio between the rates of membrane transport of FDG and glucose (R_t) was determined from the upper limiting value for the LC in hearts perfused at 5 mmol/L glucose in the presence of cytochalasin B, an inhibitor of glucose transport (22). Perfusions were conducted for 60 min. We tested also the effects of increasing concentrations of cytochalasin B on contractile performance as an indicator of exogenous energy substrate utilization. With increasing concentrations of cytochalasin B (0.25, 0.5 and 1.0 $\mu\text{mol/L}$), cardiac power decreased by $30\% \pm 8\%$ ($n = 5$), $43\% \pm 8\%$ ($n = 6$) and $55\% \pm 3\%$ ($n = 3$), respectively. The addition of lactate reversed the decline in cardiac power indicating that cytochalasin B had no cytotoxic effect. R_t was determined as the ratio between the rate of FDG retention and the rate of $^3\text{H}_2\text{O}$ production from [$2\text{-}^3\text{H}$]glucose in the presence of 0.5 $\mu\text{mol/L}$ cytochalasin B.

Determination of R_p

The ratio between the rates of phosphorylation of 2-deoxyglucose and glucose (R_p) was determined using purified hexokinase (bovine heart, Sigma type X, specific activity 20 U/mg protein), assayed as described by Easterby and O'Brien (23). We determined an effective value for the ratio, by incubating enzyme with various substrate level concentrations of glucose (14–75 $\mu\text{mol/L}$) and a tracer concentration of the tracer analog, to model the situation as it occurs in the heart. The assay volume was 0.5 mL containing 40 mmol/L Tris base (pH 7.6 at 30°C with HCl), 2 mmol/L adenosine triphosphate, 16 mmol/L MgCl_2 , 0.1 mg/mL bovine serum albumin, 3.7 kBq (0.1 μCi) [$\text{U-}^{14}\text{C}$]glucose (ICN, Costa Mesa, CA), 6.3 kBq (0.17 μCi) [$1,2\text{-}^3\text{H}$]2-deoxyglucose (ICN, 6230 Bq [30 Ci/mmol]) and glucose (14, 20, 30 or 75 $\mu\text{mol/L}$). The concentration of 2-deoxyglucose, calculated from the stated specific activity, was 11 nmol/L. Reactions were conducted without added glucose-6-phosphate or with glucose-6-phosphate added to a concentration of 10, 30, 50 or 100 $\mu\text{mol/L}$. Reactions were initiated with enzyme (5–30 ng in 10 μL) and were terminated after 10 min at 30°C by diluting an aliquot into stop mix containing Dowex 2-X8 (Bio-Rad Laboratories). The resin was washed and reaction products ([$\text{U-}^{14}\text{C}$]glucose-6-phosphate and [$1,2\text{-}^3\text{H}$]2-deoxyglucose-6-phosphate) were eluted with HCl as described by Gots and Bessman (24). A portion of the eluent was taken for dual-isotope liquid scintillation counting to determine ^3H and ^{14}C content.

Calculations, Mathematical Principles for FDG Time-Activity Curves and Statistics

The LC was calculated as the ratio between simultaneously measured rates of uptake of the glucose tracer analog and the tracer. Glucose uptake rates were determined by linear regression analysis of the slope of the cumulative $^3\text{H}_2\text{O}$ release. The result was related to the specific activity of [$2\text{-}^3\text{H}$]glucose and glucose concentration in the perfusate. Glucose uptake was expressed as $\mu\text{mol} \times \text{min}^{-1} \times \text{g dry weight}^{-1}$.

Rates of retention of the tracer analog were determined by dividing the slopes of the tissue time-activity curves of FDG ($\mu\text{Ci} \times \text{min}^{-1}$) by the radioactivity of FDG in the perfusate ($\mu\text{Ci} \times$

$\mu\text{mol glucose}^{-1}$) and the dry weight (g) and were expressed as $\mu\text{mol} \times \text{min}^{-1} \times \text{g dry weight}^{-1}$. Slopes of the time-activity curves were determined by linear regression analysis. To ensure that the FDG uptake curves had reached equilibrium, the slopes were calculated from the last 20 min of the perfusions.

The parameters required for the estimation of the LC from FDG retention were generated by graphical analysis as described by Gjedde (25,26) and Patlak et al. (27). Corresponding tissue time-activity curves and perfusate time-activity curves were combined to produce curves representing changes of volumes of distribution (V_D) as a function of the time integral of perfusate radioactivity, normalized against that radioactivity (Θ). The curves have a characteristic course that can be described by Equation 5 (28):

$$V_D = \alpha\Theta + \beta(1 - e^{-\gamma\Theta}). \quad \text{Eq. 5}$$

The coefficients α , β and γ were determined by nonlinear regression. Unidirectional (K^\ddagger) and net (K^*) clearances of FDG were calculated as:

$$K^\ddagger = \alpha + \beta\gamma \quad \text{Eq. 6}$$

and

$$K^* = \alpha, \quad \text{Eq. 7}$$

expressed in mL perfusate \times (mL tissue) $^{-1} \times \text{min}^{-1}$. To convert data to mL \times (g tissue) $^{-1} \times \text{min}^{-1}$, K^* was divided by 1.05, the value for the density of the myocardium (29).

All data are presented as mean \pm SD. Comparisons among the five groups were performed by one-way analysis of variance followed by a post hoc comparison by Scheffé's test if statistically significant differences were demonstrated. $P < 0.05$ was considered significant.

RESULTS

Cardiac Performance

Cardiac power was 8.69 ± 1.50 mW ($n = 39$) and was stable for the duration of each experiment. There were no differences in cardiac power among the groups.

Rates of FDG Retention and Glucose Uptake

Figure 1 shows a representative tissue time-activity curve of a heart perfused with 5 mmol/L glucose as the only substrate. The two tracings represent the input function (lower curve) and the tissue time-activity curve (upper curve) of FDG. The input function was stable for the duration of the experiment. The tissue time-activity curve was curvilinear during the first 20 min and reached steady state thereafter. During steady state, the uptake rate of glucose determined by FDG retention was greater than the uptake rate of glucose determined by $^3\text{H}_2\text{O}$ production from [$2\text{-}^3\text{H}$]glucose. The LC was still close to unity (Table 1). The graphical analysis of the FDG accumulation curves (see inset in Fig. 1 for an example) showed a mean K^*/K^\ddagger ratio of 0.62 ± 0.22 (Table 2), indicating that myocardial FDG uptake is determined to a large extent by both membrane transport and phosphorylation. The rate of $^3\text{H}_2\text{O}$ production from [$2\text{-}^3\text{H}$]glucose was linear during the entire experiment.

As shown in Table 1, the glucose uptake rates assessed by both FDG and [$2\text{-}^3\text{H}$]glucose were very similar at 2.5 and at 5 mmol/L. Although both the LC and the K^*/K^\ddagger ratios were

TABLE 1
Myocardial Glucose Uptake Determined Either by Production of $^3\text{H}_2\text{O}$ from [2- ^3H]Glucose or by FDG Retention and Ratio Between Uptake Rates of Tracer (FDG) and Tracee (Glucose) (LC)

Measured value	Groups				
	2.5 mmol/L glucose	5 mmol/L glucose	5 mmol/L + 40 $\mu\text{U}/\text{mL}$ glucose	5 mmol/L glucose + 4 mmol/L β -hydroxybutyrate	5 mmol/L glucose + 4 mmol/L β -hydroxybutyrate + insulin 40 $\mu\text{U}/\text{mL}$
Myocardial glucose uptake ($\mu\text{mol} \times \text{min}^{-1} \times \text{g dry}^{-1}$)					
By [2- ^3H]glucose	4.26 ± 1.03	4.91 ± 1.87	9.22 ± 3.41*	2.59 ± 1.64*	5.26 ± 1.52
By FDG retention	6.04 ± 1.50	5.20 ± 1.93	5.16 ± 2.34	1.94 ± 1.13†	2.75 ± 1.05†
LC	1.45 ± 0.31	1.14 ± 0.31	0.58 ± 0.16‡	0.75 ± 0.17‡	0.53 ± 0.17‡

* $P < 0.05$ vs. all groups.

† $P < 0.05$ vs. 5 mmol/L glucose and 2.5 mmol/L glucose, and 5 mmol/L glucose and 40 $\mu\text{U}/\text{mL}$ insulin.

‡ $P < 0.05$ vs. 5 mmol/L glucose and 2.5 mmol/L glucose.

FDG = 2-deoxy-2-[^{18}F]fluoro-D-glucose; LC = lumped constant.

Working rat hearts were perfused with Krebs-Henseleit saline at near physiological workload. Rates of myocardial glucose and FDG uptake were determined by linear regression analysis. See text for details.

increased at 2.5 mmol/L relative to values observed at 5 mmol/L perfusate glucose concentration, neither increase reached statistical significance (Table 2).

When 40 $\mu\text{U}/\text{mL}$ insulin was present in hearts perfused with 5 mmol/L glucose, the initial rates of FDG retention and of glucose uptake as measured by $^3\text{H}_2\text{O}$ production from [2- ^3H]glucose were both increased compared with hearts perfused in the absence of insulin (Fig. 2). At approximately 20 min, the FDG retention curve became less steep, whereas the $^3\text{H}_2\text{O}$ production curve remained unchanged. The rate of glucose uptake assessed with $^3\text{H}_2\text{O}$ production was greater than the assessment from FDG retention resulting in a decrease of the LC compared with hearts perfused with glucose alone (Table 1). The graphical analysis of the FDG retention curves (see inset of Fig. 2 for an example) showed a similar decrease of the K^*/K^\dagger ratios to 0.27 ± 0.09,

indicating a predominant limitation of FDG uptake by hexokinase (Table 2).

In hearts perfused with 5 mmol/L glucose plus 4 mmol/L D,L- β -hydroxybutyrate, the initial rates of FDG retention and of glucose uptake as measured by $^3\text{H}_2\text{O}$ production from [2- ^3H]glucose were both decreased in comparison with hearts perfused with 5 mmol/L glucose only (Fig. 3). At 20 min, the curves began to diverge. The slope of the FDG retention curve became linear and less steep, whereas the slope of the $^3\text{H}_2\text{O}$ production remained unchanged. Thus, compared with hearts perfused with 5 mmol/L glucose, the presence of β -hydroxybutyrate reduced the steady-state uptake rates of FDG more than the steady-state uptake of glucose, resulting in a decrease of the LC (Table 1). As with the insulin group, the K^*/K^\dagger ratio also decreased (Table 2).

TABLE 2
Myocardial Net (K^*) and Unidirectional (K_\dagger) Clearance Rates of FDG and the Transport Limitation Ratio (K^*/K_\dagger)

Measured value	Groups				
	2.5 mmol/L glucose	5 mmol/L glucose	5 mmol/L glucose + 40 $\mu\text{U}/\text{mL}$ insulin	5 mmol/L glucose + 4 mmol/L β -hydroxybutyrate	5 mmol/L glucose + 4 mmol/L β -hydroxybutyrate + 40 $\mu\text{U}/\text{mL}$ insulin
K^* ($\text{mL} \times \text{min}^{-1} \times \text{g wet}^{-1}$)	0.131 ± 0.034	0.139 ± 0.092	0.102 ± 0.044	0.049 ± 0.028*	0.061 ± 0.027*
K_\dagger ($\text{mL} \times \text{min}^{-1} \times \text{g wet}^{-1}$)	0.157 ± 0.040	0.218 ± 0.038	0.359 ± 0.095†	0.127 ± 0.037	0.215 ± 0.076
K^*/K_\dagger	0.85 ± 0.20	0.62 ± 0.22	0.27 ± 0.09*	0.38 ± 0.11‡	0.29 ± 0.10*

* $P < 0.01$ vs. 5 mmol/L glucose and 2.5 mmol/L glucose.

† $P < 0.05$ vs. all groups.

‡ $P < 0.05$ vs. 5 mmol/L glucose and 2.5 mmol/L glucose.

FDG = 2-deoxy-2-[^{18}F]fluoro-D-glucose.

Working rat hearts were perfused with Krebs-Henseleit saline at near physiological workload and rate constants were determined from FDG time-activity curves by iterative curve fitting as described in text. Transport limitation ratio quantitatively describes relative influence of transport and phosphorylation processes on overall hexose uptake as explained in text.

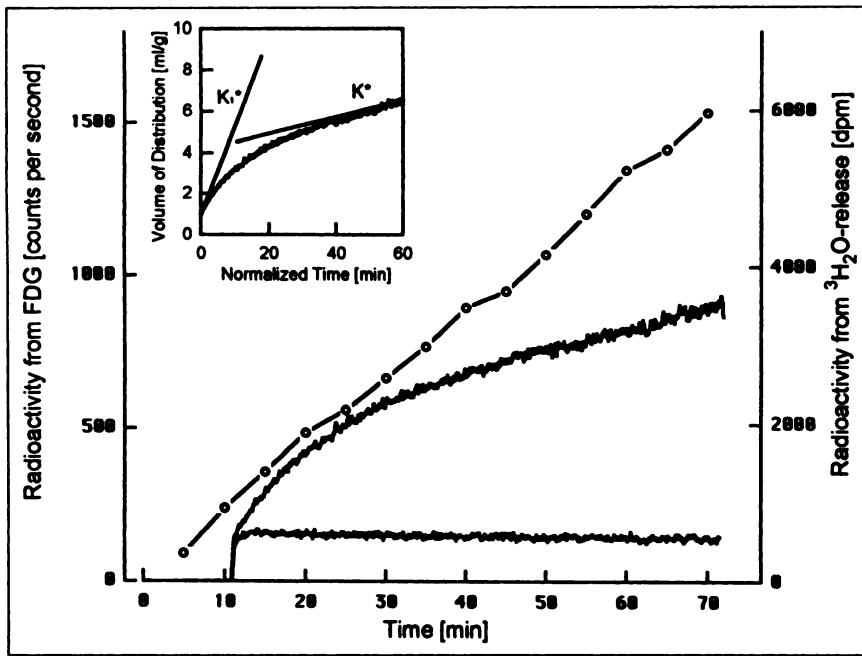


FIGURE 2. Influence of $40 \mu\text{U}/\text{mL}$ insulin on uptake of glucose and FDG. Line with open circles represents $^3\text{H}_2\text{O}$ production from $[2-^3\text{H}]$ glucose. Solid lines represent tracing curves of FDG: Upper line represents tissue time-activity curve, and lower line represents input function, which remains constant throughout experiment. Compared with hearts perfused with glucose only (Fig. 1), insulin increased glucose uptake, as measured by $^3\text{H}_2\text{O}$ production from $[2-^3\text{H}]$ glucose. Initial uptake rate of FDG was also increased, but at approximately 20 min, FDG retention curve became less steep. Compared with hearts perfused with glucose alone, steady-state uptake of glucose increased more than steady-state uptake of FDG. Graphical analysis of myocardial FDG retention (inset) shows that ratio between net transport of FDG during steady state (K^*) and initial unidirectional transport of FDG into heart (K_1^*) is decreased compared with hearts perfused with glucose alone ($K^*/K_1^* = 0.16$).

When hearts were perfused with 5 mmol/L glucose and $40 \mu\text{U}/\text{mL}$ insulin as well as 4 mmol/L D,L- β -hydroxybutyrate, the initial rates of FDG retention and of glucose uptake as measured by $^3\text{H}_2\text{O}$ production from $[2-^3\text{H}]$ glucose were unchanged compared with hearts perfused with 5 mmol/L glucose only. The diverging course of the $^3\text{H}_2\text{O}$ production

and the FDG curves after 20 min was not different from the findings when each of these compounds was present alone. Thus, compared with hearts perfused with 5 mmol/L glucose, the presence of both insulin and β -hydroxybutyrate also reduced the steady-state uptake rates of FDG while the steady-state uptake of glucose remained unchanged,

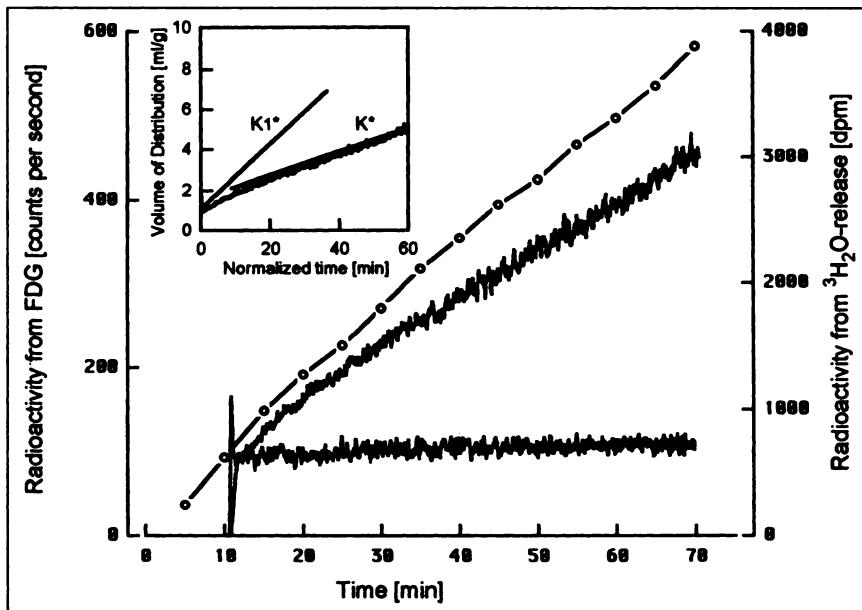


FIGURE 3. Influence of 4 mmol/L D,L- β -hydroxybutyrate on uptake of glucose and FDG. Line with open circles represents $^3\text{H}_2\text{O}$ production from $[2-^3\text{H}]$ glucose. Solid lines represent tracing curves of FDG: Upper line represents tissue time-activity curve, and lower line represents input function, which remains constant throughout experiment. Compared with hearts perfused with glucose only (Fig. 1), β -hydroxybutyrate decreases glucose uptake as measured by $^3\text{H}_2\text{O}$ production from $[2-^3\text{H}]$ glucose. Initial uptake rate of FDG was also decreased. At approximately 20 min, slope of FDG retention curve was further reduced so that $^3\text{H}_2\text{O}$ production and FDG retention curves diverged. Compared with hearts perfused with glucose alone, steady-state uptake of FDG decreased more than steady-state uptake of glucose. Graphical analysis of myocardial FDG retention (inset) shows that ratio between net transport of FDG during steady state (K^*) and initial unidirectional transport of FDG into heart (K_1^*) is decreased compared with hearts perfused with glucose alone ($K^*/K_1^* = 0.34$).

resulting in a decrease of the LC (Table 1) and the K^*/K^\dagger ratio (Table 2).

Washout Studies

Figure 4 shows FDG retention and washout curves after a change to a tracer-free perfusate at 65 min. The upper curve is a time-activity curve of a heart perfused with 2.5 mmol/L glucose as the only substrate. FDG accumulation was linear and the slope did not change during the perfusion when the tracer analog was present. At the beginning of the washout, there was a rapid fall in the radioactivity emanating from the heart, which was followed by a plateau. The fall in radioactivity was caused by disappearance of tracer from the vascular space. Subsequently, no radioactivity was observed in samples taken from the coronary effluent during plateau of the washout period (data not presented). Therefore, the plateau represents tracer irreversibly trapped in the myocardium.

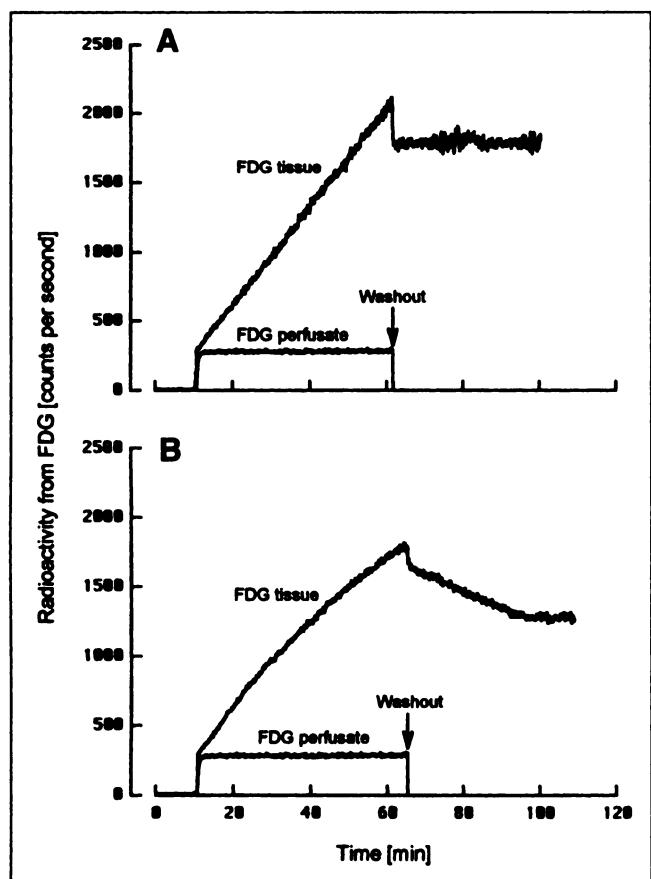


FIGURE 4. Time-activity curves of myocardial FDG retention and washout after change to tracer-free perfusate at 65 min. (A) Time-activity curve of heart perfused with 2.5 mmol/L glucose alone. FDG accumulation was linear. Washout yielded rapid fall caused by disappearance of tracer from heart cavities. Subsequent plateau represents tracer irreversibly trapped in myocardium. (B) Time-activity curve from heart perfused with 5 mmol/L glucose, 4 mmol/L D,L-β-hydroxybutyrate and 40 μ U/mL insulin. Uptake and washout curves were biphasic. Initial rapid disappearance of radioactivity was followed by continuous decline over approximately 25 min before new steady state was reached. Continuous decline represents countertransport of free intracellular FDG.

dium. The findings are consistent with an absence of glucose-6-phosphatase activity in heart muscle.

The lower curve is a representative time-activity curve of a heart perfused with 5 mmol/L glucose as well as 40 μ U/mL insulin and 4 mmol/L D,L-β-hydroxybutyrate. The FDG accumulation curve showed a biphasic configuration comparable to the findings described in Figures 2–4. The washout curve was also biphasic. The initial rapid disappearance of radioactivity was followed by a continuous decline over approximately 25 min before a new steady state was reached. Analysis of the coronary effluent showed ^{18}F radioactivity during the early part of the washout curve but not after 30 min (data not presented).

Intracellular Free Glucose and Glucose Metabolites

We also measured the extracellular and intracellular volume of distribution of glucose (Table 3). Insulin increased both intracellular free glucose and glucose-6-phosphate. Insulin also increased the glycogen content. When β-hydroxybutyrate was present as second substrate, both intracellular free glucose and glycogen increased substantially, while glucose-6-phosphate was unchanged. With the combination of insulin and β-hydroxybutyrate added to glucose, all three compounds increased compared to hearts perfused with glucose as the only substrate. The intracellular glucose content correlated inversely with the LC ($r = -0.61$, $P < 0.05$).

Ratios Between R_t and R_p

Hexose Transport Kinetics. In the presence of cytochalasin B, the K^*/K^\dagger ratio was 1 in all experiments. We obtained a value of 1.73 ± 0.22 for R_t ($n = 6$). The value was similar for a full range of cytochalasin B concentrations: At 250 nmol/L, R_t was 1.70 ± 0.10 ($n = 3$), and at 1000 nmol/L, R_t was 1.72 ± 0.16 ($n = 3$).

Hexokinase Kinetics. By using a dual-isotope radiochemical assay for hexokinase with glucose present at substrate concentration and the glucose analog (2-deoxyglucose) present at tracer concentration, we were able to determine a value for R_p at each glucose concentration tested. In the absence of added glucose-6-phosphate, the value was 0.15 ± 0.04 ($n = 4$), independent of the glucose concentration. Hexokinase was markedly inhibited by added glucose-6-phosphate; V_{max} was decreased by 96% at 100 μ mol/L. However, R_p remained unchanged after addition of glucose-6-phosphate at increasing concentrations: 10 μ mol/L, $R_p = 0.14 \pm 0.02$ ($n = 4$); 30 μ mol/L, $R_p = 0.12 \pm 0.02$ ($n = 4$); 50 μ mol/L, $R_p = 0.12 \pm 0.02$ ($n = 4$); 100 μ mol/L, $R_p = 0.13 \pm 0.04$ ($n = 4$). A value of $R_p = 0.15$ was chosen for our further calculations.

Prediction of the LC with FDG. Figure 5 shows LCs predicted from the time course of FDG and mean values of R_t and R_p (using Eq. 3) plotted against measured LCs. Note the close correlation between the predicted and measured values for all five groups ($r = 0.96$, $P < 0.001$).

TABLE 3
Myocardial Contents of Intracellular Free Glucose, Glucose-6-Phosphate and Glycogen

Measured value	Groups				
	2.5 mmol/L glucose	5 mmol/L glucose	5 mmol/L glucose + 40 μ U/mL insulin	5 mmol/L glucose + 4 mmol/L β -hydroxybutyrate	5 mmol/L glucose + 4 mmol/L β -hydroxybutyrate + 40 μ U/mL insulin
Glucose (μ mol \times g dry $^{-1}$)	ND	0.7 \pm 1.3	7.7 \pm 8.5*	6.9 \pm 9.9	9.1 \pm 2.9*
Glucose-6-phosphate (μ mol \times g dry $^{-1}$)	0.05 \pm 0.04	0.14 \pm 0.09	0.56 \pm 0.19†	0.27 \pm 0.20	0.79 \pm 0.32*
Glycogen (μ mol glucosyl units \times g dry $^{-1}$)	95 \pm 18	106 \pm 19	147 \pm 38*	148 \pm 45*	182 \pm 32†

* $P < 0.05$ vs. 5 mmol/L glucose and 2.5 mmol/L glucose.
 † $P < 0.05$ vs. 5 mmol/L glucose and 2.5 mmol/L glucose.
 ND = not detectable.
 Working rat hearts were perfused with Krebs-Henseleit saline at near physiological workload as described in text and freeze clamped at end of perfusion.

DISCUSSION

The LC is a correction factor required for the determination of MGU from tissue time-activity curves of FDG. Recent observations, both *in vitro* (6,7) and *in vivo* (9), indicate that this correction factor is influenced by modulators of MGU. Our study was undertaken to evaluate whether the changing value of the LC can be determined from

dynamic measurements of FDG retention. The results confirm that the LC changes under conditions that affect hexose transport and phosphorylation. We have now demonstrated that these changes are reflected in the time-activity curves of FDG retention, which provide a basis for individual determination of the LC.

Modeling of FDG as an analog for glucose uptake in the heart is derived from the model originally developed for use in the brain (1). Like any model of a physiological system, the mathematical description is a simplification. In addition, the biological mechanisms regulating the rate of glucose uptake differ in the brain and in the heart. The single transport step of the model represents multiple processes, including diffusion and transport across the capillary endothelium followed by transport across the cell membrane. In the brain, the major limitation of transport resides at the blood-brain barrier (1), whereas the transport-limiting step in the heart is localized at the level of facilitated membrane transport into the myocytes (20). Additionally, the competition between energy-providing substrates in heart muscle (18,30), the well-known insulin sensitivity of the heart (18,31), the dynamic state of glycogen synthesis and degradation (32) and the intracellular compartmentation of hexokinase in the heart (33) make the heart appear more complex than the brain. Despite the biologic differences between the two organs, the general principles are identical. An extension of these principles provides the framework for a model that accurately predicts rates of MGU from dynamic FDG activity curves.

The Experimental Approach

To obtain independent measurements of myocardial glucose uptake, measured by [2^3 H]glucose, and the tracer analog FDG, measured by accumulation in the tissue, we tested five distinctly different metabolic conditions in an *in vitro* working rat heart preparation. None of them can be considered physiological, but each condition was selected to

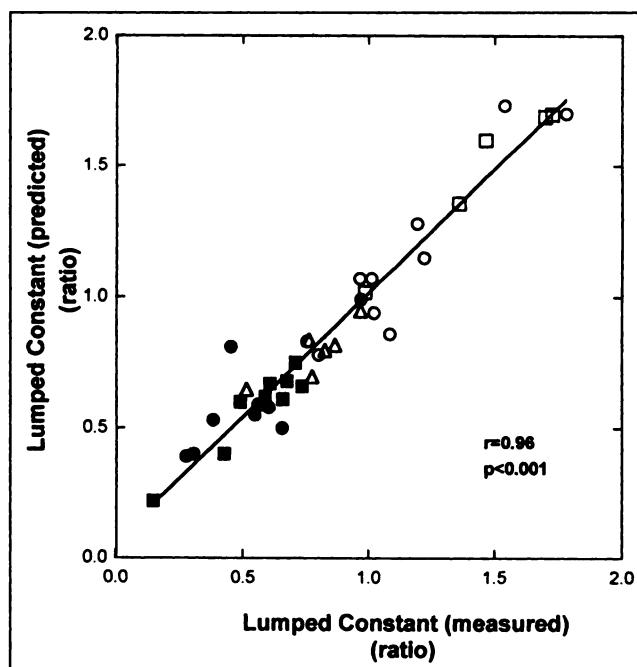


FIGURE 5. Relation between LC predicted according to Equation 3 in text and LC measured as ratio between myocardial glucose uptake with tracer ($[2^3\text{H}]$ glucose) and tracer analog (FDG). ○ = 5 mmol/L glucose; □ = 2.5 mmol/L glucose; ■ = 40 μ U/mL insulin and 5 mmol/L glucose; △ = 4 mmol/L D,L- β -hydroxybutyrate and 5 mmol/L glucose; ● = 4 mmol/L D,L- β -hydroxybutyrate, 40 μ U/mL insulin and 5 mmol/L glucose.

obtain a better understanding of the mechanisms involved in the changing tracer-tracee relation. In perfusions with glucose alone, MGU was independent of glucose concentrations (2.5 and 5 mmol/L). Accumulation of intracellular glucose in perfusions with glucose plus insulin or β -hydroxybutyrate suggests that hexokinase becomes partially rate limiting for glucose uptake as also reflected by a decrease in the K^*/K^\ddagger ratio. The change in metabolic control strength between transport and phosphorylation exposes differences for glucose and FDG, which are reflected in the changing LC.

The increase of intracellular free glucose is further evidenced by the tissue time-activity curves obtained during the washout experiments, which are consistent with an accumulation of free FDG that is subjected to countertransport during the tracer-free period. The accumulated intracellular free FDG was washed out during the first 30 min. Because there was no washout of radioactivity in hearts perfused with glucose alone and the release ceased after 30 min when a competing substrate was present, it is unlikely that dephosphorylation of FDG-6-phosphate was the cause for the prolonged washout of radioactivity from the tissue in the presence of insulin, β -hydroxybutyrate or both. These data are consistent with the reported absence of glucose-6-phosphatase in the heart muscle (34). The apparent inconsistencies with ^{31}P -nuclear magnetic resonance studies demonstrating loss of 2-deoxyglucose-6-[^{31}P]phosphate from the cells once 2-deoxyglucose is removed from the perfusion medium (35) may be explained by the incorporation of 2-deoxyglucose into glycogen (36) (unpublished observations).

The Mathematical Approach

In the graphical analysis of the time course for tracer uptake, the identification of the fitted slope parameter α (Eqs. 5 and 7) as the uptake rate constant K^* requires no assumptions other than the presence of an irreversible trapping process. However, the use of Equations 5 and 6 to estimate the transport parameter K^\ddagger involves several additional assumptions and preliminary manipulations of the counting rate data. First, the equations are strictly correct only when the delivery of tracer to the tissue is constant in time. This condition was satisfied in the current experiments. Second, the equations imply that the time course being fitted can be described by two tissue compartments corresponding to free tracer and trapped tracer. All tracer in heart tissue is assumed to have passed through the transport process K^\ddagger . The acquired time courses do not conform to this assumption, because the radioactivity signal includes tracer in the extracellular space including cardiac chambers, vasculature and interstitial fluid. In our analysis, we assumed that extracellular components were evident as the rapidly rising edge in the early counting rate data and that they remained constant over the duration of the experiment. This component was thus subtracted from the measured time-course data before fitting by Equation 5. Although the graphical presentation of the fitting results in the insets of Figures 1-3

implies that K^\ddagger is determined as the initial slope of the uptake data, its evaluation by Equations 5 and 6 actually depends on the fitting of the entire uptake curve, which provides a considerable advantage in statistical reliability. The values for K^* and K^\ddagger in this study are of the same order of magnitude as those derived from isolated rabbit interventricular septum (37,38).

In hearts perfused with 2.5 mmol/L glucose as the only substrate, the FDG retention curve was linear over the entire uptake period (Fig. 4A). This implies that the steady-state condition was present from the beginning of the FDG tracing and thus that countertransport out of tissue is negligible under these conditions of extreme transport limitation. As the countertransport rate approaches zero, the coefficient β in Equations 5 and 6 is predicted by theory also to approach zero. Thus, when the fitted slope was seen to be the same over all segments of the uptake curve, the fitting of the data to Equation 5 did not converge reliably. In this event, the parameter K^* was determined as the slope of the curve over the entire uptake period, and the K^*/K^\ddagger ratio was set to 1.

When the approach is applied in the clinical setting in humans, tracer will be administered as a bolus and tracer concentrations in plasma will be variable over time. Estimation of K^* by graphical methods remains reliable under these conditions, but the estimation of K^\ddagger from Equations 5 and 6 becomes problematic. However, K^\ddagger can be determined after the uptake data have been subjected to conventional analysis. (Our tissue time-activity curves are the correlate to the clinically generated FDG uptake curves after subtraction, for example, Patlak graphical analysis.) Furthermore, because the K^\ddagger required by the theory is that for plasma membrane transport, the critically important issue of identifying and accounting for all the contributions of extracellular tracer must be addressed.

The Lumped Constant

The LC is an operational factor defined as the ratio between the net clearance of FDG and glucose. In the model derived for calculation of the LC, both transport and phosphorylation are assumed to exhibit saturable kinetics described by the Michaelis-Menten equation (11). These kinetics allow the establishment of the quantitative relations between the tracer and tracee at each step. The relations are included as R_t and R_p in the prediction of the magnitude of the LC by Equation 3. The R_t and the R_p are ratios between Michaelis constants and are expected to be constant. The results of this study support this assumption.

Although Equation 3 denotes that R_t and R_p represent the theoretical upper and lower values for the LC, these extreme values are never attained *in vivo*. In the working rat heart, however, it is possible to assess the upper value of the LC under conditions where glucose uptake is limited by membrane transport as a way to determine the value for R_t . With the transport inhibitor, we found that the R_t was stable and the K^*/K^\ddagger ratio was 1 over a range of cytochalasin B concentrations, indicating that glucose uptake was limited

by membrane transport. The magnitude of R_t is unity (38) or above in most cells (8,16). Our value (1.73 ± 0.22) is of similar magnitude as in the brain of rats and humans (1.10–1.75) (8). The value is also in the range of the value derived from the relation between the LC and the K^*/K^\ddagger ratio of the rabbit interventricular septum (9) but is higher than the estimated ratio from the same preparation (38).

To establish a value for R_p , we performed *in vitro* experiments with purified heart hexokinase. Enzyme activity with glucose and deoxyglucose were determined simultaneously in the same reaction tube by using the two substrates labeled with different isotopes and by using a very low concentration of deoxyglucose. The only glucose analog investigated by this approach was deoxyglucose, because the availability of [^3H]deoxyglucose facilitated the determination. The value for R_p was calculated as the hexokinase-catalyzed rate of synthesis of [1,2- ^3H]2-deoxyglucose-6P relative to that of [$\text{U}-^{14}\text{C}$]glucose-6P. The value that we obtained (0.15 ± 0.04) is the effective value for R_p , because it was determined under conditions relevant to the situation we wish to model, namely, enzyme activity when the native substrate (glucose) is present at substrate concentrations, and the tracer analog (deoxyglucose or FDG) is present at very low concentration. Presumably, the effective value for R_p of FDG is similar to the value for deoxyglucose reported here, because of the chemical similarity of the two analogs (fluorine replaces hydrogen at position 2). Previous studies have documented the same disposition of deoxyglucose compared to FDG with respect to uptake and retention by heart (7), justifying the use of the value for R_p of deoxyglucose to model FDG kinetics in our heart perfusions. Because hexokinase is largely inhibited by ambient concentrations of glucose-6-phosphate in the heart and the inhibition could change the kinetic behavior of the enzyme for glucose relative to a glucose analog, we felt it was important to establish that R_p is not sensitive to inhibition of hexokinase by glucose-6-phosphate. Further, the dual-isotope approach allowed us to determine values for R_p over a range of glucose concentrations. We established that R_p is independent of glucose concentration, consistent with the derivation of R_p described by Sokoloff et al. (1). According to the Sokoloff model, as long as the concentration of the tracer analog is small, $R_p = (V_{\max}^*/K_m^*)/(V_{\max}/K_m)$ and is independent of glucose concentration.

Clinical Implications

Quantitative measurements form the basis for any investigation on the regulation of glucose uptake in the heart. Although numerous studies reported rates of myocardial glucose uptake from FDG uptake data (5,39), these rates may be inaccurate because of the use of a fixed LC in the calculations. The mathematical model used in this study suggests a way to quantitate glucose uptake *in vivo* from the FDG uptake data alone. However, two hurdles need to be overcome to master this task successfully. First, the constancy of R_p and R_t must be shown. This aspect has been addressed in this study. Second, R_p and R_t values must be

obtained for human heart. The second condition poses a greater challenge because the experimental approach from this study cannot be used in human heart. The values would have to be adapted from studies in animals. Although extrapolating values for R_p and R_t from animal studies may introduce an error, this error would be of systematic nature, in the same order of magnitude for every study, not influenced by the metabolic environment and rather small. Assessment of MGU in the human heart involves additional factors beyond the demonstration of a simple relationship between FDG kinetics and the LC, for example, partial volume correction, spill-over effects and assessment of K^* and K^\ddagger in small regions of interest. Preliminary clinical data show that these challenges are also manageable (9). Thus, the reliable quantitation of myocardial glucose uptake by FDG in human heart should be possible.

At this point, we cannot be certain whether our new model is directly applicable to diseased heart muscle. One study investigated FDG uptake in patients with chronically ischemic heart disease and dysfunctional myocardium by PET (39). The investigators demonstrated higher values of sensitivity and specificity for the prediction of recovery after revascularization by qualitative compared to quantitative analysis of FDG uptake using a fixed LC. Changes in the LC may be responsible for the demonstrated inferiority of quantitative analysis of FDG uptake for the assessment of viability compared to qualitative analysis. It is conceivable that conditions prevail during a clinical PET study in which the LC is very low (high insulin, high levels of competing substrates, reperfusion) (40), which will result in a profound underestimation of true glucose uptake. More important, FDG uptake may be below an “uptake threshold” for FDG, which has been used as a cutoff value for the diagnosis of viability (39).

CONCLUSION

Under controlled *in vitro* conditions, we have demonstrated that the variability of the LC can be predicted from its physiological dependence on membrane transport rates and hexokinase activity. Individual values of the LC can be calculated from the time course of the FDG uptake curves because incorporation of the quantitative relations between FDG and glucose, expressed by the Michaelis-Menten equation, allows unmeasurable parameters of glucose uptake to be substituted by measurable parameters of FDG uptake. We propose that, in the case of the heart, the term “lumped constant” may be a misnomer and should be replaced by the term “quotient” because the correction factor is variable.

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