Kinetic Differences and Similarities Among 3 Tracers of Myocardial Glucose Uptake

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Two glucose tracer analogs, uniformly labeled [^{14}C]2-deoxyglucose ([U-^{14}C]2DG) and FDG, are widely used to assess myocardial glucose uptake. Despite the similar electron configuration of the fluorine and hydrogen atoms, uptake of the 2 tracer analogs may not be the same because of their different electronegativity. **Methods:** To test this hypothesis, we determined glucose uptake in isolated rat hearts simultaneously from the accumulation of [U-^{14}C]2DG radioactivity in the tissue, by continuous monitoring of FDG accumulation with a pair of coincidence detectors and by cumulative release of ^{3}HOH from [2-^{3}H]glucose. A first group of hearts was perfused at physiologic workload with Krebs-Henseleit buffer containing 10 mmol/L glucose; a second group, with the buffer containing 5 mmol/L glucose plus 0.4 mmol/L oleate and 1 mU/mL insulin. Third and fourth groups were subjected to ischemia (i.e., a 75% reduction in coronary flow) and reperfused. For the third group, the buffer contained 5 mmol/L glucose; for the fourth, 5 mmol/L glucose plus 0.4 mmol/L oleate. **Results:** No difference in the total amount of tracer accumulation in any group was seen between the 2 tracer analogs. The ratio [±SD] of [U-^{14}C]2DG to FDG ranged from 0.93 ± 0.09 to 1.31 ± 0.11. However, both tracer analogs paralleled glucose uptake in the absence of insulin but underestimated glucose uptake significantly in the presence of insulin. Changes in 2DG uptake with ischemia and reperfusion could be detected only with FDG. **Conclusion:** Although uptake of [U-^{14}C]2DG equals uptake of FDG quantitatively, acute changes in 2DG uptake (and, thus, in the tracer–tracee relationship) are detectable only with the fluorine-labeled tracer.

Key Words: isolated working rat heart; positron counting; autoradiography; insulin; ischemia; reperfusion; lumped constant

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U nder strictly controlled experimental conditions in vitro, the kinetics of myocardial glucose uptake with radioactive tracers are most reliably assessed using tritium-labeled glucose in the C$_2$ position. The method is based on release of the ^{3}H atom in the form of ^{3}HOH in the phosphoglucoisomerase reaction (*1*). Determination of myocardial glucose uptake with this tracer is considered the gold standard and is used mainly in isolated heart systems (*2–5*). However, [2-^{3}H]glucose is not the appropriate tracer to assess glucose uptake in vivo because this tracer is also used by other glycolytically active organ systems (e.g., red blood cells).

Two-deoxyglucose (2DG), either radioactively labeled or in conjunction with ^{31}P nuclear magnetic resonance spectroscopy, has instead been used to assess the glucose uptake of various organs with a high metabolic rate in vivo (*6–9*). 2DG is, like glucose, transported and phosphorylated but, unlike glucose, not further metabolized. Accumulation of 2DG over time is proportional to glucose uptake. Kinetic differences between glucose and 2DG uptake are measured by an experimentally obtained correction factor (lumped constant [LC]) (*10*). Uniformly labeled [^{14}C]2-deoxyglucose ([U-^{14}C]2DG) is widely used for assessment of glucose uptake in brain, heart, and skeletal muscle (*7,11–13*) by autoradiography. The fluorine-labeled 2DG, FDG, has superior properties for dynamic assessment of glucose uptake and has received widespread acceptance for PET studies of glucose uptake in the brain and heart (*8,14*). The differences between the 2 glucose tracer analogs are the replacement of the hydrogen atom in the C$_2$ position of [U-^{14}C]2DG by the positron-emitting ^{18}F and the lack of ^{14}C labeling in FDG. Despite the similar electron configuration of the fluorine and hydrogen atoms, uptake of the 2 tracer analogs may not be the same because of their different electronegativity. We therefore compared the 2 tracer analogs with the true glucose tracer, [2-^{3}H]glucose, in isolated working rat hearts. [2-^{3}H]glucose was used to determine LC. We found that [U-^{14}C]2DG quantitatively equaled FDG uptake and that changes in LC affected both tracer analogs alike. However, dynamic changes in 2DG uptake and LC were detectable only with FDG as the tracer analog.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (275–300 g) were obtained from Harlan (Indianapolis, IN). Animals had free access to food and water until the beginning of the experiments. The Animal Welfare Committee of the University of Texas-Houston Health Science Center approved the use of the animals and the experimental protocol.

Materials

Chemicals were obtained from Fisher Scientific (Lexington, MA) or Sigma Chemical Co. (St. Louis, MO). Enzymes and

cofactors for metabolite assays were obtained from Boehringer Mannheim (Indianapolis, IN) or Sigma. Regular human insulin (Humulin R) was obtained from Eli Lilly and Co. (Indianapolis, IN).

Radioisotopes

The positron-emitting glucose tracer analog, FDG (specific activity > 185,000 GBq/mmol), was prepared by the method of Hamacher et al. (15) at the University of Texas-Houston Health Science Center cyclotron facility. High-performance liquid chromatography–purified [2-³H]glucose and [U-¹⁴C]2DG were obtained from Amersham Corp. (Arlington Heights, IL). The purity of the ³H-labeled tracers was ascertained by measuring the intrinsic ³HOH content.

Working Heart Preparation

The preparation procedure has been described in detail (16). Working hearts were perfused at 37°C with 200 mL recirculating Krebs-Henseleit buffer containing 5 mmol/L or 10 mmol/L glucose or 5 mmol/L glucose plus 0.4 mmol/L sodium oleate (Intergen Co., Purchase, NY) bound to 1% bovine serum albumin (Cohn fraction V), which was free of fatty acids and equilibrated with a mixture of 95% O_2 and 5% CO_2. These substrate concentrations were chosen so as to evaluate the 3 tracers under conditions most relevant for practical use. The total perfusate [Ca^{2+}] was 2.5 mmol/L. The experiments were performed with a preload of 15 cm H_2O and an afterload of 100 cm H_2O. In the experiments involving ischemia, the afterload varied between 100 cm H_2O and 35 cm H_2O, in accord with the perfusion protocol. The hearts were beating spontaneously at an average rate of 300 beats per minute. Aortic flow and coronary flow were measured every 5 min. Heart rate and systolic and diastolic aortic pressures were measured continuously with a 3-F transducer (Millar Instruments, Houston, TX) and a MacLab physiologic recording system (AD Instruments, Milford, MA). Cardiac performance was expressed as cardiac power (the product of cardiac output and mean aortic pressure) in milliwatts as described earlier (2). The perfusion chamber was placed between a pair of coincidence detectors for the detection of positron annihilation.

Perfusion Protocol

Hearts in groups 1 and 2 were perfused for 40 min. After 5 min, the radioactive tracers FDG (14.8 MBq), [2-³H]glucose (370 kBq), and [U-¹⁴C]2DG (370 kBq) were added to 200 mL recirculating perfusate. Group 1 comprised 7 hearts perfused with Krebs-Henseleit buffer containing 10 mmol/L glucose as a substrate. In group 2 (5 hearts), the perfusate contained 5 mmol/L glucose, 0.4 mmol/L oleate, and 1 mU/mL insulin. Hearts in groups 3 and 4 were perfused for 70 min. After 5 min, 14.8 MBq FDG, 370 kBq [U-¹⁴C]2DG, and 370 kBq [2-³H]glucose were added, as for groups 1 and 2. At 20 min, the afterload was lowered from 100 to 35 cm H_2O for 30 min. The afterload was then readjusted to 100 cm H_2O for the final 20 min of the perfusion protocol. This low-flow ischemic protocol has been described in detail (17). Group 3 comprised 4 hearts perfused with Krebs-Henseleit buffer containing 5 mmol/L glucose as the only substrate. In group 4 (6 hearts), the perfusate contained both 5 mmol/L glucose and 0.4 mmol/L oleate as substrates.

The final 5 min of all perfusions was performed with tracer-free, nonrecirculating buffer to wash out radioactivity from the extracellular space. At the end of the perfusions, all hearts were freeze clamped with aluminum tongs cooled to the temperature of liquid nitrogen.

Measurement of Radioactivity

FDG. Tissue accumulation of the positron-emitting FDG was counted on a second-to-second basis by a pair of coincidence detectors placed on opposite sides of the heart (2). Positron annihilation between the detectors was measured with a fast–slow coincidence system connected to a personal computer for data acquisition. FDG radioactivity in the perfusate was continuously measured by β counting of a portion of the arterial side of the recirculating perfusate. All counts were decay corrected to the time at which FDG was added. The system was calibrated with a heart-shaped model (bar phantom) containing a known amount of radioactivity. A calibration factor (Hz/μCi) was obtained from the decay curve of the bar phantom and used to calculate glucose uptake rates (μmol/min/g dry weight) from the slopes of the time–activity curves. The slopes of FDG uptake were obtained through linear regression analysis of the tissue time–activity curves. In groups 3 and 4, linear regression analysis was performed on the slope portions that represented the time intervals before ischemia, during ischemia, and after ischemia and on the entire curve for comparison with [U-¹⁴C]2DG uptake.

³H and ¹⁴C. Dual-label counting of these isotopes was performed on a 1900TR liquid scintillation analyzer (Packard Instruments, Meriden, CT) using the manufacturer's method of spectral index analysis. Glucose uptake was determined by the rate of ³HOH production from [2-³H]glucose (1). Release of ³HOH into the perfusate was analyzed in 5-min intervals. ³HOH was separated from [2-³H]glucose in the perfusate by anion exchange chromatography on AG 1 × 8 resin (BioRad Laboratories, Hercules, CA) (18). The amount of ³HOH in the perfusate was plotted against time, and the slopes of the desired intervals were used to calculate glucose uptake rates, which were expressed as μmol/min/g dry weight.

Uptake of [U-¹⁴C]2DG uptake was assessed from the amount of ¹⁴C radioactivity that had accumulated in the tissue during the experiments. Rates of 2DG uptake were calculated from the total activity accumulated divided by the time during which [U-¹⁴C]2DG was present and the specific activity of [U-¹⁴C]2DG/mol glucose in the perfusate.

Calculation of LCs for 2DG and FDG

LC was calculated for both 2DG and FDG by dividing the rate of [U-¹⁴C]2DG uptake by the rate of glucose uptake as determined from release of ³HOH from [2-³H]glucose (LC_{2DG}) and by dividing the rate of FDG uptake by the rate of glucose uptake (LC_{FDG}). For protocols 3 and 4, the LC_{FDG} was also determined before ischemia, during ischemia, and during reperfusion.

Tissue Analysis

The frozen tissue was ground under liquid nitrogen. A portion of the pulverized tissue (100 mg) was dissolved in 1 mL Solvable (Packard) and counted for its ¹⁴C radioactivity after the addition of 10 mL scintillation cocktail (Packard). Another portion was weighed and then dried in an oven to a constant weight to determine the wet-to-dry weight ratio.

Perfusate Samples

Samples of the perfusate (1 mL) were withdrawn every 5 min and stored on ice until assayed for glucose and lactate with a glucose–lactate analyzer (2300 STAT; YSI Inc., Yellow Springs,

OH). The samples were analyzed for the specific activity of [2-³H]glucose, [U-¹⁴C]2DG, and FDG and for the ³HOH content.

Statistical Analysis

All data are presented as mean ± SD (SEM). Statistical comparison was by 1-way repeated-measures ANOVA with posthoc comparison by the Newman-Keuls test (*19*). Differences were considered statistically significant when P was 0.05. NS indicates no significant difference.

RESULTS

Table 1 shows rates of myocardial glucose uptake as measured by [2-³H]glucose, [U-¹⁴C]2DG, and FDG for all 4 groups. The values for groups 3 and 4 represent composite rates reflecting the average rate of glucose or 2DG uptake before, during, and after ischemia. Rates of glucose uptake for groups 1, 3, and 4 were the same for all 3 tracers. In the presence of insulin (group 2), both 2DG and FDG underestimated glucose uptake. With 2DG, glucose uptake was underestimated by 54%, with FDG, by 46%.

Table 2 shows LC$_{2DG}$ and LC$_{FDG}$, as well as the ratio of 2DG over FDG uptake. LCs in groups 1, 3, and 4 were not significantly different from one another and were near the value of 1. Significantly lower LCs for both tracer analogs represent underestimation of glucose uptake by the tracer analogs in the presence of insulin (group 2). The ratios of 2DG over FDG uptake ranged from 0.93 ± 0.09 to 1.31 ± 0.11 and were not significantly different among the 4 groups.

Figure 1 shows myocardial FDG uptake as a function of 2DG uptake. A strong correlation is seen between the glucose uptake rates measured with the 2 tracer analogs. The correlation coefficient was 0.9174 (P < 0.001), and the slope of the graph was close to unity, with a value of 0.956.

Because the composite 2DG and FDG uptake equaled the composite myocardial glucose uptake in groups 3 and 4, in which steady-state conditions were changed by the initiation of ischemia and reperfusion, we investigated whether these changes may have affected the dynamics of glucose and 2DG uptake differentially. We determined the relationship between FDG and glucose uptake (LC$_{FDG}$) in these groups before, during, and after ischemia separately.

TABLE 1
Average Myocardial Glucose Uptake for Entire Experiment as Assessed by [2-³H]glucose, 2DG, and FDG

Group no.	[2-³H]glucose (μmol/min/g dry wt)	[U-¹⁴C]2DG (μmol/min/g dry wt)	FDG (μmol/min/g dry wt)
1	4.62 ± 0.45	3.59 ± 0.70	4.34 ± 0.68
2	3.47 ± 0.51	1.52 ± 0.18*	1.86 ± 0.24†
3	4.49 ± 0.59	4.22 ± 0.49	3.81 ± 0.35
4	2.78 ± 0.34	2.24 ± 0.37	2.62 ± 0.34

*P < 0.01 vs. [2-³H]glucose.
†P < 0.05.
wt = weight.
Values are mean ± SEM for 4–7 experiments per group.

TABLE 2
Ratios of 2DG over [2-³H]Glucose Uptake (LC$_{2DG}$), FDG over [2-³H]Glucose Uptake (LC$_{FDG}$), and [U-¹⁴C]2DG over FDG Uptake (2DG/FDG) for the 4 Groups

Group no.	LC$_{2DG}$	LC$_{FDG}$	[U-¹⁴C]2DG/FDG
1	0.94 ± 0.12	0.77 ± 0.13	1.31 ± 0.11
2	0.55 ± 0.06*	0.45 ± 0.05*	1.23 ± 0.06
3	0.91 ± 0.18	1.02 ± 0.20	0.93 ± 0.09
4	0.97 ± 0.12	0.83 ± 0.14	1.28 ± 0.17

*P < 0.05 in direct comparison with group 1.
Values are mean ± SEM for 4–7 experiments per group.

Figure 2 shows the true rates of glucose and FDG uptake for groups 3 and 4 before, during, and after ischemia. FDG uptake in group 3 (Fig. 2A) decreased slightly with ischemia and reperfusion but was not significantly different from glucose uptake, which remained unchanged. FDG uptake in group 4 (Fig. 2B) was not different from glucose uptake before and during ischemia. During reperfusion, FDG uptake decreased significantly whereas tritiated water release remained unchanged. Thus, LC$_{FDG}$ decreased from 1.16 ± 0.16 to 0.10 ± 0.10 (P < 0.01), a finding that is in keeping with our earlier observations (*20*).

DISCUSSION

We investigated kinetic differences and similarities between 3 tracers of myocardial glucose uptake, [2-³H]glucose, [U-¹⁴C]2DG, and FDG. The 2 differently labeled radioactive glucose tracer analogs, [U-¹⁴C]2DG and FDG,
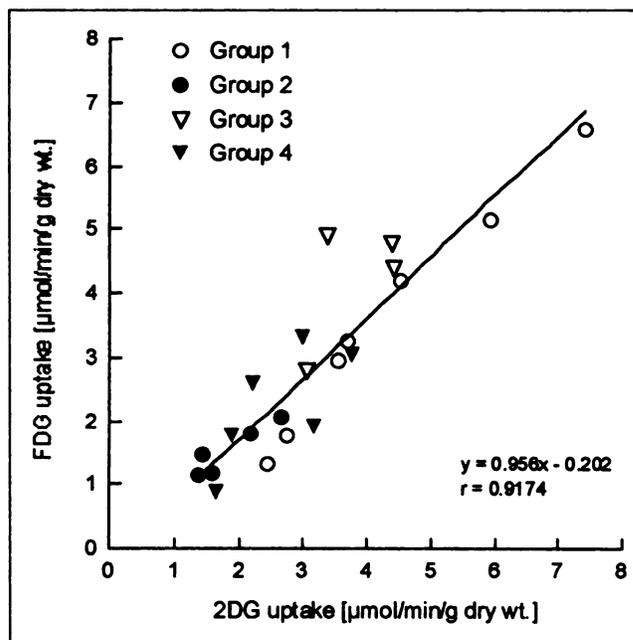

FIGURE 1. Glucose uptake as assessed by FDG (FDG uptake) as function of glucose uptake as assessed by 2DG (2DG uptake) of experiments from all 4 groups.
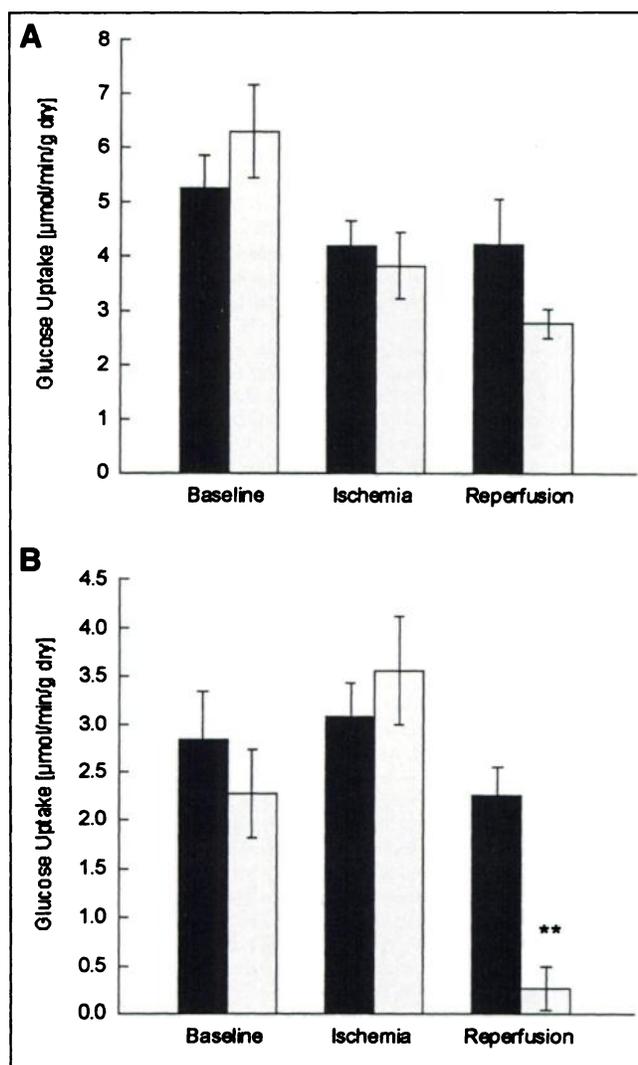
FIGURE 2. Myocardial glucose (black bars) and FDG (white bars) uptake before ischemia (baseline), during ischemia, and during reperfusion of hearts perfused with Krebs-Henseleit buffer containing glucose (5 mmol/L) as only substrate (A) and of hearts perfused with buffer containing glucose (5 mmol/L) and oleate (0.4 mmol/L) as substrates (B). Note significant underestimation of glucose uptake during reperfusion in B. Values are mean ± SEM; n = 4 (A) and 6 (B). **P < 0.01 compared with glucose uptake.

equaled each other quantitatively for the assessment of glucose uptake, but acute changes in 2DG uptake were detectable only with FDG. Because the dynamics of glucose and 2DG uptake may vary with experimental conditions, quantification of glucose uptake with the tracer analogs requires proper values for the LC for the specific experimental conditions. However, [14]C- and ^{18}F-labeled 2DG do not differ as tracers for glucose uptake under steady-state conditions.

The results of this study are of direct importance for both clinical and experimental studies using radioactively labeled 2DG. Although [U-^{14}C]2DG is used in experimental studies (7,11–13), FDG has a wide range of clinical applications

(8,14). Combined use of the 2 tracers has also been reported (21,22). A comparison of these studies is possible only if the kinetics of tracer uptake are known for both tracer analogs. Despite the similar electron configuration of fluorine and hydrogen atoms, uptake of the 2 tracer analogs may not be the same because of their different electronegativity. We show in this study that possible differences between the 2 tracer analogs are negligible in assessments of glucose uptake. Thus, studies using FDG or [U-^{14}C]2DG are directly comparable if the remaining experimental conditions are similar. This conclusion agrees with another report that also shows identical uptake of the 2 tracers (21). However, because glucose uptake with [U-^{14}C]2DG is bound to be an end-point determination, steady-state conditions must prevail while the tracer is present if true rates of glucose uptake are to be assessed.

Based on the radioactive nature of the tracers, it is obvious that FDG, with the possibility of exogenous detection of radioactivity, has better time resolution than do ^{14}C-labeled tracers. Our goal was to compare the uptake kinetics of the 2 tracer analogs side by side. We used [2-^{3}H]glucose to determine LCs. In this respect, the results in groups 3 and 4 are important because they show that even the composite uptake rates of the 2 tracer analogs, which were obtained under conditions in which the steady state was changed twice (ischemia and reperfusion), equaled each other.

LCs correct for the kinetic differences in glucose and 2DG uptake. We reported earlier that LC decreases with insulin (23,24) and with reperfusion after low-flow ischemia (20). A decrease in LC, as observed during reperfusion in this study, leads to underestimation of glucose uptake. The calculated total FDG uptake for the entire duration of the experiments in group 4 (composite rate) was not different from the composite glucose uptake. This observation seems to be at odds with the observation that FDG uptake was not different from glucose uptake before and during ischemia. This apparent discrepancy is caused by the different durations of nonischemic and ischemic perfusion. The tracers were present for 60 min. During half that time, the heart was subjected to ischemia, and FDG uptake was slightly higher than glucose uptake (NS). The significant underestimation of glucose uptake during reperfusion was based on only a 15-min monitoring period. Thus, the contribution of this period to total glucose and FDG uptake was relatively small. If the reperfusion had been longer, total FDG uptake would have decreased and probably reached statistical significance. Thus, the similarities of FDG and 2DG uptake with glucose uptake in group 4 may be a feature specific to the experimental design rather than a representative result. Our goal was not to establish the quantitative relationship between uptake of tracer analogs and uptake of a true tracer but, rather, to establish the quantitative relationship between the 2 tracer analogs, which showed the same kinetic behavior independent of the perfusion protocol and the relation to glucose.

We previously described the influence of ischemia and reperfusion on LCs (20). We also noted that the effect of

reperfusion on LCs differed when the substrate composition in the perfusate was changed. When glucose was the only substrate, glycolytic activity was higher (glucose uptake higher in group 3 than in group 4) and transport appeared to be rate limiting at all times, a factor associated with a high LC value (20). During ischemia the heart relies mainly on glucose as a substrate. If during reperfusion fatty acids are present in the perfusate, fatty acid oxidation can resume and would inhibit glycolysis. In this case, hexokinase would become rate limiting for glucose uptake, a factor associated with a low LC value (22). This case would explain the LC_{FDG} decrease in group 4 during reperfusion. In contrast, one may also conceive that decreased FDG uptake during reperfusion in group 4 was caused by release of ischemically accumulated free FDG. Although the slopes of FDG during reperfusion were stable for up to 1 h, further studies are warranted to investigate this possibility. However, this possibility has no bearing on comparison of the kinetics of 2DG and FDG uptake.

CONCLUSION

Despite differences between the molecular structure of [U-^{14}C]2DG and FDG, the tracers are quantitatively equal in myocardial uptake, i.e., under steady-state conditions the analogs can be used interchangeably for the determination of glucose uptake. In contrast to [U-^{14}C]2DG, FDG has the ability to show changes in the steady state. However, for quantification of glucose uptake, determination of the proper LC for the experimental conditions is important.

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