Effect of Hyperinsulinemia on Myocardial Fluorine-18-FDG Uptake

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This study was performed to evaluate the effect of insulin on myocardial kinetics of ^{18}F-fluorodeoxyglucose (FDG) and glucose in patients with ischemic heart disease. **Methods:** Twelve male patients (age range 54–79 yr; mean age 69 ± 8 yr) were studied during the fasting awake state. Patients with diabetes and previous myocardial infarction of the left anterior descending vascular bed were excluded from the study. Patients were injected with a 185-MBq (5-mCi) bolus of FDG during arterial and coronary sinus catheterization. Thirty minutes after FDG injection, paired basal arterial and coronary sinus blood samples were taken for the measurement of FDG and glucose uptake. Thereafter, a primed (100 mU · m^{-2} · min^{-1} for 10 min) continuous (50 mU · m^{-2} · min^{-1} infusion of insulin was administered for 60 min using the euglycemic clamp technique, and blood samples were repeated. Blood samples also were taken periodically for the measurement of arterial free fatty acids and insulin. **Results:** Euglycemic insulin infusion lowered arterial concentrations of free fatty acids, reducing myocardial extraction of free fatty acids by 85% and stimulated uptake of glucose and FDG. Myocardial glucose and FDG extraction fractions (%) increased from 1 ± 1 and 2 ± 2 at baseline to 8 ± 2 and 10 ± 3 during insulin infusion, respectively. The lumped constant value was estimated to be 1.44 ± 0.14 (r = 0.87) for the fasted state, 0.99 ± 0.07 (r = 0.74) during insulin infusion and 1.00 ± 0.05 (r = 0.92) when both groups of data were pooled together. **Conclusion:** The data obtained in this study show that FDG uptake quantitatively traces glucose uptake during physiological hyperinsulinemia in patients with ischemic heart disease.

Key Words: fluorine-18-fluorodeoxyglucose; insulin; lumped constant; tracer kinetics

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PET with ^{18}F-fluorodeoxyglucose (FDG) is widely recognized to be the gold standard for the noninvasive detection of myocardial viability. In spite of methodological and environmental metabolic problems, the assumption that FDG uptake qualitatively reflects myocardial glucose use appears to be valid in most clinical conditions (1,2). Many patients who undergo myocardial viability assessment have comorbid diseases that may be associated with insulin resistance. Accordingly, Knutti et al. (3) suggested that the use of euglycemic insulin clamp improves the image quality of myocardial studies with FDG, especially in diabetic patients. This involves simultaneous infusion of insulin and glucose, which promotes glucose uptake in insulin-sensitive tissues. This procedure, however, is invasive and would add an order of complexity and cost to a routine clinical study. Therefore, patients who are referred for a cardiac PET study with FDG in the clinic are generally given an oral glucose load to promote the secretion of endogenous insulin, which will stimulate glucose uptake in insulin-sensitive tissues.

Serum glucose is then measured as an indication of circulating insulin, and insulin is sometimes administered to ensure that serum glucose has reached a level of 120 mg% or less (4).

The basic rationale for using FDG for the assessment of myocardial glucose uptake is well established. FDG competes with glucose for facilitated transport sites (5) and for phosphorylation by hexokinase (6). However, the phosphorylation product FDG-6-phosphate, unlike glucose-6-phosphate, is not metabolized any further. Like other phosphorylated compounds, FDG-6-phosphate can leave the cytosol only by hydrolysis back to FDG. Because phosphatase activity is low in the heart (7), FDG-6-phosphate is essentially trapped inside the cytosol. A correction factor called the lumped constant (LC) is defined to equate FDG uptake to glucose uptake because FDG is a glucose analog. The LC can be estimated experimentally by the ratio of steady-state fractional extraction of FDG to that of glucose. Although FDG can compete specifically with glucose, its affinities for transporter molecules and for hexokinase are different from those of glucose. Thus, the LC value can be altered with changes in the relative control strengths of the transport and phosphorylation steps. The LC value has been shown to change with insulin in the rat heart (8–10) but also has been reported to be the same with insulin in isolated perfused rabbit septum (11) and not to vary significantly over a wide range of metabolic rates in the canine heart (12). The effect of insulin on the LC value therefore remains inconclusive. Moreover, the effect of insulin on FDG uptake relative to glucose has not been examined directly in humans. Thus, the goal of this study was to compare directly the effect of insulin on myocardial kinetics of FDG and glucose in patients with ischemic heart disease using an euglycemic insulin clamp and arterial-coronary sinus catheterization.

MATERIALS AND METHODS

Study Population

Study subjects were 12 men (age range 54–79 yr) referred for elective cardiac catheterization to evaluate chest pain. Significant atherosclerosis (defined as a >50% luminal narrowing) was found in an average of 2.3 ± 1.3 vessels, and left ventricular ejection fraction was estimated to be 49% ± 8%. Patients with diabetes and unstable angina were excluded from the study. Furthermore, no patients had wall motion abnormalities, resting perfusion defects or previous myocardial infarction of the left anterior descending vascular bed, the region principally sampled by the coronary sinus catheter. No subject had chest pain or electrocardiographic evidence of myocardial ischemia in the 12 hr before the study or during the study protocol. The protocol for this study was approved by the Human Studies Subcommittee of the VA Connecticut Healthcare Systems (West Haven, CT), and informed consent was obtained in writing from each patient.
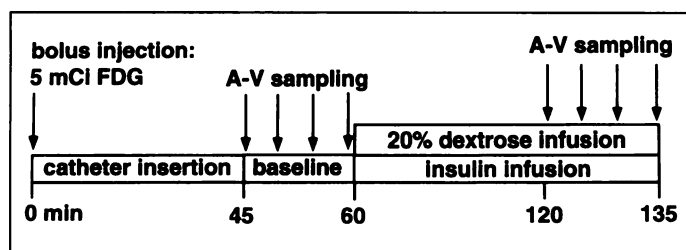
FIGURE 1. A schematic representation of the experimental protocol for studies with a bolus injection of 5 mCi (185 MBq) ^{18}F-FDG. FDG was administered at time 0. Four pairs of arteriovenous (A-V) blood samples were taken every 5 min at 45 min postinjection during the baseline. At 60 min, the euglycemic hyperinsulinemic clamp was initiated by the simultaneous infusion of 20% dextrose and insulin for an hour. Four more pairs of arteriovenous blood samples were then taken every 5 min to complete the protocol.

Radioisotope

FDG (specific activity >185 TBq [5000 Ci]/mmol), a glucose analog labeled with the positron-emitting radionuclide ^{18}F, was prepared in an 11 Mev negative ion medical cyclotron (CTI, Knoxville, TN). The chemical synthesis developed by Hamacher et al. (*13*) was carried out in an automated central processing control unit as described by Padgett et al. (*14*). The radiochemical purity was determined to be 98.6% ± 2.2% by high-performance liquid chromatography using a radial compression NH_2 cartridge (Waters, Milford, MA). The mobile phase was 10% water in acetonitrile and was run at 4.0 ml/min at room temperature.

Experimental Protocol

The protocol for these experiments is depicted in Figure 1. All studies were performed after a 12- to 16-hr fast. One hundred eighty-five MBq (5 mCi) FDG was injected into the patient over 10 sec at time 0 before arterial and coronary sinus catheterization. During the period between 0 and 45 min, a six-French introducer sheath (C.R. Bard, Inc., Billerica, MD) was inserted into the right femoral artery for blood sampling. Through an eight-French introducer sheath in the right internal jugular vein, a six-French sampling catheter was advanced under fluoroscopy into the coronary sinus. This catheter was placed at the junction of the coronary sinus and great cardiac vein, sufficiently proximal in the coronary sinus to avoid admixture of right atrial blood during sampling. Four arterial-venous paired samples were taken at 5-min intervals from 45 to 60 min during the baseline period. The 45-min starting time for sampling was chosen because the PET scans normally began 45 min after a bolus of FDG was injected into the patient to allow blood pool radioactivity to clear to a low level and to have tissue ^{18}F radioactivity predominantly in the form of FDG-6-phosphate, which is essentially trapped in tissue. These two factors resulted in better image contrast. Our objective was to follow a standard clinical PET protocol, and thus results obtained in this study could be related to clinical PET imaging. In addition, Sokoloff et al. (*15*) showed in their rat brain studies with [^{14}C]deoxyglucose that cerebral extraction fraction can be greatly overestimated in the first 10 min postinfusion of the tracer due to the initial equilibration of [^{14}C]deoxyglucose in tissue. Therefore, the extraction fraction measurement in this study did not initiate until 45 min postinjection to minimize potential measurement errors. Plasma FDG radioactivity varies less with time after 45 min of injection, providing a situation that is closer to the constant infusion paradigm. At 60 min, a primed (100 mU · m^{-2} · min^{-1} for 10 min), continuous (50 mU · m^{-2} · min^{-1}) infusion of regular insulin (Humulin, Eli Lilly, Indianapolis, IN) was initiated. The euglycemic hyperinsulinemic clamp was applied as described previously (*16*). During insulin infusion, whole blood glucose concentration was maintained at slightly above the fasting level with a variable rate infusion of 20% dextrose (the euglycemic clamp technique). Four

TABLE 1
Arterial Concentration of Plasma Substrates and Insulin During Baseline and Insulin Infusion

Substrate/hormone	Baseline	Insulin infusion
Glucose (mmol/liter)	4.6 ± 0.4	4.8 ± 0.3
Free fatty acids (mmol/liter)	0.86 ± 0.12	0.28 ± 0.03*
Insulin (nmol/liter)	0.091 ± 0.049	1.86 ± 0.35*

* p < 0.05 versus baseline.
Values are mean ± s.d.

more arterial-venous paired samples were taken at 5-min intervals after 60 min of insulin infusion.

Measurement of Substrate Concentrations, Insulin and FDG Radioactivity

Whole blood glucose was measured immediately with an automated glucose analyzer (Yellow Springs Instruments Corp., Yellow Springs, OH), which uses the glucose oxidase method. Whole blood glucose measurements were used to adjust the glucose infusion rate during the euglycemic hyperinsulinemia protocol. Plasma glucose, which was used to correlate with plasma FDG radioactivity, was determined enzymatically using a spectrophotometer (Milton Roy Co., Rochester, NY) (*17*). Plasma insulin was measured with a radioimmunoassay, and free fatty acids were measured fluorometrically (*18*). FDG radioactivity was determined by counting 3 ml of plasma samples in an automated well-counter (Packard, Meridien, CT). Counts were decay corrected to the beginning of the counting time and adjusted by the sample weight.

Data Analysis

The percentage of glucose and free fatty acids extraction fractions were calculated by dividing the arterial-coronary sinus concentration difference by the arterial concentration. Radioactivity counts with decay correction were used instead of concentration for the calculation of the percentage of FDG extraction fraction. All four calculated FDG extraction fractions were then averaged for each time period, basal and hyperinsulinemia. Because whole blood glucose levels were maintained at steady state with the clamp technique, results obtained from all four arterial and coronary sinus plasma glucose samples were averaged, respectively, for each time period to increase the measurement accuracy. The coefficient of variation was found to be less than 3%. Glucose concentration also was measured in whole blood and did not differ significantly from that measured in plasma. The LC values were estimated by the slope obtained from correlating FDG extraction fractions with glucose extraction fractions using linear regression. Because FDG competes specifically with glucose for the same transporter and phosphorylation enzyme, FDG will not be extracted into the heart if glucose uptake reduces to zero. Thus, the regression was constrained to have a zero intercept. Because of the small extraction fractions of FDG and glucose at baseline, the LC also was estimated for the pooled data obtained from both baseline and insulin infusion.

Data are presented as mean ± s.d. A two-tailed t-test was used to determine the statistical significance between individual values obtained during baseline and euglycemic hyperinsulinemia. Significance was set at the 0.05 level.

RESULTS

Glucose, Free Fatty Acids and Insulin Concentrations

Arterial concentrations of glucose, free fatty acids and insulin in these patients after an overnight fast during baseline and euglycemic hyperinsulinemia are summarized in Table 1. Fasting arterial glucose concentrations remained fairly constant
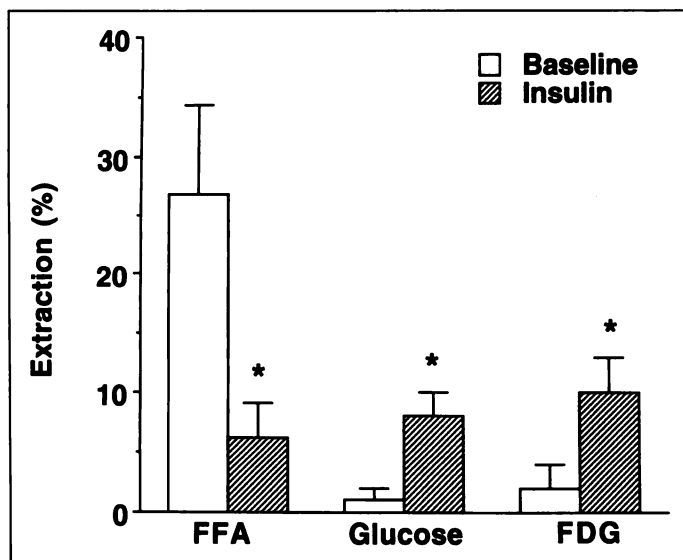
FIGURE 2. Percentage of myocardial extraction of free fatty acids, glucose and ^{18}F-FDG at baseline and during infusion of insulin and 20% dextrose. *Significantly different from baseline values, $p < 0.05$.

FIGURE 3. Plot of normalized counts for arterial plasma ^{18}F-FDG radioactivity as a function of time. All samples were decay corrected and normalized to the first sampling point, which was at 45 min. Each error bar represents the standard deviation of the corresponding time.

throughout the entire study, and arterial insulin concentration increased 20-fold, indicating that the euglycemic hyperinsulinemic clamp was successful. Fasting plasma free fatty acids concentrations decreased by a factor of three after an hour of insulin and glucose infusion. These results are similar to those obtained from previous experiments using the same clamp technique (16).

Myocardial Extraction of Free Fatty Acids, Glucose and FDG

As shown in Figure 2, in the basal fasting state, the heart extracts a large percentage of free fatty acids and a small percentage of glucose and FDG from arterial blood. Both glucose and FDG extractions increased markedly and proportionately after insulin administration. By contrast, myocardial extraction of free fatty acids declined by 77%. This degree of decline was similar to what was observed previously using the same euglycemic hyperinsulinemic clamp technique (16). The data in Table 1 and Figure 2 confirm that myocardial free fatty acids extraction in patients is directly proportional and myocardial glucose extraction inversely proportional to arterial free fatty acids concentration.

Relationship Between Myocardial Extractions of FDG and Glucose

Arterial plasma FDG counts normalized to the first sample were plotted against time in Figure 3. Each point represents the means, with the error bars representing the standard deviation. The error bars include the variation in the injected dose and the time delay between sampling and counting. The 3-ml plasma samples contained about 50,000 counts/min at baseline and about 12,000 counts/min during insulin infusion. Insulin infusion caused arterial plasma FDG counts to decrease at a rate of 1%/min, presumably by stimulating uptake of FDG into insulin-sensitive tissues.

The LC as defined by Sokoloff et al. (15) is determined by the ratio between steady-state extraction fractions of deoxyglucose and glucose. Note that the glucose extraction fraction is expressed in the denominator for the LC, but some literature findings have been reported with the glucose extraction fraction in the numerator instead. This study was not performed at steady-state plasma FDG radioactivity because its primary purpose was to compare the myocardial extractions of FDG and
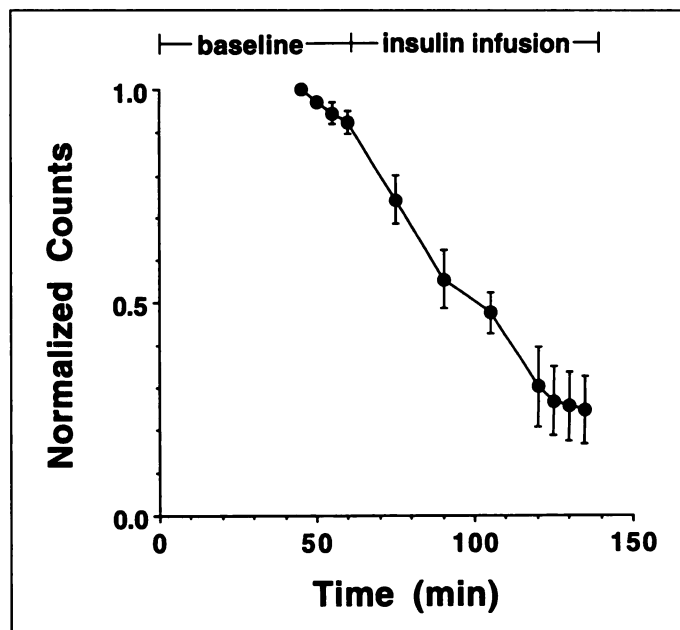
glucose in insulinized patients in the clinical setting. However, plasma samples were obtained at times sufficiently distant from the FDG bolus (basal 45–60 min; insulin infusion 120–135 min) that arterial FDG counts varied only modestly during the sampling intervals (Fig. 3), whereas the euglycemic insulin clamp procedure ensured that plasma glucose concentration was indeed constant. Thus, the ratios of baseline and insulin-infused FDG/glucose myocardial extraction fractions should closely approximate LC values in these patients. As shown in Figure 4, FDG extraction highly correlated with glucose extraction in these patients and the slope of the correlation (1.00 ± 0.05, $r = 0.92$) is an approximation of the FDG/glucose LC value for humans. Data scattered about the regression line in Figure 4
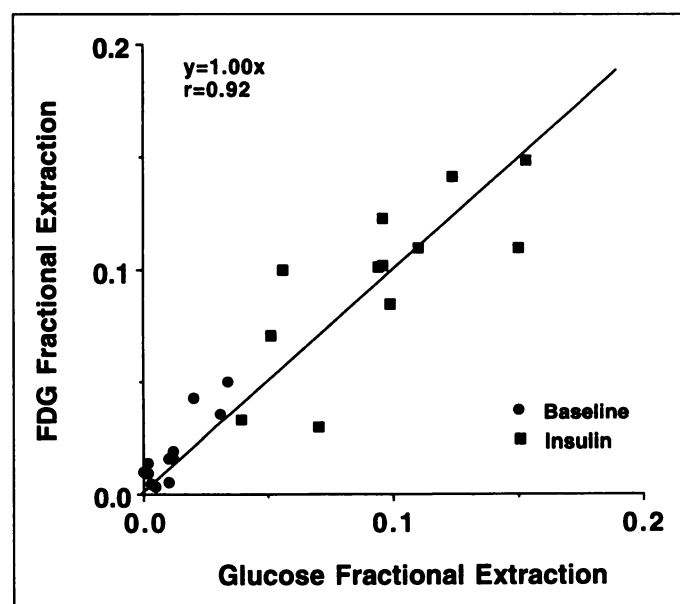

FIGURE 4. Plot of relation between ^{18}F-FDG fractional extraction and glucose fractional extraction during baseline and infusion of insulin and 20% dextrose. Each square or circle represents an average of all four measurements taken during baseline or insulin-glucose infusion for an individual patient. Some data are overlapped for baseline values.

probably reflects the fact that plasma FDG counts and glucose concentrations were measured using completely different analytical techniques and that the determination of fractional extractions using arteriovenous substraction would undoubtedly magnify any analytical differences. Our data were not sufficient to determine to what extent this scatter was measurement dependent or physiological.

DISCUSSION

This study was undertaken to determine whether uptake of the glucose tracer analog FDG into the heart, relative to glucose uptake, would be altered by the administration of insulin in patients with ischemic heart disease. We found that there was no significant disparity between extraction of glucose and that of FDG by the heart during maximal physiological stimulation by insulin.

Cunningham and Cremer (19) suggested that the theoretical value for the LC for FDG should be 0.89 because the affinity of FDG for both the transporter and hexokinase are higher than those of ^{14}C-labeled 2-deoxyglucose in the rat brain (5) and the LC for ^{14}C-labeled 2-deoxyglucose was found to be 0.48. The estimation of the LC value in this study (1.00 ± 0.05, r = 0.92) was in good agreement with their speculation. When the basal and insulin-clamped data were processed separately, the slope was 1.44 ± 0.14 (r = 0.87) for the fasted state and 0.99 ± 0.07 (r = 0.74) during insulin infusion. Because the glucose and FDG extraction fractions at baseline were less than 3%, the correlation would naturally be less accurate because of analytical imprecision. In addition, the correlation was better for the combined data. Therefore, the LC value probably approached unity for the fasted state, and the administration of insulin did not alter the LC value significantly. In other words, myocardial extraction of FDG follows that of glucose closely across the entire physiological insulin range in humans. Note that only the results obtained during insulin infusion are relevant to the clinical setting, where a glucose load is generally used to stimulate the secretion of insulin, which in turn stimulates myocardial glucose uptake. Using an isolated perfused working rat heart model, Ng et al. (8) demonstrated that the LC value was reduced from 0.94 ± 0.06 during perfusion with 5 mM glucose to only 0.33 ± 0.03 with the addition of 10 mU/ml insulin. Using the same model, Russell et al. (10) reported that the addition of insulin caused the LC value to fall from 1.96 ± 0.31 to 0.80 ± 0.30. Hariharan et al. (9), from the same laboratory, later reported that the addition of insulin into the perfusate changed the LC value from 0.57 ± 0.07 to 0.35 ± 0.08. Krivokapich et al. (11), however, did not find the LC value to be significantly different from 0.60 when insulin was added in the isolated perfused rabbit septum. The hearts in the working model are usually perfused at a rate of 15 ml/min, whereas the isolated septa are perfused at a rate of 1.5 ml/min, which is more compatible with myocardial blood flow in humans. In addition, these isolated hearts were perfused with glucose as the only substrate. The changes in the LC values observed in the isolated working heart model were probably due to a higher demand on glucose use, which caused both the transport and phosphorylation steps to operate near the saturation levels. The human heart, on the other hand, is supplied with multiple substrates as alternative fuels, and, consequently, myocardial glucose use is not easily manipulated to operate near the maximal capacity. Therefore, the data obtained in this study suggest that the relative control strengths of the transport and phosphorylation steps are less relevant in humans than in an isolated perfused rat heart.

The mean LC value of 0.66 was obtained by Ratib et al. (12)

for the canine myocardium using the arterial-coronary sinus sampling technique. The dietary state of the dogs involved in these studies was not controlled to achieve a wide range of myocardial glucose metabolic rates; thus, the dogs were probably not studied in the fasted state. The LC value for the brain using labeled deoxyglucose was reported to be 0.483 in the rat (15) and 0.344 in the monkey (20). Therefore, the reason for the difference between the LC value of 1.0 obtained in this study and the one derived in canine myocardium may be attributable to species differences. Another possible explanation for the difference in the LC value is that the canine experiments were not carried out in either the fasted state or the hyperinsulinemic state.

Bøtker et al. (21), examining myocardial FDG uptake indirectly using PET imaging and kinetic modeling in healthy volunteers, estimated that LC varied from 0.36 during somatostatin infusion to 1.04 during hyperinsulinemic euglycemic clamp. They further estimated that LC increased linearly within the physiological insulin range (70–525 pmol/ml) and did not increase further at supraphysiological insulin levels. The mean plasma insulin level for this study was 1860 pmol/ml, which is slightly above the upper physiological range. Note that their LC values were calculated using a constrained method developed by Kuwabara et al. (22) for the estimation of cerebral metabolic rate of glucose using FDG. This method assumes a fixed value of transport and phosphorylation ratios for FDG and glucose for all conditions. In addition, this method requires the estimation of two parameters, the unidirectional transfer rate constant and the phosphorylation rate constant of FDG, by fitting the time-activity curve with a compartmental model. The process of parameter estimation will undoubtedly introduce more errors to the estimated LC values. Therefore, the accuracy of the LC values estimated by the constrained method for the heart requires validation with direct measurements. Our results obtained using direct arterial–venous balance measurements from patients with ischemic heart disease in this study support their findings that LC should be close to 1.0 at upper range physiological insulin levels. Nonetheless, our results did not show a significant difference in the LC between the basal state and the insulin state.

Greenberg et al. (23) found that the LC increased in ischemia as well as in postischemic tissue in the brain in a model of focal cerebral ischemia in the cat. Using an extracorporeally perfused, intact, working pig heart model, Liedtke et al. (24) reported that labeled deoxyglucose appears to be an inaccurate marker of glycolytic flux in reperfusion after exposures to mild-to-moderate regional ischemia. Therefore, it is unknown whether the same LC value can be used for both normal and ischemic myocardium.

In a ^{31}P nuclear magnetic resonance study, Hoerter et al. (25) found that insulin increased the rate of degradation of 2-deoxy-glucose-6-phosphate in the Langendorff-perfused rat heart. If this were the case in the human heart, some of the ^{18}F activity in the coronary sinus in the insulin-infused state could have included FDG dephosphorylated from FDG-6-phosphate. Myocardial extraction fractions of FDG would have been underestimated. However, using the isolated perfused rabbit septum, Krivokapich et al. (11) found that the dephosphorylation rate constant of FDG-6-phosphate was unaltered in the presence of 5 mU/ml and 25 mU/ml insulin. Furthermore, Ng et al. (8) reported that ^{18}F radioactivity accumulated linearly for at least 40 min in rat hearts perfused with various glucose concentrations and the presence or absence of 10 mU/ml insulin, indicating that glucose-6-phosphatase activity in the heart is minimal and insulin does not increase the degradation rate of

FDG-6-phosphate. This is consistent with the general belief that glucose-6-phosphatase activity is low in cardiac tissue (7). Furthermore, high phosphatase activity in itself would not invalidate the use of FDG as a tracer for measuring glucose transport and phosphorylation rates as the LC can be determined empirically because the LC incorporates the extent of phosphatase activity against glucose-6-phosphate.

In this study, we measured myocardial fractional extractions of glucose and FDG at baseline and during hyperinsulinemia. Clinical imaging protocols, however, measure myocardial uptake of FDG, which is the product of FDG extraction, arterial concentration and myocardial blood flow. Thus, changes in myocardial blood flow during insulin infusion will affect FDG uptake independent of changes in FDG extraction. We have previously shown (16) that hyperinsulinemia of the degree used in this study increases myocardial blood flow by 10%. Although myocardial blood flow was not measured in this study, any change would have affected myocardial FDG and glucose uptake equally and therefore would not have affected the estimation of the LC values. In addition, Sokoloff et al. (15) reported that increase in cerebral blood flow did not change the LC in their rat brain studies with [^{14}C]deoxyglucose.

Study Limitations

Note that myocardial glucose extraction in the fasted state being so small is difficult to be measured accurately with our method. Our results, however, are consistent with those of Ferrannini et al. (26), who performed metabolic studies routinely using the same method. Therefore, our results are credible, although accurate measurements are inherently difficult to obtain with our method. Also note that LC in the fasted state is not clinically relevant because clinical PET studies with FDG for the heart are routinely acquired in the insulin-stimulated state. Thus, it is more important to determine the LC under insulin stimulation, which our data clearly show is about 1.0.

To determine the LC value accurately, both plasma FDG and glucose must be kept at steady state during the study. Plasma glucose levels in this study were maintained at steady state using the euglycemic clamp technique. Plasma FDG, however, could not reach steady state because it was administered as a bolus to simulate the clinical situation; therefore, the concentrations of free FDG and its rates of phosphorylation in the tissues never reached a steady state. Therefore, the LC values determined in this study are an approximation. The primary objective of this study was to demonstrate the ability of FDG to track glucose uptake quantitatively in the heart across the physiological range of insulin concentrations encountered during clinical imaging in patients with ischemic heart disease. Because the administration of insulin enhances image quality for myocardial studies with FDG, our finding that the LC value approaches unity during physiological hyperinsulinemia would imply that images obtained with FDG may be translated into quantitative glucose uptake measurements.

CONCLUSION

Many studies using FDG in the heart assume a value of 0.66 for the LC. This value was obtained from studies in control animals, and it is not known whether it is applicable to the normal or ischemic human myocardium. The data obtained in this study, however, show that FDG uptake quantitatively traces glucose uptake during physiological hyperinsulinemia in patients with ischemic heart disease. Therefore, a LC value of 1.0 should be applied to the normal myocardial sectors for patients with ischemic heart disease in clinical FDG studies using the glucose load approach or the euglycemic insulin clamp tech-

nique. Additional studies, however, are necessary to determine the specific LC value for ischemic myocardium in humans.

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