Comparison of Fluorine-18-Fluorodeoxyglucose and Tritiated Fluoromisonidazole Uptake during Low-Flow Ischemia

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Fluorine-18-fluoromisonidazole (FMISO) is trapped in hypoxic but viable canine myocardium. Because of the potential for its use as a marker of myocardial viability, we compared FMISO activity to [^{18}F]fluorodeoxyglucose (FDG) activity in the same myocardial samples from eight dogs subjected to 3 hr of moderate regional myocardial ischemia. **Methods:** Tritiated FMISO was injected 15–30 min after onset of regional ischemia (40%–70% reduction in systolic wall thickening) which was maintained for 3 hr. FDG was injected after 2 hr of ischemia. Myocardial blood flow (MBF) was measured by the radiolabeled microsphere technique at the time of each radiotracer injection. At 3 hr of ischemia, the heart was excised and cut into short-axis slices. One slice encompassing both ischemic and normal tissue was cut into 64 samples. FMISO and FDG activity in each sample were normalized to the mean normal zone activity and further expressed as a function of regional MBF. **Results:** FMISO uptake was consistently greater than FDG uptake, although this was significantly different only for MBF, between 40%–60% of normal. When analyzed relative to endocardial-epicardial location, endocardial FMISO uptake was significantly greater in all hypoperfused samples. **Conclusion:** These results suggest that FMISO is as sensitive as FDG for detecting myocardial ischemia and could be used for identification of viable myocardium.

Key Words: myocardial viability; fluorine-18-fluorodeoxyglucose; myocardial blood flow; fluorine-18-fluoromisonidazole

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Several studies indicate that chronic left ventricular wall motion abnormalities may be reversed by coronary revascularization (*1–3*). Recent data (*4*) suggest that 30%–50% of dysfunctional myocardial segments are potentially viable. From these observations, the concept of persistent ischemia as a basis for chronic regional dysfunction has emerged (*5,6*). This concept has been termed hibernating myocardium and has prompted the search for noninvasive methods for detecting chronically hypoperfused but viable myocardium. The underlying metabolic abnormalities responsible for hibernating myocardium are unknown. There has not been direct evidence that human myocardium can remain in a hypoxic state chronically. Furthermore, the concept of hibernating myocardium has been questioned recently (*7*). A method for direct in vivo identification of hypoxic myocardium would enable us to address this issue.

Strategies for detecting viable myocardium based on the reversibility of stress perfusion defects using ^{201}Tl (*8*) or increased [^{18}F]FDG uptake relative to myocardial blood flow (FDG/flow mismatch) (*3,4*) have shown promise but are not entirely satisfactory. Both methods have positive predictive values of 75%–85% with similar negative predictive values (*8*). Tamaki and et al. (*4*) found that 20% of segments preoperatively predicted to be scar by PET FDG/flow matching demonstrated improved function postoperatively. Furthermore, of the hypoperfused segments that demonstrated FDG uptake preoperatively, 24% continued to have FDG uptake postoperatively in spite of patent saphenous vein grafts. Technical considerations in relating ventricular function studies to PET images might account for some of the discrepancies. It is also possible, however, that the FDG method is not sensitive to the level of myocardial ischemia/hypoxia present in these cases, or that the methods of analysis used were suboptimal. Neither Tillisch et al. (*3*) nor Tamaki (*4*) quantitated FDG uptake or flow. Furthermore, the FDG method is sensitive to plasma glucose levels. Prellwitz et al. (*9*) suggested that diabetic patients are more likely to have a suboptimal study than nondiabetic patients, unless careful attention is paid to the patients' metabolic state at the time of the PET study.

We, and others, have demonstrated that the 2-nitroimidazole, fluoromisonidazole (FMISO), is selectively trapped in hypoxic myocardium (*10–14*). Following intravenous injection, FMISO is initially distributed throughout the total body water space, with subsequent clearance from the normal myocardium directly as a function of plasma clearance (*10*). Because its retention is dependent on active electron transport enzymes, it is not trapped in necrotic tissue (*14*). When labeled with the positron emitter, ^{18}F, the trapped FMISO can be imaged with PET and

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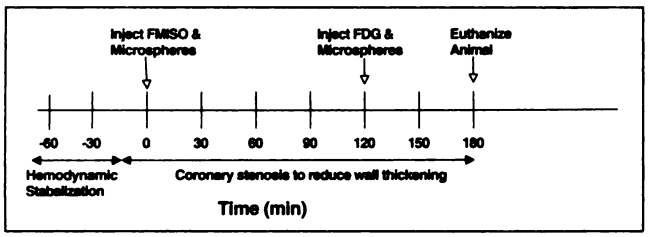

FIGURE 1. Graphical representation of the study protocol.

its uptake occurs in myocardial regions with reversible dysfunction (*12,13*).

Because the uptake and retention mechanisms of FMISO and FDG differ, we postulated that important differences might exist in the relative sensitivities of the two agents for the detection of myocardial ischemia. The purpose of this study was to compare the trapping of FMISO to FDG uptake in an animal model of moderate ischemia not associated with infarction. This model demonstrates immediately reversible regional dysfunction following reperfusion (*12*).

METHODS

Animal Surgery and Instrumentation

After a 12-hr fast, nine mongrel dogs (20–35 kg) were initially anesthetized with thiamyl sodium (15 mg/kg), intubated, mechanically ventilated to maintain arterial blood gases within a physiologic level and maintained on halothane as an anesthetic agent. Through a left thoracotomy, the animals were instrumented with a left atrial line and a mechanical or hydraulic occluder on the midportion of the left anterior descending coronary artery (LAD). Doppler crystals were sutured to the epicardium in the distribution of the LAD and circumflex coronaries for measurement of systolic wall thickening (*15*).

Experimental Protocol

After hemodynamic stabilization (15–30 min), wall thickening, heart rate and systolic blood pressure were recorded. To standardize the recording of ventricular function and eliminate the effect of respiratory variation, the strip chart recorder was started every 15 min by a timer to run at 100 mm/sec. As soon as the recorder had started, the respirator was stopped at end expiration and 10–12 cardiac cycles were recorded and the respirator restarted. The fifth beat after the respirator stopped was used as the starting point to determine EKG RR interval, systolic blood pressure and wall thickness. Heparin (2000–3000 units) was administered intravenously and the coronary occluder was then adjusted so as to reduce anterior wall thickening by approximately 40%–70%. The protocol is summarized in Figure 1. Plasma glucose determinations were made during the control period and at hourly intervals during the ischemic period. After stabilization of reduced wall thickening (15–30 min), 2–4 $\times$ 10^6 radiolabeled microspheres (15 μm diameter labeled with ^{113}Sn or ^{103}Ru) were injected into the left atrium, along with simultaneous withdrawal of blood from the ascending aorta, for 1 min to measure regional myocardial blood flow (*16*). This was followed by an intravenous injection of tritiated FMISO (approximately 0.01 mCi/kg animal weight) (*17*).

In seven of the nine animals, wall thickening was maintained within the 40%–70% reduction range for 2 hr by occasional adjustment of the occluder. In two animals, there was a decrease in wall thickening which seemed stable after 20 min and thus FMISO and microspheres were injected. Over the next 15–30 min, however, wall function returned to control values despite of complete occlusion of the LAD coronary artery. Presumably, this occurred as collateral vessels opened. Two hours after the initial microsphere injection, a second microsphere was injected for measurement of regional myocardial blood flow. This was followed by an injection of 2–4 mCi of [^{18}F]FDG intravenously over a 1-min period. The reduced level of wall thickening was maintained for an additional hour, at which time the animal was euthanized by a overdose of potassium chloride.

The heart was rapidly excised, rinsed free of blood and the left ventricular cavity was filled with an expandable polyurethane foam which rapidly hardened to maintain its shape. A 1.5-cm thick slice was cut perpendicular to the long-axis of the heart and encompassing the sites of both echo crystals. This slice was then cut into 16 equal radial sectors and each of these sectors was then cut into four epicardial to endocardial samples for weighing and counting. The average weight for 576 samples was 0.55 $\pm$ 0.22 g.

Data Analysis

RR interval, systolic blood pressure and systolic wall thickening were measured from the calibrated strip chart recordings. The peak of the R wave was used to define end-diastole and end-systole at the time of the aortic pressure diacrotic notch. Systolic wall thickening was calculated by determining the absolute thickening difference between end-diastole and end-systole and dividing that difference by the end-diastolic thickness. Five consecutive beats were averaged at each time point for wall thickening, heart rate and blood pressure determinations. Percent systolic wall thickening at each time point was subsequently referenced to the baseline value and expressed as a percentage of baseline.

The tissue samples were initially counted in a gamma counter with a 3-inch NaI crystal, along with standards for each of the microspheres and ^{18}F. After the ^{18}F had decayed for $\geq$10 half-lives, the samples were recounted for gamma activity from the microspheres. All counts were corrected for spillover using a matrix inversion technique (*18*). To accurately count tritium, it was necessary to separate the microspheres from the tissue samples. To accomplish this separation, tissue samples were frozen at -70°C and crushed with an aluminum mortar and stainless steel pestle chilled to -70°C. Aliquots (30–40 mg) were placed in chilled, preweighed microfuge tubes and reweighed. The samples were homogenized in distilled water at 1:2 (w/v), microfuged for 10 min and 0.5 ml of their supernatant was transferred to scintillation vials. One milliliter of Soluene 350 (Packard) was added to each vial and the vials were vortexed and allowed to stand for 30 min.

Hydrogen peroxide (0.05 ml 30%) was added to each vial and the vials swirled and allowed to stand for 24 hr. Hionic-fluor scintillation cocktail (Packard) was added to each of the vials before they were counted for gamma radioactivity (Packard Autogamma) and beta decay (Packard 1600TR) because leaching of radioisotope from the microspheres interfered in tritium counting. This microsphere spillover into the liquid scintillation spectrum was determined by back calculating the contribution of each gamma radionuclide to each of the beta regions. A set of efficiency standards was prepared for ^{113}Sn and ^{103}Ru using Soluene 350 treated blood as a quenching agent. These efficiency standards were counted in the same liquid scintillation counter and an efficiency curve was calculated for each of the gamma radionuclides in three beta windows. The quantity of each of the gamma

radionuclides in each sample was measured with the gamma counter and then the contribution of each of the gamma radionuclides to each of the beta regions was calculated using the efficiency curves described above. Those contributions were subtracted from the counts in the corresponding beta regions. The quantity of each of the beta radionuclides in each sample was calculated from the remaining beta spectrum and the beta radionuclide efficiency curves for each of those regions.

Microsphere activity was converted to myocardial blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) using the standard reference organ technique (*16*). Fluorine-18-FDG and ^{3}H-FMISO activity were expressed as counts per minute per gram and disintegrations per minute per gram, respectively, in each of the tissue samples.

To facilitate comparison between the animals, flow, FMISO and FDG values in each sample were normalized as a decimal fraction of the mean "normal myocardial" activity for each animal. Average normal zone blood flow was calculated by averaging the respective values for the two separate microsphere injections from 12 myocardial tissue samples located in the center of the anatomically normal zone. "Normal" FMISO and FDG activity was calculated by averaging the activity in each of the same 12 samples used for the normal blood flow determination. To examine the effect of endocardial or epicardial location on the uptake of the two compounds, the sample weights and activities for the two innermost and outermost samples from each sector were added together and averaged; the data were expressed as above.

In the clinical setting, absolute FDG activity may be lower in an ischemic but viable myocardial region than in a normal region in the same heart. The activity may be relatively greater, however, than the corresponding flow to that region. In the normal region, FDG and flow will be balanced. To achieve the greatest sensitivity and specificity for identification of viable myocardium in human studies, regional PET FDG activity is frequently expressed as a ratio to the FDG in a known normal region or to a normal file and then this ratio compared to the corresponding regional myocardial blood flow [either in absolute terms or normalized to a normal region or to a normal file (*19,20*)]. To express our results in a comparable manner, we divided the normalized FDG activity in each piece by its corresponding absolute flow and plotted the results as a function of the normalized (ischemic-to-normal ratio) myocardial blood flow. For comparison, the FMISO data have also been expressed in the same manner, although this could potentially introduce apparent FMISO uptake in normal regions. This artifact occurs because FMISO concentration in normal tissue at late times (more than 1 hr) is driven by the partition coefficient of FMISO, resulting in relatively low concentration and very little variation (approximately 20%) compared with the approximately fivefold heterogeneity in myocardial blood flow in normal myocardium (*21*). This would result in a fivefold range of FMISO-to-flow ratios.

Statistical Analysis

Differences between control hemodynamic and plasma glucose levels and the average for the ischemic period for each of these variables were compared using a paired t-test (two-tailed). Systolic wall thickening during ischemia as a percent of control was compared between the LAD and circumflex regions using the Wilcoxon Signed-Rank test. Because of the small number of samples with low flow, the data were grouped for each 0.2 interval in flow from zero to maximal flow for the comparison between FMISO and FDG uptake. FMISO and FDG activity in each of the myocardial blood flow ranges were compared using analysis of variance.

TABLE 1
Physiologic Measures

Parameter	Control	Ischemia
Heart rate (bpm)	101.6 ± 12.4	97.9 ± 11.9
Systolic blood pressure (mmHg)	101.6 ± 13.1	96.3 ± 9.3*
Systolic wall thickening		
LAD (% of control)	—	65.3 ± 20.1
Circumflex (% of control)	—	82.8 ± 19.1†
Plasma Glucose (mg/dl)	64 ± 14	68 ± 11

*$p < 0.05$ vs. control.
†$p = 0.05$ vs. LAD.

RESULTS

Control heart rate, systolic blood pressure, systolic wall thickening and plasma glucose levels are shown in Table 1 along with the average values for the ischemic period.

The difference between LAD and circumflex wall thickening is at the limits of statistical significance ($p = 0.05$, Table 1). The lack of a larger difference is likely due to two animals (as noted in the Methods section) in which there was an initial reduction in wall thickening in the ischemic (LAD) region, however, thickening returned to control levels with time, despite complete occlusion of the coronary artery. Presumably, this occurred because of the opening of collaterals.

Uptake of FMISO and FDG for all of the samples ($n = 576$) normalized only to their respective value in the normal region is shown as a function of the normalized myocardial blood flow in Figure 2. The greater FMISO activity in the

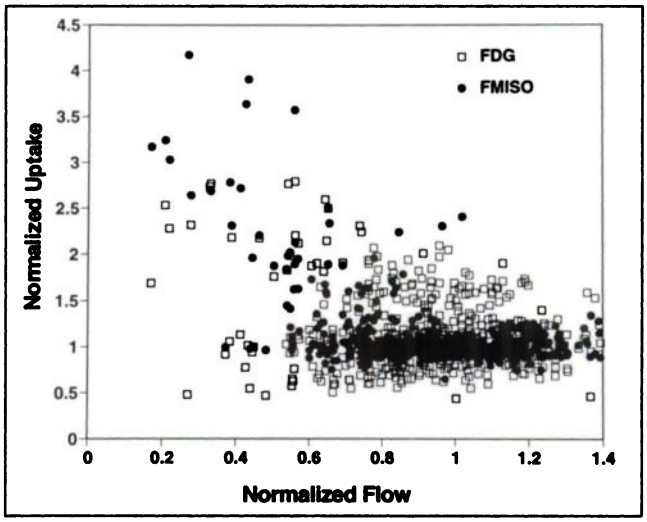

FIGURE 2. The individual data points for FMISO and FDG in Figure 2 have been grouped according to myocardial blood flow intervals of 0.20 units. The statistical comparisons are between FMISO and FDG for a given flow interval.

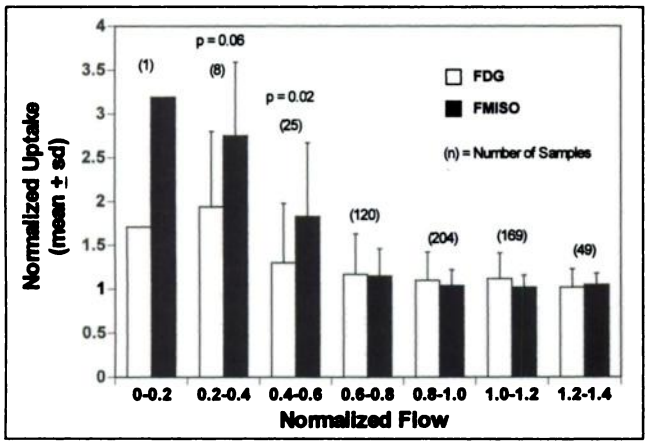

FIGURE 3. FMISO and FDG uptake in 576 myocardial samples normalized to the activity in 12 samples taken from the center of the anatomically normal zone. Uptake is plotted as a function of myocardial blood flow in the same samples normalized to the average flow in the same 12 normal samples.

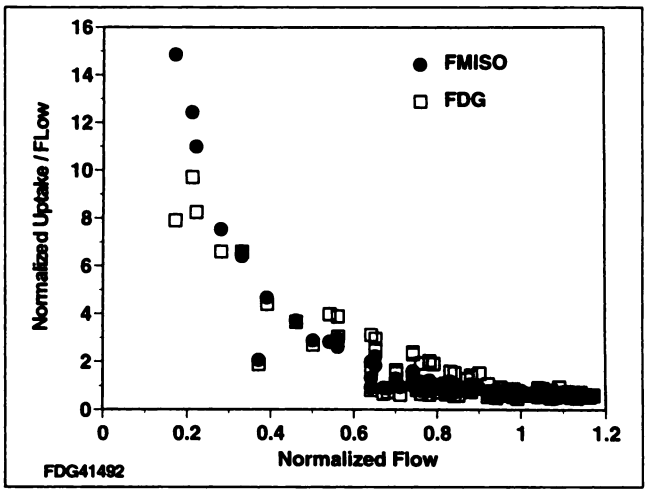

FIGURE 5. FMISO and FDG activity in a single experiment. Tissue activity in each sample has been normalized to the corresponding absolute myocardial blood flow in each sample. This method is the same as that used for clinical FDG viability studies.

low flow regions relative to the FDG uptake is apparent. Figure 3 shows the normalized FMISO activity and FDG uptake grouped according to increments in myocardial blood flow. Again, the greater FMISO uptake is apparent, although this was significantly different from FDG only for the 0.4–0.6 flow range. The relationship between normalized FMISO and FDG uptake in the same samples at three levels of myocardial blood flow is shown in Figure 4. As shown, in the most ischemic regions (normalized MBF < 0.5), the uptake of FMISO was greater than FDG uptake in 14 of 16 samples. Mean FMISO uptake in these 16 samples was 2.52 ± 1.01 compared to 1.52 ± 0.82 for FDG ($p < 0.002$).

When normalized FMISO and FDG uptake in each sample were expressed as ratios to the corresponding absolute flow, in the manner frequently used for analysis of clinical FDG-PET images, uptake increased as flow decreased. This is shown for a single animal in Figure 5 and for all animals in Figure 6. There was a trend for the FMISO uptake to be greater than FDG, but this was statistically significant only for one range of flows (0.4–0.6) (Fig. 6).

When the data were examined in terms of endocardial versus the epicardial half of the myocardial wall, we found the same increased uptake of both tracers with decreasing flow. Uptake of both compounds, however, was higher in those samples lying within the endocardial half, suggesting the ischemic insult was greater in the endocardium.

DISCUSSION

Following injection, FMISO is initially distributed throughout the total body water space, with subsequent clearance from the normal myocardium directly as a function of plasma clearance (*10*). Because its retention is de-

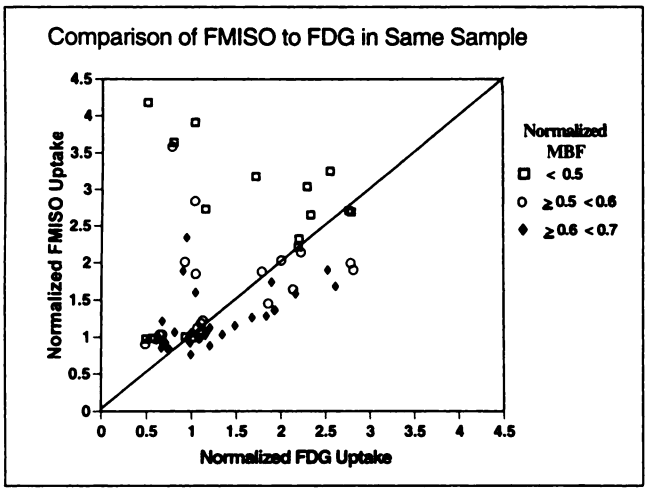

FIGURE 4. Normalized FMISO and FDG uptake in the same sample are compared for all samples whose normalized myocardial blood flow (MBF) was within the indicated ranges.

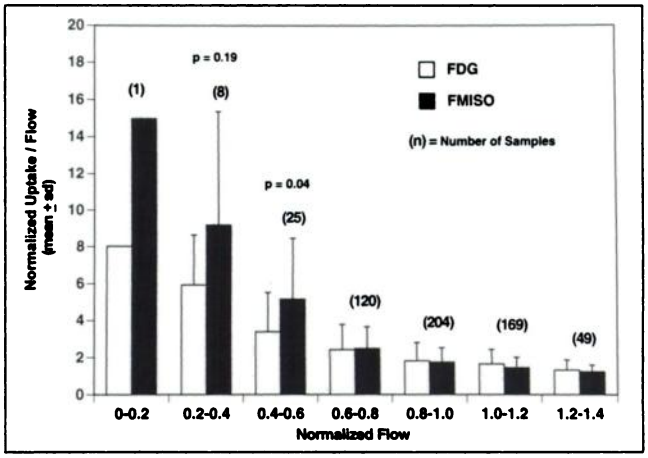

FIGURE 6. Flow-normalized FMISO and FDG activity as described for Figure 5 for all 576 tissue samples are expressed in terms of 0.20 intervals of flow. Statistical comparisons are between FMISO and FDG for a given flow interval.

pendent on active electron transport enzymes, it is selectively trapped in hypoxic myocardium (*10–14*) but not in necrotic tissue (*14*). Thus, the increased FMISO uptake in areas of moderate myocardial ischemia observed in this study is consistent with previous work by our group and others (*12,13*). The finding of most interest is the trend toward greater uptake of FMISO than FDG. This suggests that FMISO might be an alternative to FDG for determining myocardial viability. FMISO may be particularly advantageous in patients with diabetes in whom FDG images tend to be of poorer quality (*9*).

Another potential advantage of FMISO over FDG to identify chronically ischemic myocardium is that FMISO uptake, expressed in absolute terms, is elevated even in areas of very low flow, whereas absolute FDG uptake is not. In practical terms, this suggests that FMISO imaging studies may not require a separate flow study, as is routinely done for FDG to identify areas of FDG flow mismatch (*19,20*) or to derive quantitative estimates of glucose utilization (*7*).

It is not surprising that both the FMISO and FDG activity was greatest in the endocardial half of the left ventricular wall. It has long been recognized that the endocardium is more sensitive to hypoperfusion than the epicardium. This greater endocardial uptake also supports the wall thickening data that the ischemic burden was relatively mild and would have been reversible.

There are potential limitations to this study. Myocardial blood flow was only measured at two time points and the average of these two were assumed to represent flow throughout the 3 hr of ischemia. The validity of this assumption cannot be tested. There was little change, however, in the hemodynamic parameters or wall thickening during this period, suggesting that there should have been little change in flow. If change occurred, it was probably a decrease in flow along with the trend for decrease in metabolic demands in both the normal and ischemic zones, as suggested by the trend in wall thickening changes. Furthermore, since FMISO and FDG were measured in the same pieces, any changes in absolute flow would not affect the relative differences in tracer trapping or uptake. The difference between FMISO and FDG uptake may have been underestimated because the experiment was terminated after only 3 hr of ischemia. We have previously shown a progressive increase in FMISO uptake in ischemic myocardium over a 4-hr period (*10,12*). Thus, by sampling at 3 hr, we may have underestimated FMISO activity in the ischemic samples, whereas FDG sampling for a similar time used for clinical imaging protocols resulted in optimized FDG data. Also, because of the 2-hr time difference between injection of FMISO and FDG, different anaerobic conditions may have existed. There was no significant difference in MBF between the time of the FMISO and FDG injections, although there was a trend for MBF to be lower at the time of the FDG injection. This could have resulted in greater uptake of FDG than would have occurred had FDG been injected at the time of the FMISO injection. On the other side of the ischemia issue, there was an insignificant decrease in blood pressure with a constant heart rate over this time which would reduce myocardial work, thus potentially decreasing ischemia and lessening FDG uptake. This could also reduce FMISO uptake.

FDG uptake may have been underestimated because of a potential inhibition of glucose uptake through a heparin-induced release of lipoprotein lipase, with subsequent increase in the plasma free fatty acids. Nuutila et al. (*22*) recently reported that a 20-fold increase in plasma fatty acids produced by an infusion of heparin (3000 IU infused over 3.5 hr) and Intralipid (200 ml of 20%) resulted in only a 30% decrease in myocardial glucose uptake as measured by PET. It seems unlikely that heparin alone, as used in our study (2000–3000 IU given ≥2 hr before the FDG injection), would increase free fatty acids to the level noted above. We did not, however, measure plasma fatty acid levels and thus cannot exclude the possibility that FDG uptake was artificially depressed relative to what would have been expected for the level of ischemia present.

The animals used in this study were fasted for 8–12 hr which clearly suppressed plasma glucose levels and presumably the insulin levels. Both of these conditions would have the tendency to decrease FDG uptake. We used the average FDG activity in anatomically normal myocardial regions, however, to normalize the values in the individual samples both in the ischemic and normal regions. Unless there is an unrecognized relative greater inhibition of FDG uptake in ischemic compared to normal regions because of the fasting state, there should not have been an underestimation of FDG uptake.

Independent of the effect of basal glucose levels on FDG uptake, if flow had been reduced to the point of preventing washout of inhibitory metabolic intermediates, FDG uptake could have been underestimated. Given the relative mild nature of the ischemia, it seems unlikely that this phenomenon occurred to any great extent, although this issue cannot be addressed by our data. Similarly, high plasma lactate levels also could have suppressed FDG uptake. Although plasma lactate levels were not measured, given the relatively mild ischemia and the small ischemic regions, the amount of lactate spilling over into the systemic circulation should have been small and the lactate effect minimal or nonexistent.

CONCLUSION

FMISO trapping during moderate myocardial ischemia is at least equal to, if not greater than, that for [^{18}F]FDG. FMISO is a potentially attractive alternative to FDG for several reasons:

1. FMISO is taken up only by cells that are hypoxic but still retain their viability.
2. FMISO probably does not require normalization to myocardial blood flow for determination of tissue viability.

3. FMISO is probably unaffected by tissue pH or basal metabolic conditions such as plasma fatty acid or glucose.
4. FMISO requires only a simple analysis of tissue-to-blood ratios. Finally, we observed FMISO uptake in clinically stable patients (*23*); however, studies comparing FMISO and FDG uptake in the same patient population prior to coronary revascularization are necessary to determine the relative role of the two compounds in identifying viable myocardium. FMISO is unlikely to be useful in clinical conditions of transient ischemia, such as might occur during exercise, because of the need for ischemia/hypoxia to be sustained during the interval from radiopharmaceutical injection through imaging to observe trapping.

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