
Absolute Quantification of Regional Cerebral Glucose Utilization in Mice by ^{18}F -FDG Small Animal PET Scanning and $2\text{-}^{14}\text{C}$ -DG Autoradiography

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The purpose of this study was to evaluate the feasibility of absolute quantification of regional cerebral glucose utilization (rCMR_{glc}) in mice by use of ^{18}F -FDG and a small animal PET scanner. rCMR_{glc} determined with ^{18}F -FDG PET was compared with values determined simultaneously by the autoradiographic $2\text{-}^{14}\text{C}$ -DG method. In addition, we compared the rCMR_{glc} values under isoflurane, ketamine and xylazine anesthesia, and awake states. **Methods:** Immediately after injection of ^{18}F -FDG and $2\text{-}^{14}\text{C}$ -DG into mice, timed arterial samples were drawn over 45 min to determine the time courses of ^{18}F -FDG and $2\text{-}^{14}\text{C}$ -DG. Animals were euthanized at 45 min and their brain was imaged with the PET scanner. The brains were then processed for $2\text{-}^{14}\text{C}$ -DG autoradiography. Regions of interest were manually placed over cortical regions on corresponding coronal ^{18}F -FDG PET and $2\text{-}^{14}\text{C}$ -DG autoradiographic images. rCMR_{glc} values were calculated for both tracers by the autoradiographic $2\text{-}^{14}\text{C}$ -DG method with modifications for the different rate and lumped constants for the 2 tracers. **Results:** Average rCMR_{glc} values in cerebral cortex with ^{18}F -FDG PET under normoglycemic conditions (isoflurane and awake) were generally lower (by 8.3%) but strongly correlated with those of $2\text{-}^{14}\text{C}$ -DG ($r^2 = 0.95$). On the other hand, under hyperglycemic conditions (ketamine/xylazine) average cortical rCMR_{glc} values with ^{18}F -FDG PET were higher (by 17.3%) than those with $2\text{-}^{14}\text{C}$ -DG. Values for rCMR_{glc} and uptake (percentage injected dose per gram [%ID/g]) with ^{18}F -FDG PET were significantly lower under both isoflurane and ketamine/xylazine anesthesia than in the awake mice. However, the reductions of rCMR_{glc} were markedly greater under isoflurane (by 57%) than under ketamine and xylazine (by 19%), whereas more marked reductions of %ID/g were observed with ketamine/xylazine (by 54%) than with isoflurane (by 37%). These reverse differences between isoflurane and ketamine/xylazine may be due to competitive effect of ^{18}F -FDG and glucose uptake to the brain under hyperglycemia. **Conclusion:**

We were able to obtain accurate absolute quantification of rCMR_{glc} with mouse ^{18}F -FDG PET imaging as confirmed by concurrent use of the autoradiographic $2\text{-}^{14}\text{C}$ -DG method. Underestimation of rCMR_{glc} by ^{18}F -FDG in normoglycemic conditions may be due to partial-volume effects. Computation of rCMR_{glc} from ^{18}F -FDG data in hyperglycemic animals may require, however, alternative rate and lumped constants for ^{18}F -FDG.

Key Words: small animal PET; mouse; ^{18}F -FDG; anesthesia; cerebral glucose metabolism

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Recent advances in the technology of PET have made it possible to image small animals such as rats and mice (1,2). PET imaging of mice provides unique opportunities to study in vivo normal physiology and pathophysiology of various disease models, particularly because transgenic manipulation of mice has created phenotypes with the potential to link specific genes to molecular and organ functions of both nondiseased and diseased animals (3). The major advantage of small animal PET studies over necropsy studies is that the former allows within-subject longitudinal studies (3,4).

A glucose analog, ^{18}F -FDG, is the most frequently used radiopharmaceutical in clinical PET and animal PET studies. ^{18}F -FDG PET uptake measurements in regions of interest (ROIs), expressed as percentage ^{18}F -FDG uptake of the injected dose per gram (%ID/g) of tissue, are simple and easy to obtain. However, these ^{18}F -FDG uptake measurements may not accurately reflect true regional rates of glucose utilization because ^{18}F -FDG uptake can be affected by plasma glucose levels and other factors independent of the rate of glucose utilization. Fully quantitative PET studies require information on the time course of tracer delivery to the tissue. This "input function" is best obtained from

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direct arterial blood sampling during the PET study (4). Repeated blood sampling is readily feasible in rats (5) but very difficult in the mouse because of the small size of the vessels (~1 mm) (6) and the total blood volume (2–3 mL) (4,7). Moore et al. (5) first reported quantitative measurements of regional cerebral glucose utilization ($rCMR_{glc}$) in rats by using ^{18}F -FDG and microPET. There are also reports of determinations of brain glucose utilization in genetically manipulated mice with the $2\text{-}^{14}C$ -DG autoradiographic technique (8,9). Quantitatively accurate determinations of $rCMR_{glc}$ in mice with ^{18}F -FDG and small animal PET have not yet been reported, however.

For the purposes of this study, we used ^{18}F -FDG as a well-studied radioligand to assess feasibility and limitations of small animal PET studies in the mouse brain. Values for $rCMR_{glc}$ obtained with ^{18}F -FDG PET were compared with those obtained concurrently with the autoradiographic $2\text{-}^{14}C$ -DG method (10). PET imaging of small animals unfortunately requires animal immobilization during scanning, and anesthesia is often used to achieve this immobilization, even though anesthesia is known to affect $rCMR_{glc}$ (11,12). Therefore, we also examined and compared the values for $rCMR_{glc}$ obtained under isoflurane and ketamine/xylazine-mixed anesthesia and with those obtained in awake mice. We have used various anesthetics to obtain variability in the outcome measures of $rCMR_{glc}$ obtained with ^{18}F -FDG PET and thereby provide a wider range of values to correlate with the gold-standard autoradiographic $2\text{-}^{14}C$ -DG method.

MATERIALS AND METHODS

Animal Preparation

All animal procedures were performed in strict accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals and approved by the National Institute of Mental Health (NIMH) Animal Care and Use Committee. Two-month-old wild-type male BALB/c mice weighing 28–34 g were obtained from Taconic Farms. The animals were housed in groups of 5 and allowed food and water ad libitum during their holding time. Because of their small glycogen reserve and rapid metabolism, the animals were not fasted and were provided with food and water until the morning of the experiment (13).

After anesthetizing the animals with 1% isoflurane, a 35-cm polyethylene catheter (PE 10; Intramedic, Clay Adams) (dead space: 0.02 mL) was inserted into the left femoral artery, and a 30-gauge needle (Beckton Dickinson) attached to a 30-cm polyethylene catheter (dead space: 0.02 mL) was inserted into the tail vein of all mice. The catheters and needles were secured with tissue adhesive (3M Vetbond). Mice were allowed free movement throughout a 3-h period of recovery from the anesthesia and surgery before initiation of the $rCMR_{glc}$ measurement.

Values for $rCMR_{glc}$ were determined in animals under 3 conditions: anesthetized with 1% isoflurane and 100% O_2 administered by inhalation through a nose cone ($n = 7$); anesthetized by an intramuscular injection of a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture ($n = 7$); and awake animals ($n = 6$).

Physiologic Variables

Mean arterial blood pressure was measured with a Digi-Med Blood Pressure Analyzer (model 400; Micro-Med). Hematocrit was determined by centrifugation of arterial blood samples in a Micro-Centrifuge (Thomas Scientific). Arterial plasma glucose content was measured in a Beckman Glucose Analyzer 2 (Beckman Instruments). Body temperature was maintained in awake mice by warming the environment with a heating lamp. Body temperature in the anesthetized animals was monitored by a rectal temperature probe and maintained at $36.5^{\circ}C$ – $37.5^{\circ}C$ by a heating lamp and heating pad throughout the procedure.

Small Animal PET Scanner

We used the National Institutes of Health Advanced Technology Laboratory Animal Scanner (ATLAS) small animal PET scanner with an effective transaxial field of view of 6.0 cm and a 2-cm axial field of view. The scanner contains 18 dual-layered phoswich detector modules of Ce-doped $Lu_{18}Gd_{0.2}$ (7 mm) and GSO (Ce-doped Gd_2SiO_5) (8 mm) (Hitachi). This phoswich design allows depth-of-interaction (DOI) detection while preserving a sensitivity of 2.7% (100–650 keV) (14). PET images were reconstructed by single-slice rebinning and 2D-ordered-subset expectation maximization (SSRB/2D OSEM) algorithm (5 iterations and 18 subsets), achieving a 1.5-mm full width at half maximum (FWHM) resolution at the center (15). The reconstructed voxel size was $0.28 \times 0.28 \times 1.125$ mm. Coronal section images were created for subsequent data analysis. Image data were not corrected for attenuation or scatter.

$rCMR_{glc}$ Measurement Procedures

The procedure was initiated by injection of a mixture of 7.77–14.43 MBq of ^{18}F -FDG and 0.13–0.15 MBq of $2\text{-}^{14}C$ -DG through the tail-vein catheter (dual-tracer injection). We used Y connector for the tracer injection (volume: 0.05–0.22 mL) followed by saline flush (volume: 0.07 mL). Timed arterial samples of approximately 35 μ L were collected before and at 15, 30, and 45 min after the injection. The blood samples were collected into heparin-treated tubes and immediately centrifuged, radioactivity was determined in 5 μ L of plasma, and glucose was measured in 10 μ L of plasma. Additional arterial samples of approximately 10 μ L were drawn at 0–1 (6 continuous samples), 2, 5, and 10 min for radioactivity measurements. For blood collection, we first took 0.03 mL of heparinized saline and blood for dead space followed by each blood sampling. We returned the each blood sample for dead space to avoid excessive blood loss. The catheter was flushed with 0.03 mL of heparinized saline after each sample. During the blood sampling, hemostatic forceps were used to clamp the end of the catheter. Hematocrit was measured at 0 and 30 min, and mean arterial blood pressure was measured at 0 and 25 min. Plasma ^{18}F -FDG concentration was assayed in an automated γ -well counter (Perkin Elmer Life Sciences) on the day of the experiment, and plasma $2\text{-}^{14}C$ -DG concentration was measured in a liquid scintillation counter (Global Medical Instrumentation) for ^{14}C on the following day. At the end of the period for arterial sampling (45 min), the animal was euthanized and then a PET scan was obtained. Euthanasia was performed with an intravenous ketamine injection and cervical dislocation. Although the major advantage of small animal PET is its capability of allowing live-animal imaging experiments, euthanasia was performed before the PET scan for the following reasons: no effective physiologic restraints were available to immobilize awake animals during the PET scanning; alternative use of anesthesia for immobilization may

affect $rCMR_{glc}$ because of potential washout of ^{18}F -FDG during the 15-min imaging period; and the $2\text{-}^{14}C$ -DG autoradiographic method (10), which was used as the standard of comparison, does not include a delay period after the 45-min experimental period that was required for PET scanning at the end of the period. This choice of immobilization allowed us to evaluate not only the PET quantification of $rCMR_{glc}$ relative to the $2\text{-}^{14}C$ -DG method but also the effects of anesthesia on $rCMR_{glc}$ during ^{18}F -FDG uptake period compared with the awake condition. The duration of the PET scan was 15 min, after which the brain was removed, frozen in isopentane maintained at $-40^{\circ}C$ with dry ice, and stored at $-80^{\circ}C$ until sectioning. Brains were cut into $20\text{-}\mu m$ coronal sections in a cryostat at $-22^{\circ}C$. The frozen brain sections were thaw-mounted on glass cover slips, immediately dried on a hot plate at about $60^{\circ}C$, and autoradiographed together with calibrated ^{14}C -methylmethacrylate standards on Kodak EMC-1 x-ray film (9).

Calculation of $rCMR_{glc}$ and FDG Uptake

Absolute $rCMR_{glc}$ values for both tracers were calculated by the operational equation of the autoradiographic $2\text{-}^{14}C$ -DG method (10) with adjustments of the rate and lumped constants for the plasma glucose concentrations and for differences in kinetic characteristics between the 2 tracers. Rate constants and lumped constants used in the computation were those previously determined in the rat. We used the following kinetic parameters and lumped-constant (LC) values for $2\text{-}^{14}C$ -DG (10,16) and ^{18}F -FDG (5,17), respectively: for animals in the normoglycemic range (6.0–19.7 mmol/L) (5,10,17), $k_1 = 0.189$ and 0.302 min^{-1} , $k_2 = 0.245$ and 0.392 min^{-1} , $k_3 = 0.052$ and 0.068 min^{-1} , and $LC = 0.481$ and 0.625 ; and for animals in the hyperglycemic range (16) (19.7–30.9 mmol/L), $k_1 = 0.127$ and 0.203 min^{-1} , $k_2 = 0.235$ and 0.376 min^{-1} , $k_3 = 0.042$, and 0.055 min^{-1} , and $LC = 0.400$ and 0.520 .

The effects of hyperglycemia on these rate constants for ^{18}F -FDG have not yet been published. Therefore, we adjusted the rate constants for ^{18}F -FDG for the degree of hyperglycemia in proportion to the ratios of their published values for $2\text{-}^{14}C$ -DG under hyperglycemic and normoglycemic conditions (16).

ROIs ($3\text{--}5\text{ mm}^2$) were manually placed over 4 bilateral cortical regions (frontal, parietal, temporal, and occipital) on the coronal ^{18}F -FDG PET images (slice thickness: 1 mm) (Fig. 1). Because of

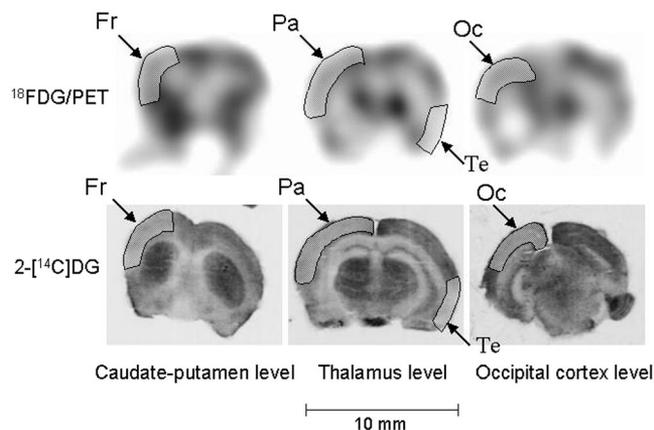


FIGURE 1. Representative coronal ^{18}F -FDG PET and $2\text{-}^{14}C$ -DG autoradiographic images of brain sections of unanesthetized mouse with ROIs delineated on them. Fr = frontal cortex; Pa = parietal cortex; Te = temporal cortex; Oc = occipital cortex.

the lower spatial resolution and greater partial-volume effects of the PET images than those of the $2\text{-}^{14}C$ -DG autoradiograms, the boundaries of the ROIs ($3\text{--}5\text{ mm}^2$) were defined by the PET images and drawn on the corresponding $2\text{-}^{14}C$ -DG autoradiograms. Furthermore, because the $2\text{-}^{14}C$ -DG autoradiograms were derived from brain sections with a slice thickness of 0.02 mm , 50 contiguous serial sections totaling 1 mm in thickness, equivalent to that of the PET slices, were used, and the ROIs were placed on 12 technically optimal slices with minimum damage of tears, folds, or bubbles in bilateral cerebral hemispheres of the 50 contiguous serial sections. Two slices with highest ROI values and another 2 slices with lowest ROI values were then discarded. Thus, ROI values were obtained by averaging a total of 8 autoradiograms (corresponding to a summed thickness of 0.16 mm) (Fig. 1). Identification of anatomic structures was guided by the stereotactic mouse brain atlas (Fig. 1) (18). A calibration factor was determined by using a 3-cm diameter cylinder phantom with known activity-enabling-ROI counts per pixel to be converted to μCi (kBq)/g tissue, with the assumption that tissue density is 1 g/cm^3 . These values were divided by the injection dose (μCi [kBq]) to obtain an image-ROIs-derived ^{18}F -FDG %ID/g of tissue. We performed linear regression analyses to determine the correlation between $rCMR_{glc}$ values determined with the ^{18}F -FDG small animal PET and those with $2\text{-}^{14}C$ -DG autoradiography. We determined differences in $rCMR_{glc}$ values between the ^{18}F -FDG and $2\text{-}^{14}C$ -DG methods for the 3 conditions (i.e., isoflurane anesthesia, ketamine/xylazine anesthesia, and the unanesthetized awake state) by paired t tests. Mean cortical $rCMR_{glc}$ determined with $2\text{-}^{14}C$ -DG and ^{18}F -FDG and the %ID/g uptake values among the 3 conditions were compared by unpaired t tests. The level of statistical significance was designated as $P < 0.05$.

RESULTS

Physiologic Variables

Arterial blood pressures were significantly lower and arterial plasma glucose concentrations were significantly higher in isoflurane anesthetized mice than in awake mice, but the glucose concentrations were within a normoglycemic range ($6.4\text{--}15.9\text{ mmol/L}$) in both groups (Table 1). Arterial plasma glucose concentrations in the ketamine/xylazine-treated mice, however, were hyperglycemic ($20.0\text{--}27.2\text{ mmol/L}$), except for 2 mice (17.3 and 19.6 mmol/L) (Table 1). None of the other physiologic values differed statistically significantly among the isoflurane and ketamine/xylazine anesthetized and awake groups (Table 1).

Comparison of $rCMR_{glc}$ Values Obtained with ^{18}F -FDG Small Animal PET and $2\text{-}^{14}C$ -DG Autoradiography

^{18}F -FDG PET images were of adequate quality to allow visual identification of individual cortical regions (Fig. 1). The shape of the plasma time-activity curves for ^{18}F -FDG and $2\text{-}^{14}C$ -DG was nearly identical, although the magnitude of activity was different between the 2 tracers (Fig. 2). For normoglycemic mice (isoflurane anesthetized and awake), average cortical values for $rCMR_{glc}$ determined with ^{18}F -FDG correlated highly with those of $2\text{-}^{14}C$ -DG ($y = 0.83x + 3.6$, $r = 0.98$, $r^2 = 0.95$, $P < 0.001$) (Fig. 3) but were lower than those obtained with $2\text{-}^{14}C$ -DG by 8.3% (Table 2). This slight underestimation of $rCMR_{glc}$ by the

TABLE 1
Physiologic Variables Before Measurement of Cerebral Glucose Utilization in Mice

Anesthesia type/state	Age (d)	Body weight (g)	Mean arterial blood pressure (mm Hg)	Hematocrit (%)	Arterial plasma glucose concentration (mmol/L)
Isoflurane (<i>n</i> = 7)	207 ± 48	31 ± 2	69 ± 8*	40 ± 2	11.4 ± 2.2†
Ketamine/xylazine (<i>n</i> = 7)	180 ± 31	31 ± 2	87 ± 13	42 ± 4	21.9 ± 3.3*
Awake (<i>n</i> = 6)	196 ± 38	30 ± 2	101 ± 18	38 ± 5	8.3 ± 2.2

**P* < 0.01.
†*P* < 0.05.
All values are expressed as mean ± SD.

¹⁸F-FDG PET method was likely due to partial-volume effects (see the Discussion section). Although our calculations of rCMR_{glc} by the ¹⁸F-FDG PET as well as the 2-¹⁴C-DG methods included adjustments of the rate constants and LC for hyperglycemia in the animals under ketamine and xylazine anesthesia, the average values for rCMR_{glc} obtained with PET overestimated those obtained with 2-¹⁴C-DG by 17.3% (Table 2).

Comparison of rCMR_{glc} and FDG Uptake (%ID/g) Values Under Isoflurane and Ketamine and Xylazine Anesthesia and Awake State

Average values for cortical rCMR_{glc} determined with 2-¹⁴C-DG were significantly lower under both isoflurane (28.0 ± 9.7 μmol/100 g/min) and ketamine and xylazine anesthesia (41.9 ± 7.5 μmol/100 g/min) than those in the awake animals (67.6 ± 11.1 μmol/100 g/min). The reductions were more marked with isoflurane (by 59%) than with ketamine and xylazine (by 26%) (Fig. 4A). Likewise, average cortical rCMR_{glc} determined with ¹⁸F-FDG PET in both the isoflurane (26.0 ± 6.5 μmol/100 g/min) and ketamine and xylazine anesthetized (48.8 ± 5.3 μmol/100 g/min) groups were significantly lower than those in the awake group (60.5 ± 8.8 μmol/100 g/min), with the reductions under isoflurane greater (by 57%) than under ketamine and xylazine (by 19%) (Fig. 4B). However, average FDG uptake

(expressed as %ID/g and not corrected for the plasma input function) were significantly lower with both isoflurane (6.6 ± 1.6) and ketamine and xylazine (4.8 ± 0.9) conditions than in the awake (10.4 ± 1.3) condition, with more marked reductions with ketamine and xylazine (by 54%) than with isoflurane (by 37%) (Fig. 4C).

DISCUSSION

The present study shows that in vivo ¹⁸F-FDG PET imaging in mice allows absolute quantification of average rCMR_{glc} with an accuracy comparable with that obtained with the 2-¹⁴C-DG autoradiographic method. It should be noted, however, that this is true only of average values within PET-defined ROIs that are relatively large compared with that possible with 2-¹⁴C-DG autoradiography, which provides much finer spatial resolution and quantitative determinations within structures with different rates within those ROIs. This study determined rCMR_{glc} in vivo in mice concurrently with both ¹⁸F-FDG PET and the 2-¹⁴C-DG autoradiographic method and is an extension of prior studies in the literature (8,9). Moore et al. reported that quantitative ¹⁸F-FDG microPET determinations of rCMR_{glc} in rats were only modestly correlated with those obtained with 2-¹⁴C-DG autoradiography (*r* = 0.47, *P* < 0.05) values (5). The reason for the higher correlation (*r* = 0.98) in our studies in mice is probably due to methodologic differences between our study and theirs. We injected ¹⁸F-FDG and 2-¹⁴C-DG simultaneously and drew timed arterial samples to determine simultaneously the input functions for both tracers. The mice were killed 45 min after injection, imme-

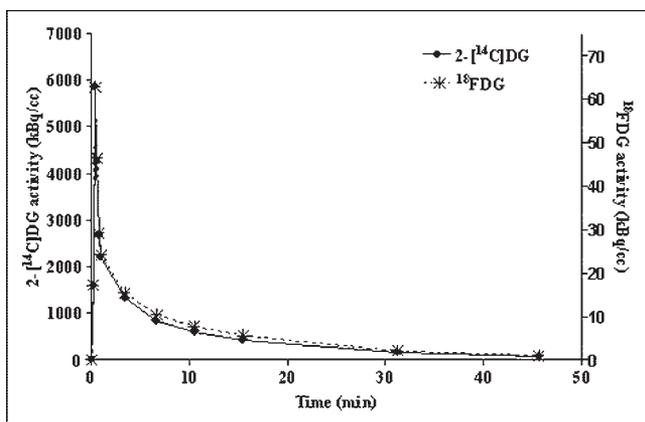


FIGURE 2. Representative time-activity curves of ¹⁸F-FDG and 2-¹⁴C-DG in unanesthetized mouse.

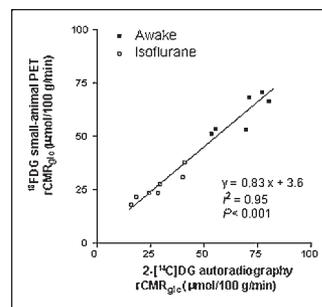


FIGURE 3. Comparison of weighted-average rCMR_{glc} (μmol/100 g/min) determined by ¹⁸F-FDG small animal PET and 2-¹⁴C-DG autoradiography in cortical ROIs of normoglycemic, awake and isoflurane anesthetized mice.

TABLE 2

Mean \pm SD of rCMR_{glc} (μ mol/100 g/min) in Each Cerebral Cortex Under Isoflurane, Ketamine/Xylazine, and Awake

Region and anesthesia	2- ¹⁴ C-DG autoradiography	¹⁸ F-FDG PET	% diff.
Frontal cortex			
Isoflurane	25.1 \pm 7.5	24.1 \pm 6.0	-4.0
Ketamine/xylazine	40.3 \pm 5.6	48.1 \pm 4.1	+19.5**
Awake	70.3 \pm 9.6	64.5 \pm 9.4	-8.3*
Parietal cortex			
Isoflurane	26.4 \pm 10.3	26.3 \pm 6.1	-0.5
Ketamine/xylazine	42.3 \pm 6.1	49.3 \pm 7.2	+16.6
Awake	74.0 \pm 13.5	61.2 \pm 6.6	-17.3*
Temporal cortex			
Isoflurane	29.7 \pm 10.2	24.7 \pm 6.0	-16.8*
Ketamine/xylazine	42.7 \pm 9.8	51.4 \pm 4.0	+20.4
Awake	53.0 \pm 10.5	53.5 \pm 8.2	+0.9
Occipital cortex			
Isoflurane	30.9 \pm 11.7	28.9 \pm 8.9	-6.5
Ketamine/xylazine	42.1 \pm 10.2	47.4 \pm 8.4	+12.5
Awake	73.0 \pm 12.4	62.8 \pm 11.7	-13.9*

P* < 0.05; *P* < 0.01; comparison between individual 2-¹⁴C-DG and ¹⁸F-FDG values (paired *t* test).

% diff. = percent difference between means of 2-¹⁴C-DG and ¹⁸F-FDG determinations.

diately after drawing the last blood sample. The brain was scanned with the small animal PET scanner and then removed, frozen, and autoradiographed. The determinations of rCMR_{glc} with the 2 procedures were performed simultaneously during the same period of uptakes of both tracers and, therefore, under identical physiologic conditions. In contrast, Moore et al. (5) conducted the ¹⁸F-FDG microPET study on the day of the surgery for head injury and the 2-¹⁴C-DG autoradiographic study on the following day. Although their colleagues at the University of California, Los Angeles, had previously claimed that the magnitude of the cerebral metabolic depression does not differ significantly between days 1 and 2 after head injury (19), the values for rCMR_{glc} that Yoshino et al. obtained showed considerable variability between days 1 and 2 (19). Therefore, measurements of rCMR_{glc} on different days with the head injury model may not be as precise as simultaneous measurements in normal animals. In addition, different tracer injections and blood sampling procedures in the study by Moore et al. might account for the lower correlation between their ¹⁸F-FDG microPET study and 2-¹⁴C-DG autoradiographic study. The slight underestimation (by 8.3%) of rCMR_{glc} determined with the ¹⁸F-FDG PET method compared with that obtained with the 2-¹⁴C-DG method is likely due to the lower spatial resolution of PET. First, the slice thickness of the PET scans was about 1 mm, whereas that of the autoradiographed slices was about 0.02 mm. Second, the FWHM of PET was 1.5 mm, whereas that of the autoradiograms was approximately 0.1 mm. Therefore, although we

placed comparable cerebral cortical ROIs on both ¹⁸F-FDG and 2-¹⁴C-DG images, the same ROIs on the ¹⁸F-FDG images probably included greater contributions from white matter than the 2-¹⁴C-DG images because of lesser distinction of the cortical boundary from the subcortical white matter, which was due to the greater partial-volume effects with PET than with autoradiography. To examine further the effect of lower spatial resolution on the computed rCMR_{glc}, we blurred a 2-¹⁴C-DG autoradiogram so that it had a similar spatial resolution to that of the ¹⁸F-FDG PET images and found that this reduced the recovered activity in the ROIs over the parietal cortex by 15% below that obtained with the original unblurred autoradiogram. This underestimation is similar to the percentage difference between rCMR_{glc} determined in the parietal cortex with 2-¹⁴C-DG autoradiography and with ¹⁸F-FDG (17%) (Table 2).

Xylazine, an α_2 -adrenergic receptor agonist, reduces circulating insulin because of direct stimulation of α_2 -adrenergic receptors on pancreatic islet cells, thereby reducing insulin release and leading to elevated plasma glucose concentrations (20,21). Consequently, plasma glucose levels in our ketamine and xylazine anesthetized mice were in a hyperglycemic range (i.e., almost 3 times the normal level). On the other hand, isoflurane-associated hypoinsulinemia is generally mild (20,22), and plasma glucose levels in the isoflurane anesthetized group, though statistically significantly higher than those of awake mice, were within a normoglycemic range. Our observed effects of anesthesia on plasma glucose levels are consistent with those of previous reports (11,12). Because glucose competitively inhibits 2-¹⁴C-DG and ¹⁸F-FDG transport across the blood-brain barrier and its phosphorylation by hexokinase, plasma glu-

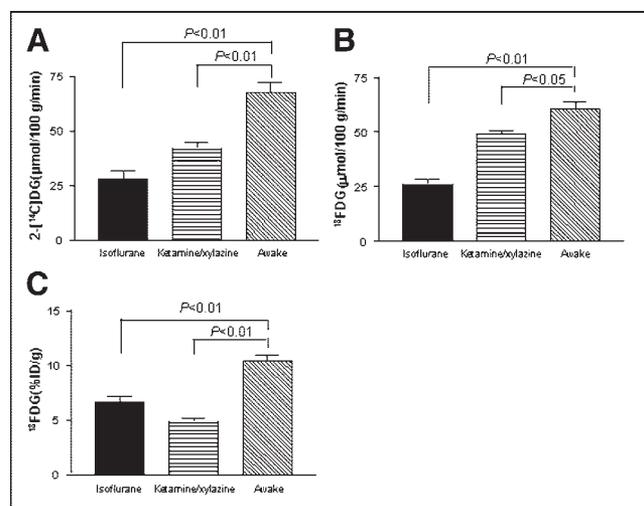


FIGURE 4. Comparison of weighted-average cortical rCMR_{glc} (μ mol/100 g/min) determined by 2-¹⁴C-DG autoradiography (A) and ¹⁸F-FDG PET (B) and of ¹⁸F-FDG uptake (%ID/g) (C) in mice under isoflurane or ketamine/xylazine anesthesia and awake mice.

cose levels can influence the computation of $rCMR_{glc}$. Orzi et al. (16) reported that the kinetic parameters, k_1 and k_3 , for $2\text{-}^{14}\text{C}\text{-DG}$ decrease with increasing arterial plasma glucose concentrations. The effects of hyperglycemia on these rate constants for $^{18}\text{F}\text{-FDG}$ have not yet been published (17). Therefore, for the purpose of the present studies we adjusted the rate constants for $^{18}\text{F}\text{-FDG}$ for the degree of hyperglycemia in proportion to the ratios of their published values for $2\text{-}^{14}\text{C}\text{-DG}$ under hyperglycemic and normoglycemic conditions (16). Compared with values obtained with the $2\text{-}^{14}\text{C}\text{-DG}$ autoradiography, however, $^{18}\text{F}\text{-FDG}$ PET underestimated $rCMR_{glc}$ in both isoflurane anesthetized and awake mice with plasma glucose levels in the normoglycemic range. This underestimation is probably not due entirely to inaccuracies in the rate and lumped constants. It is likely that partial-volume effects also contributed to this underestimation. Partial-volume effects cannot, however, explain the overestimation of $rCMR_{glc}$ obtained with $^{18}\text{F}\text{-FDG}$ PET in the cortical regions of the hyperglycemic mice under ketamine and xylazine anesthesia. It is clear that more definitive evaluation of the kinetic constants for $^{18}\text{F}\text{-FDG}$ in normoglycemic and hyperglycemic mice is needed.

Our findings by both the $^{18}\text{F}\text{-FDG}$ PET and autoradiographic $2\text{-}^{14}\text{C}\text{-DG}$ procedures of reduced $rCMR_{glc}$ in mice under isoflurane anesthesia are consistent with the approximately 40%–60% reductions previously found with the $2\text{-}^{14}\text{C}\text{-DG}$ autoradiographic method in rats (23,24). On the other hand, the reductions in $rCMR_{glc}$ we observed with both techniques during ketamine and xylazine anesthesia may appear to be inconsistent with the findings of others who reported that ketamine alone produces a variety of effects among the various structures of the brain (e.g., reductions in components of the sensory pathways and increases in those of the limbic system) (11,25). This apparent discrepancy may be attributed to our use of the combination of ketamine and xylazine mixture in contrast to ketamine alone used in the other studies (11,25). Ketamine is the most common dissociative anesthetic used in rodents (26) and when used alone in rats and mice, it causes muscle rigidity and incomplete anesthesia. To compensate for this problem, combinations of ketamine and xylazine have been used as tranquilizers (26), and this mixture produces excellent sedation and relaxation (27). A combination of ketamine with diazepam, another supplement, also causes a generalized decrease of $rCMR_{glc}$ in rats (11), similar to our findings with that of the ketamine and xylazine mixture in the mouse brain. In addition, the greater decrease in $^{18}\text{F}\text{-FDG}$ %ID/g in the ketamine and xylazine mixture than in isoflurane may be due to the competitive inhibition of $^{18}\text{F}\text{-FDG}$ uptake with increased glucose concentrations in plasma as produced with the xylazine effect (16,21). The rank order of plasma glucose values was inversely related to FDG uptake, such that uptake with ketamine and xylazine < isoflurane < awake. Failure to take arterial glucose into account in measuring brain uptake of labeled glucose or its

analogs is only one example. Studies of the effects of some physiologic tests on something related to cerebral blood flow by either functional MRI or PET is even more serious. Cerebral blood flow is much more sensitive to several blood constituents than to functional activity in brain tissue (e.g., pH, P_{CO_2}), and these can readily be changed by the test conditions but would not be perceived without blood sampling.

Limitations and $rCMR_{glc}$ Quantification

In the present study, we used $^{18}\text{F}\text{-FDG}$ as a well-studied radioligand to assess feasibility and limitations of small animal PET in the mouse brain. Here, several limitations of the present study that are related to the accuracy of $rCMR_{glc}$ measurements are summarized to alert researchers who plan to use genetically modified mice with small animal PET on the appropriate use of this evolving technology. There are several factors that can affect the quantitative accuracy of $rCMR_{glc}$ measurements.

First, to clarify the terminology, “accuracy” was used in the present study to refer to how close the $^{18}\text{F}\text{-FDG}$ PET $rCMR_{glc}$ measurements were to those of $2\text{-}^{14}\text{C}\text{-DG}$ autoradiography, which was considered to be the true $rCMR_{glc}$ values in our study. In this context, we showed that PET measurements of $^{18}\text{F}\text{-FDG}$ $rCMR_{glc}$ in mice were reasonably accurate compared with the concurrent autoradiographic measurements of $2\text{-}^{14}\text{C}\text{-DG}$ $rCMR_{glc}$ in the same mice under the same physiologic conditions during the $^{18}\text{F}\text{-FDG}$ and $2\text{-}^{14}\text{C}\text{-DG}$ uptake period. However, despite relatively large ROIs, $^{18}\text{F}\text{-FDG}$ $rCMR_{glc}$ measurements underestimated those of $2\text{-}^{14}\text{C}\text{-DG}$ by 10%. This underestimation of $^{18}\text{F}\text{-FDG}$ $rCMR_{glc}$ measurements appears to have resulted from the larger partial-volume effect with PET than with autoradiography. Although ROIs for the striatum and thalamus that were visible on PET images (Fig. 1) can be defined, these small in vivo ROIs would have greater partial-volume effects than the ex vivo autoradiographic ROIs. Thus, the spatial resolution of current small animal PET systems poses a major limitation of PET imaging for the small mouse brain. Despite this underestimation of $^{18}\text{F}\text{-FDG}$ $rCMR_{glc}$ measurements, we believe that these $^{18}\text{F}\text{-FDG}$ $rCMR_{glc}$ measurements reflect more accurately true glucose utilization than another commonly used glucose utilization outcome measure—namely, $^{18}\text{F}\text{-FDG}$ %ID/g.

Second, kinetic parameter and LC values affect the $rCMR_{glc}$ measurements. Currently, there are no established parameter or LC values for mice. In the present study, we used rat values under normoglycemic conditions reported in the literature for both $^{18}\text{F}\text{-FDG}$ and $2\text{-}^{14}\text{C}\text{-DG}$. Although these rat values did not affect our accuracy of $^{18}\text{F}\text{-FDG}$ $rCMR_{glc}$ measurements relative to $2\text{-}^{14}\text{C}\text{-DG}$ $rCMR_{glc}$ measurements, these values can certainly affect the accuracy of true $rCMR_{glc}$ measurements in the mouse brain. Under hyperglycemic condition, however, there are no established rat $^{18}\text{F}\text{-FDG}$ parameter values. Therefore we attempted to scale

^{18}F -FDG rate constants in proportion to the values for $2\text{-}^{14}\text{C}$ -DG. However, under hyperglycemia (ketamine/xylazine), ^{18}F -FDG rCMR_{glc} measurements were higher than those of $2\text{-}^{14}\text{C}$ -DG by 17%. This finding was contrary to what we had expected in view of the greater partial-volume effect for the PET. Therefore, in view of the absence of well-established literature values for these parameters for ^{18}F -FDG under hyperglycemia, we speculate that the parameter and LC values used under hyperglycemia may not be correct. This means that we would need to establish appropriate ^{18}F -FDG rate constant values under hyperglycemia, warranting future experiments addressing this issue.

Third, the physiologic conditions during ^{18}F -FDG or $2\text{-}^{14}\text{C}$ -DG uptake period can affect rCMR_{glc} . Our study design ensured that ^{18}F -FDG and $2\text{-}^{14}\text{C}$ -DG measurements had an identical physiologic condition during the tracer uptake period. To use the $2\text{-}^{14}\text{C}$ -DG rCMR_{glc} measurement as the gold standard in evaluating the accuracy of ^{18}F -FDG rCMR_{glc} measurement, we killed the animal after the uptake period in the present experimental design.

Last, measurement error in plasma input function can affect the accuracy of the ^{18}F -FDG and $2\text{-}^{14}\text{C}$ -DG rCMR_{glc} measurement relative to the true value. The current study design did not allow us to directly evaluate this accuracy. However, the plasma ^{18}F -FDG or $2\text{-}^{14}\text{C}$ -DG concentrations are directly measured, and, at least in our hands, the errors in their measurements would primarily be pipetting and counting errors. These errors should be small and would probably contribute insignificantly to the computed rCMR_{glc} values compared with the effects of the errors in the measurements of tissue concentrations, particularly with PET. Indirectly, this rCMR_{glc} measurement inaccuracy that is due to error in measurement of plasma input function may be reflected by the small difference of intersubject variability of rCMR_{glc} measurements between $2\text{-}^{14}\text{C}$ -DG and ^{18}F -FDG. The coefficients of variation of rCMR_{glc} measurement in the sample of normoglycemic mice ($n = 13$) for ^{18}F -FDG and $2\text{-}^{14}\text{C}$ -DG were as follows: 46% (^{18}F -FDG) and 49% ($2\text{-}^{14}\text{C}$ -DG), which suggest that the error in plasma measurements, if any, was not any greater for ^{18}F -FDG than for $2\text{-}^{14}\text{C}$ -DG.

CONCLUSION

We assessed the feasibility and limitations of small animal PET studies in the mouse brain with ^{18}F -FDG small animal PET scanning. Concurrent determinations of rCMR_{glc} with the $2\text{-}^{14}\text{C}$ -DG autoradiographic method and ^{18}F -FDG PET imaging with the ATLAS small animal scanner demonstrated that the PET imaging technique allows accurate absolute quantification of rCMR_{glc} in mice within the limits of the resolution possible with PET. Because of the limited spatial resolution of PET, the ROIs are necessarily larger than those possible with autoradiography and encompass a mixture of cerebral structures, with various rates of rCMR_{glc} clearly visible in the autoradiograms. If the

same low-resolution ROIs that are possible with PET are also applied to the autoradiographic images, the computed rCMR_{glc} is the weighted average of the rCMR_{glc} in the tissue components within the ROI. The present studies demonstrated that ^{18}F -FDG PET can provide accurate estimates of the average rCMR_{glc} within these ROIs. The anesthetic effects described here with ^{18}F -FDG PET apply only to this specific radioligand and must be assessed for any new probe that is examined.

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