Impact of Animal Handling on the Results of ^{18}F-FDG PET Studies in Mice

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Small-animal PET scanning with ^{18}F-FDG is increasingly used in murine models of human diseases. However, the impact of dietary conditions, mode of anesthesia, and ambient temperature on the biodistribution of ^{18}F-FDG in mice has not been systematically studied so far. The aim of this study was to determine how these factors affect assessment of tumor glucose use by ^{18}F-FDG PET and to develop an imaging protocol that optimizes visualization of tumor xenografts. **Methods:** Groups of severe combined immunodeficient (SCID) mice were first imaged by microPET with free access to food, at room temperature (20°C), and no anesthesia during the uptake period (reference condition). Subsequently, the impact of (a) fasting for 8–12 h, (b) warming the animals with a heating pad (30°C), and (c) general anesthesia using isoflurane or ketamine/xylazine on the ^{18}F-FDG biodistribution was evaluated. Subcutaneously implanted human A431 epidermoid carcinoma and U251 glioblastoma cells served as tumor models. **Results:** Depending on the study conditions, ^{18}F-FDG uptake by normal tissues varied 3-fold for skeletal muscle, 13-fold for brown adipose tissue, and 15-fold for myocardium. Warming and fasting significantly reduced the intense ^{18}F-FDG uptake by brown adipose tissue observed under the reference condition and markedly improved visualization of tumor xenografts. Although tumor ^{18}F-FDG uptake was not above background activity under the reference condition, tumors demonstrated marked focal ^{18}F-FDG uptake in warmed and fasted animals. Quantitatively, tumor ^{18}F-FDG uptake increased 4-fold and tumor-to-organ ratios were increased up to 17-fold. Ketamine/xylazine anesthesia caused marked hyperglycemia and was not further evaluated. Isoflurane anesthesia only mildly increased blood glucose levels and had no significant effect on tumor ^{18}F-FDG uptake. Isoflurane markedly reduced ^{18}F-FDG uptake by brown adipose tissue and skeletal muscle but increased the activity concentration in liver, myocardium, and kidney. **Conclusion:** Animal handling has a dramatic effect on ^{18}F-FDG biodistribution and significantly influences the results of microPET studies in tumor-bearing mice. To improve tumor visualization mice should be fasted and warmed before ^{18}F-FDG injection and during the uptake period. Isoflurane appears well suited for anesthesia of tumor-bearing mice, whereas ketamine/xylazine should be used with caution, as it may induce marked hyperglycemia.

PET with the glucose analog ^{18}F-FDG (^{18}F-FDG PET) is increasingly used in murine models of human diseases. Specifically, ^{18}F-FDG PET is rapidly gaining importance for monitoring progression and transformation of tumors in mice (*1*), biologic characterization of tumor tissue (*2*), and studying the effectiveness of anticancer drugs (*3,4*). Furthermore, ^{18}F-FDG is frequently used as a reference standard when evaluating other imaging agents in mice (*3–9*). For human ^{18}F-FDG PET studies, standard protocols have been established that optimize contrast between tumor and normal tissues (*10,11*). Protocols for animal imaging, on the other hand, vary widely (*1–9,12*).

Despite their rapid growth, malignant tumor xenografts frequently exhibit only modestly higher ^{18}F-FDG uptake than most normal tissues. For example, we found in a recent study that A431 tumor xenografts (volume doubling time <1 wk) were only barely visible in ^{18}F-FDG PET studies (*4*). Upon more careful review we realized that glucose metabolic activity of various background tissues was high, thereby possibly masking glucose metabolic activity of the tumors. We hypothesized that the dietary state, ambient temperature, or muscle activity might influence tumor detectability in small animals by changing ^{18}F-FDG uptake of normal tissues. These factors are known to significantly affect the biodistribution of ^{18}F-FDG in humans. Because mice have approximately 7-fold higher basal metabolic rates per body weight than humans (*13*), the effect of dietary state and ambient temperature on ^{18}F-FDG biodistribution may be even more pronounced than in humans. Preliminary studies by Akhurst et al. (*14*) have suggested that isoflurane anesthesia and fasting may improve biodistribution of ^{18}F-FDG for tumor imaging. However, to our knowledge, no systematic studies on this issue have been published so far. The aim of this study was therefore to investigate how ^{18}F-FDG biodistribution and tumor detectability could be manipulated in mice by altering dietary state, ambient temperature, and mode of anesthesia.

MATERIALS AND METHODS

Animal Preparation

All animal handling was performed in accordance with and approved by the University of California Animal Research Committee guidelines. Eight- to 10-wk-old male severe combined immunodeficient (C.B.-17 *Scid/Scid*) mice were obtained from Taconic Farms.

Tumor Model

The human epidermoid carcinoma cell line A431 (*15*) was acquired from the American Type Culture Collection. The human glioma cell line U251 (*16*) was obtained from Dr. Charles Sawyer's laboratory, Department of Medicine, UCLA, Los Angeles, CA. Both were cultivated in Dulbecco's modification of Eagle's medium supplemented with 10% fetal bovine serum. All animal manipulations were performed under sterile conditions. Cells growing exponentially in vitro were trypsinized, resuspended in phosphate-buffered saline and Matrigel (Collaborative Research), and injected subcutaneously into the right shoulder area of SCID mice ($\sim10^6$ cells per mouse). Mice were imaged when tumor diameter was at least 5 mm.

Measurement of Physiologic Parameters

Rectal temperature was measured with a thermistor probe. One group of animals was kept under isoflurane anesthesia and on a heating pad for 60 min. The heating pad we used is a plastic pad (41 × 31 cm), with water-filled chambers (Baxter Healthcare Corp.). Warm water of a defined temperature is continuously being pumped through the chambers. Another group of animals was kept under isoflurane anesthesia at room temperature. To avoid severe hypothermia, this experiment was stopped in this group after 30 min. Serum glucose levels were assayed in fasted and nonfasted conscious mice before and after isoflurane and ketamine anesthesia for 60 min. Blood samples (~10 μL per mouse) were collected from the tail vein and glucose concentration (2 samples per condition) was measured using the Freestyle glucose meter by TheraSense.

Influence of Animal Preparation and Handling on Biodistribution of ^{18}F-FDG

To determine the impact of dietary state, ambient temperature, and anesthesia on the biodistribution of ^{18}F-FDG, groups of 3–6 mice each were studied under the experimental conditions summarized in Table 1. At the time of PET mice were 10–12 wk old

TABLE 1
Summary of Study Conditions

n	Fasting	Warming	Anesthesia during uptake period*
6	No	No	No
6	No	Yes	No
6	Yes	No	No
6	Yes	Yes	No
6	Yes	Yes	No, also conscious during ^{18}F-FDG injection
3	No	Yes	Isoflurane
6	Yes	Yes	Isoflurane
3	Yes	Yes	Ketamine

*No anesthesia indicates reference condition.
n = number of animals per experiment.

with an average body weight ± SD of 24.2 ± 2.4 g. ^{18}F-FDG (7.4 MBq [200 μCi] in 0.2 mL) was injected intraperitoneally after a short (~5 min) isoflurane (2% in 100% oxygen) anesthesia period unless otherwise indicated in Table 1. PET was started 60 min after ^{18}F-FDG injection. As a reference condition, we imaged the mice with no special preparation—that is, mice not fasted and kept conscious at room temperature—during the uptake period. The biodistribution of ^{18}F-FDG during all other conditions (Table 1) was compared with this reference condition. For the fasting condition, mice were deprived of food for 8–12 h before ^{18}F-FDG injection. Mice had access to drinking water at all times. Warming was achieved by placing the entire cage, including 5 or 6 animals, on the heating pad kept at 30°C. Warming was started at least 30 min before ^{18}F-FDG injection and continued throughout the uptake and imaging period. To evaluate the influence of anesthesia on ^{18}F-FDG biodistribution, mice were either conscious during the uptake period or anesthetized by either isoflurane inhalation anesthesia (2% in 100% oxygen, IsoFlo; Abbott Laboratories) or intraperitoneal injection of a ketamine/xylazine solution (200 mg/kg ketamine and 10 mg/kg xylazine; Fort Dodge Animals Health, Division of Wyeth).

microPET was performed with the P4 microPET scanner (Concorde Microsystems Inc.). The characteristics of this device have been reported previously (*17*). In brief, the device has a ring diameter of 26 cm and a 7.8-cm axial field of view. The intrinsic spatial resolution ranges from 1.56 to 2.01 mm, with a mean of 1.75 mm. The reconstructed resolution is 1.8-mm full width at half maximum in the center of the field of view and 3 mm at 4-cm radial offset. For the PET scans the mice were kept under isoflurane anesthesia and placed in an imaging chamber with an ambient temperature of 30°C (*18*). ^{18}F-FDG was synthesized by a previously described method (*19*) that is routinely used in our facility. Quality control procedures were similar to the ones given by Hung (*20*).

Image Reconstruction

Images were reconstructed using filtered backprojection without scatter or attenuation correction. We chose a ramp filter with a cutoff frequency of 0.5 and a zoom of 5 to give a voxel size of 0.379 mm³. For cross-calibration of the dose calibrator and the microPET scanner, a 3.5-cm cylinder phantom filled with a known concentration of ^{18}F-FDG was imaged. From this scan a system calibration factor was derived by dividing the known activity concentration in the phantom by the measured mean counts per voxel in the reconstructed PET images.

Quantitative Image Analysis

Regions of interest were manually drawn over the following organs: brain, brown adipose tissue, heart, liver, paraspinal muscle, kidney, Harderian glands, and subcutaneous tumors. Tracer uptake by various organs was quantified as standardized uptake values (SUVs) using the formula: SUV = tissue activity concentration (Bq/mL)/injected dose (Bq) × body weight (g).

Intravenous Versus Intraperitoneal Injection of ^{18}F-FDG

Because of the very small caliber of the murine tail veins, partial paravenous injection is common if ^{18}F-FDG is administered by tail vein injection (intravenous). This could have significantly biased our comparison of the biodistribution of ^{18}F-FDG under various conditions. Therefore, we used intraperitoneal injection of ^{18}F-FDG for our experiments evaluating the influence of animal handling on ^{18}F-FDG biodistribution. To compare the

biodistribution of ^{18}F-FDG after intravenous or intraperitoneal injection, a dynamic PET scan of 60-min duration (12 × 5 s, 4 × 1 min, 1 × 5 min, 5 × 10 min) was acquired in 12 fasted and warmed mice bearing U251 xenografts; half had ^{18}F-FDG injected intravenously and half had intraperitoneal injections. In a second experiment we compared intravenous and intraperitoneal injection of ^{18}F-FDG in not-fasted and not-warmed mice that were not kept under anesthesia during the uptake period. In these animals, we acquired 10-min static images 60 min after injection of ^{18}F-FDG. Thus, comparison of intravenous and intraperitoneal injection of ^{18}F-FDG was performed for the 2 most diverse experimental conditions studied (fasted, warmed, and anesthesia vs. not fasted, not warmed, and no anesthesia).

Statistical Analysis

Results are presented as mean ± 1 SD. Differences among the experimental groups in the SUVs of the various tissues and tumor-to-organ ratios were statistically evaluated by ANOVA and Bonferroni post hoc tests. Statistical significance was established at the 95% level.

RESULTS

Changes in Physiologic Parameters During Anesthesia and PET

When animals were kept under anesthesia for 30 min at room temperature, the mean rectal temperature dropped from 32.1°C ± 0.8°C to 24.1°C ± 0.3°C. This dramatic decline of body temperature was avoided when mice were kept on a heating pad (body temperature after 30 and 60 min of anesthesia, 35°C ± 0.7°C and 35°C ± 0.7°C, respectively).

Serum glucose levels averaged 122 ± 21 mg/dL in the nonfasted state and 73 ± 34 mg/dL in the fasted state. One hour of isoflurane anesthesia caused a modest increase of blood glucose levels to 147 ± 33 mg/dL. A similar effect was observed in fasted animals (blood glucose, 104 ± 49 mg/dL after 1 h of anesthesia). Anesthetizing the mice with ketamine/xylazine markedly increased the serum glucose level in the fasted as well as in the nonfasted animals (335 ± 73 mg/dL and 363 ± 59 mg/dL, respectively).

Influence of Warming and Fasting on Biodistribution of ^{18}F-FDG in Mice Without Anesthesia During Uptake Period

Figure 1 shows typical examples for PET scans acquired under the various conditions in nonanesthetized animals. The results of the quantitative data analysis are summarized in Figure 2. Under the reference condition (no warming and no fasting, Fig. 1E), the highest ^{18}F-FDG uptake was seen in brown fat (SUV, 4.9 ± 1.1), Harderian glands (SUV, 2.2 ± 0.5), skeletal muscle (SUV, 2.0 ± 0.26), and myocardium (SUV, 1.7 ± 0.5).

Warming of the animals (Fig. 1A) reduced ^{18}F-FDG uptake of brown fat by 56% ($P = 0.0001$) and in skeletal muscle by 28% ($P = 0.0001$, Fig. 2). Fasting (Fig. 1B) reduced ^{18}F-FDG uptake of skeletal muscle by 31% ($P = 0.001$) and of brown fat by 32% ($P = 0.006$). Combining warming and fasting (Fig. 1C) reduced ^{18}F-FDG uptake of

brown fat by 67% ($P = 0.0001$) and of skeletal muscle by 47% ($P = 0.0001$) as compared with the reference condition. ^{18}F-FDG uptake by brown fat was significantly lower after combined warming and fasting than after fasting alone ($P = 0.001$). Conversely, ^{18}F-FDG uptake by skeletal muscle was significantly lower after combined warming and fasting than after warming alone ($P = 0.04$). The effects of warming and fasting on ^{18}F-FDG uptake by skeletal muscle were offset when no anesthesia was used during ^{18}F-FDG injection. Under this condition (Fig. 1D), ^{18}F-FDG uptake by muscle was not significantly different from the reference condition (SUV, 2.2 ± 0.4, $P = 0.23$). As expected, there was also a significant increase in cerebral ^{18}F-FDG uptake when no anesthesia was used for ^{18}F-FDG injection (SUV, 3.3 ± 0.9, $P = 0.04$).

^{18}F-FDG uptake by Harderian glands was not significantly influenced by warming or fasting but was markedly increased when no anesthesia was used for ^{18}F-FDG injection (SUV, 8.6 ± 1.9, $P < 0.001$). None of the other analyzed organs (kidney, myocardium, liver) showed significant differences in ^{18}F-FDG uptake in conscious mice. For myocardium and kidney, this was caused mainly by a large interindividual variability of tracer uptake within all study conditions. In contrast, mean liver ^{18}F-FDG uptake showed little variability within and across the different study conditions (Fig. 2).
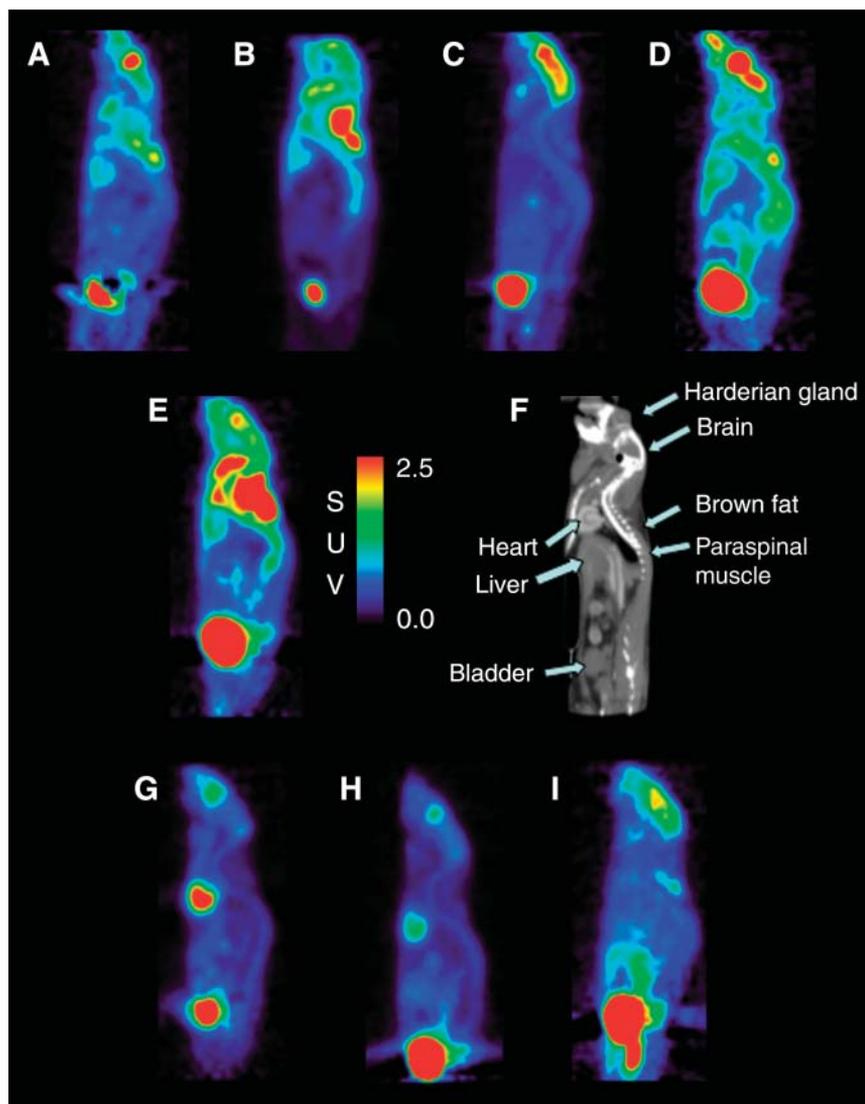
^{18}F-FDG Biodistribution in Anesthetized Mice

Isoflurane Anesthesia. Isoflurane anesthesia in nonfasted (Fig. 1G) mice caused a 5.5-fold increase of myocardial ^{18}F-FDG uptake, when compared with the reference condition ($P = 0.0001$, Fig. 3). Isoflurane anesthesia also caused a significant increase in ^{18}F-FDG uptake by liver and kidneys ($P < 0.002$, Fig. 3). In contrast, ^{18}F-FDG uptake in brown fat and skeletal muscle was markedly reduced (−92% and −67%, respectively, $P = 0.0001$).

When mice were fasted before isoflurane anesthesia (Fig. 1H), ^{18}F-FDG uptake by the myocardium was reduced by a factor of 3 compared with nonfasted animals, but still remained higher (1.7-fold, $P = 0.03$) than in the reference condition (no anesthesia, no fasting). Under isoflurane anesthesia, fasting had no significant effect on ^{18}F-FDG uptake by brown fat and muscle (Fig. 3).

Ketamine/Xylazine Anesthesia. Ketamine/xylazine anesthesia (Fig. 1I) had a similar effect as isoflurane on the ^{18}F-FDG uptake in brown fat, skeletal muscle, kidneys, and liver (Fig. 3). Interestingly, ketamine/xylazine had the opposite effect on myocardial ^{18}F-FDG uptake than isoflurane. Isoflurane increased myocardial ^{18}F-FDG uptake up to 5.5-fold, whereas ketamine/xylazine decreased myocardial ^{18}F-FDG uptake by 62%.

Comparison of Intravenous Versus Intraperitoneal Injection of ^{18}F-FDG

Figure 4 shows the time course of ^{18}F-FDG accumulation by various tissues after intraperitoneal and intravenous injection in fasted and warmed mice anesthetized by

FIGURE 1. Typical examples of biodistribution of ^{18}F-FDG under various conditions. Images show sagittal sections through mice. A contrast-enhanced microCT scan is shown for anatomic reference. (A) Not fasted, warmed, no anesthesia. (B) Fasted, not warmed, no anesthesia. (C) Fasted, warmed, no anesthesia. (D) Fasted, warmed, no anesthesia, conscious injection. (E) Reference conditions: not fasted, not warmed, no anesthesia. (F) microCT, sagittal view for anatomic reference. (G) Not fasted, warmed, isoflurane. (H) Fasted, warmed, isoflurane. (I) Fasted, warmed, ketamine.

isoflurane ($n = 6$ per group). Though tracer uptake is slower after intraperitoneal injection, all organs and the U251 tumors reach comparable activity concentrations within 60 min after injection. Similarly, no significant differences were found for tissue ^{18}F-FDG uptake of not-fasted and not-warmed mice that were not kept under anesthesia during the uptake period (Table 2, $n = 4$ per group). Thus, these data indicate that at 60 min after injection ^{18}F-FDG biodistribution is comparable for intravenous and intraperitoneal injection.

Impact of Warming, Fasting, and Anesthesia on Tumor Uptake of ^{18}F-FDG

In summary, our data in nontumor–bearing mice indicated that warming, fasting, and isoflurane anesthesia were likely to improve the biodistribution of ^{18}F-FDG for tumor imaging. We therefore evaluated whether fasting, warming, and isoflurane anesthesia significantly improve visualization of A431 and U251 tumor xenografts by reducing background activity. Groups of 3 (A431) and 6 (U251) tumor-

bearing mice each were imaged under the reference condition as well as after combined warming and fasting with and without isoflurane anesthesia. Because of the marked increase in blood glucose levels caused by ketamine/xylazine, no further experiments were performed with this form of anesthesia. At the time of imaging tumor size was 5–11 mm and did not show a significant difference among the 3 groups of animals. Figure 5 illustrates the biodistribution of ^{18}F-FDG in mice bearing A431 xenografts. Though tumors did not show focal ^{18}F-FDG above background activity under the reference condition, they demonstrated marked focal ^{18}F-FDG uptake, when animals were fasted and warmed. The average SUV of the tumors under reference conditions was 0.5 ± 0.1. In fasted, warmed, and not-anesthetized animals the tumor SUV was 3.7 times higher (1.8 ± 0.6, $P = 0.008$). Keeping fasted and warmed mice under isoflurane during the uptake period increased tumor SUV to the same level (1.60 ± 0.47, $P = 0.04$ for comparison with the reference condition).
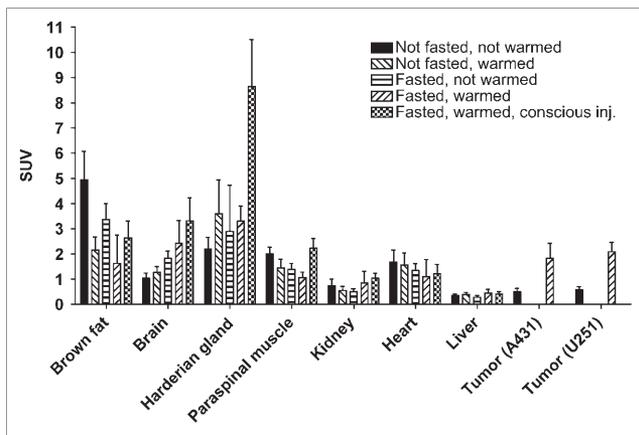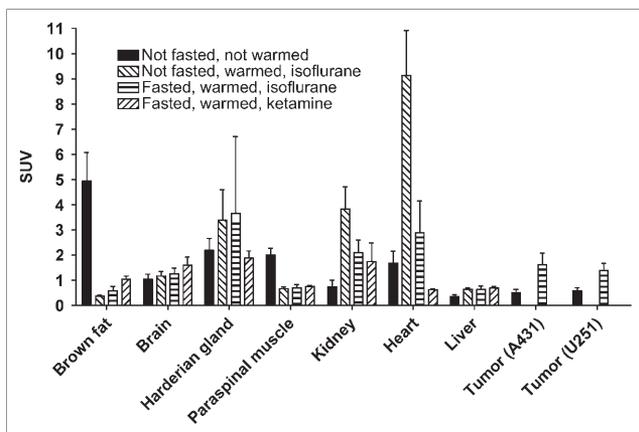
FIGURE 2. Biodistribution of ^{18}F-FDG in mice that were not anesthetized during uptake period for various studied conditions. Error bars show SD.

To assess image contrast, we calculated various tumor-to-organ ratios as shown in Table 3. Tumor-to-organ ratios were lowest under the reference condition. Warming and fasting (without isoflurane anesthesia) significantly improved the tumor-to-muscle ratio (7.9-fold, $P = 0.008$) and the tumor-to-brown fat ratio (17.4-fold, $P = 0.01$). A similar increase in ^{18}F-FDG uptake was seen in warmed, fasted, and anesthetized animals. Under this condition, the tumor-to-muscle ratio increased 8.3-fold ($P = 0.01$) and the tumor-to-brown fat ratio increased 15.5-fold ($P = 0.04$).

Experiments with mice bearing U251 xenografts provided similar results (Table 3). There was a highly significant increase in the tumor-to-muscle and tumor-to-brown-fat ratios in fasted and warmed mice ($P < 0.001$ in anesthetized and in not-anesthetized mice).

DISCUSSION

This study demonstrates that animal handling has a profound impact on the biodistribution of ^{18}F-FDG in mice


FIGURE 3. Biodistribution of ^{18}F-FDG in mice that were anesthetized during uptake period. Reference condition (not fasted, not warmed, no anesthesia during uptake period) is shown as a comparison. Error bars show SD.

and significantly influences the visualization of xenotransplanted tumors. Varying fasting state, body temperature, and mode of anesthesia caused >10-fold changes in the ^{18}F-FDG uptake of normal organs. Tumor ^{18}F-FDG uptake varied by a factor of 3.7. Depending on the conditions used for animal handling, tumors were either barely visible in the microPET studies or demonstrated marked focal ^{18}F-FDG uptake.

The influence of blood glucose and insulin levels on ^{18}F-FDG biodistribution is well known from human PET studies and previous studies in rats using tissue sampling to assess regional ^{18}F-FDG uptake (21–23). Because ^{18}F-FDG competes with glucose for intracellular transport and phosphorylation, tumor ^{18}F-FDG uptake decreases with increasing blood glucose levels. Furthermore, insulin markedly increases ^{18}F-FDG uptake by skeletal muscles and myocardium though it has generally no effect on ^{18}F-FDG uptake of cancer cells. Therefore, tumor ^{18}F-FDG uptake and image contrast are lower in the nonfasted state (high insulin and glucose levels) than in the fasted state (low insulin and glucose levels).

More recently the effect of ambient temperature on the ^{18}F-FDG uptake by brown adipose tissue has been described in patients (24). Our data show that the ambient temperature has a much more pronounced effect on ^{18}F-FDG biodistribution in mice. For mice the so-called zone of thermoneutrality lies between 30°C and 34°C (25). At this temperature body temperature is controlled by heat convection and no active processes are needed to maintain body temperature. At room temperature (21°C) mice need to generate heat by activation of brown adipose tissue and muscle activity to maintain a stable body temperature. Accordingly, metabolic rates have been shown to be 67% higher at room temperature (15 W/kg) than at the zone of thermoneutrality (9 W/kg) (25). Consistent with these previous observations, mice that were kept at 30°C showed markedly lower ^{18}F-FDG uptake by brown fat and muscle in our study (Fig. 2). Because the zone of thermoneutrality varies between different mouse strains (25) and we were unable to find specific data for SCID mice, we arbitrarily selected an ambient temperature of 30°C for our experiments. At higher temperatures a further reduction in ^{18}F-FDG uptake by brown adipose tissue might have been achieved. However, keeping mice above the zone of thermoneutrality represents a considerable heat stress. For example, C57BL/6J mice exposed to 39.5°C for 4 h demonstrate dehydration, hypoglycemia, and renal tubular necrosis (26). Therefore, we selected the lower end of the zone of thermoneutrality for our experiments.

^{18}F-FDG uptake by brown fat was also reduced by fasting the animals overnight (Figs. 1 and 2). It is known from previous studies that feeding increases the metabolic activity of brown fat in rodents. This is considered to represent a mechanism for stabilization of body weight: excess caloric intake is converted to heat by the brown adipose tissue. Conversely, overnight fasting has been shown to decrease
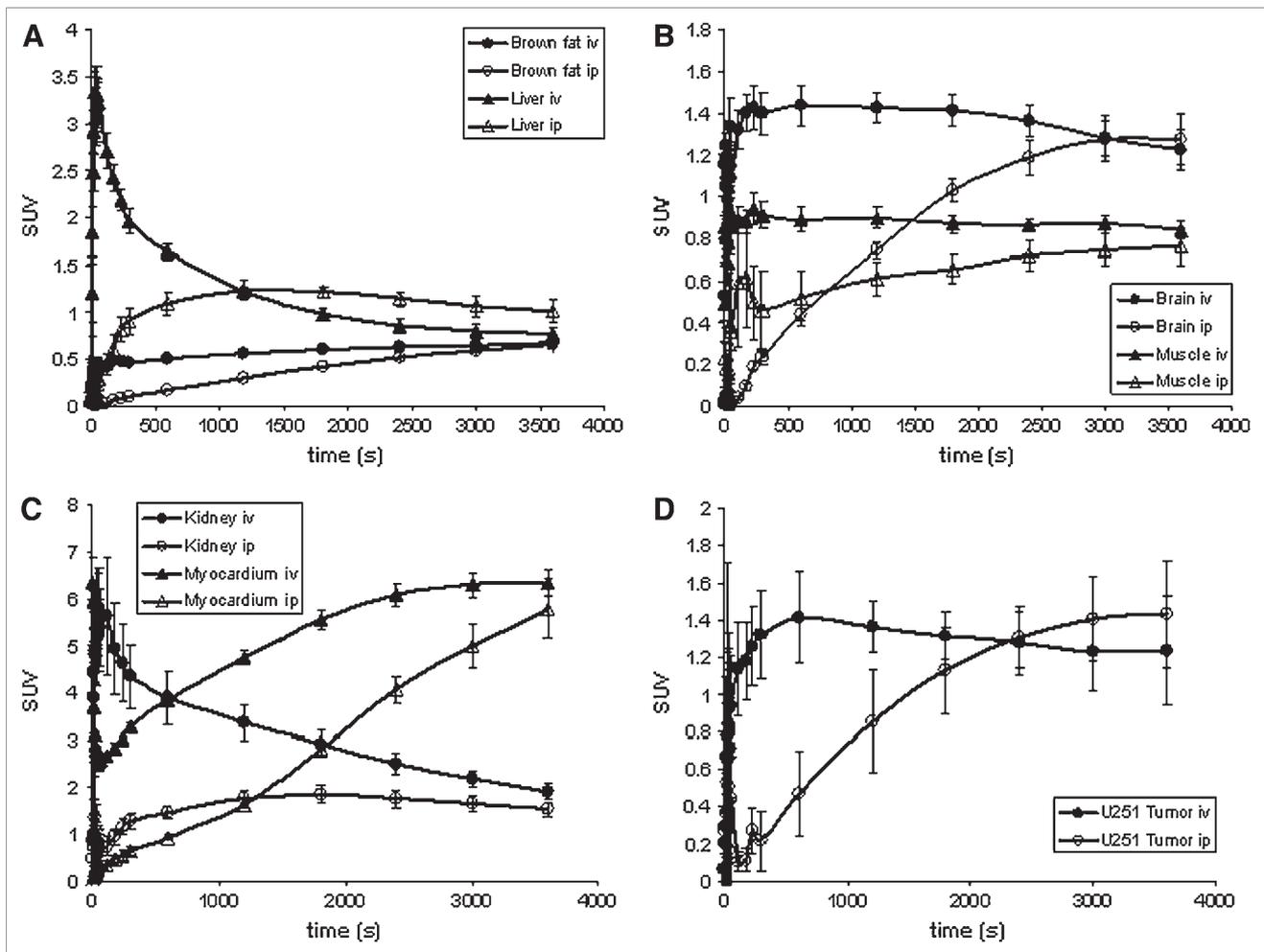
FIGURE 4. (A–D) ^{18}F-FDG uptake of various normal tissues and U251 xenografts after intravenous (iv) and intraperitoneal (ip) injection of ^{18}F-FDG ($n = 6$ per group). Error bars shown as SEs of the mean.

perfusion of brown adipose tissue as well as heat production (*27*).

In addition to its effect on ^{18}F-FDG uptake by normal tissues, warming and fasting lead to a >3-fold increase in tumor ^{18}F-FDG uptake. This finding is likely explained by a combination of lower plasma glucose levels and decreased ^{18}F-FDG uptake by normal organs.

The effect of anesthesia on ^{18}F-FDG biodistribution has very recently been studied by Lee et al. for ketamine/xylazine and pentobarbital (*28*). In the present study we extended these observations to isoflurane. Both xylazine and isoflurane are known to suppress insulin secretion (*28–30*).

However, the effects of xylazine appear to be much more pronounced, as xylazine induced marked hyperglycemia (>300 mg/dL) even when mice were fasted for at least 8 h. Fasting mice for 20 h before ^{18}F-FDG injection has been shown to attenuate xylazine-induced hyperglycemia (*28*). However, prolonged fasting leads to weight loss and may therefore be impractical, when animals need to be repeatedly imaged within a short period of time—for example, for treatment monitoring. In contrast to xylazine, isoflurane anesthesia caused only a modest increase in blood glucose levels in fasted and nonfasted animals, suggesting that its effect on insulin secretion is relatively mild.

TABLE 2
^{18}F-FDG Uptake of Various Tissues 60 Minutes After Intravenous and Intraperitoneal Injection of ^{18}F-FDG ($n = 4$ per group)

Injection	Brown fat	Brain	Muscle	Kidney	Myocardium	Liver
Intravenous	5.7 ± 0.5	1.7 ± 0.2	2.3 ± 0.02	0.9 ± 0.08	2.2 ± 0.05	0.53 ± 0.04
Intraperitoneal	6.5 ± 1.9	2.1 ± 0.7	3.5 ± 0.8	1.3 ± 0.9	2.7 ± 0.09	0.60 ± 0.2

Data are expressed in g/mL (SUV). No anesthesia was used during uptake period.
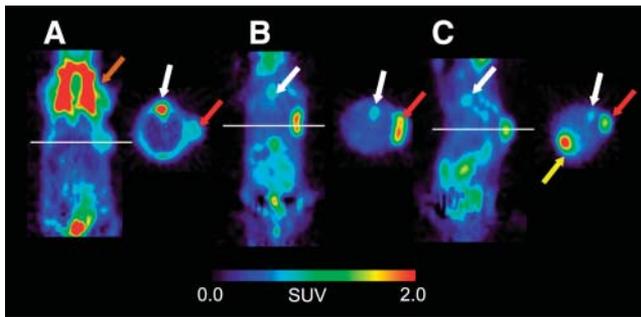
FIGURE 5. Tumor ^{18}F-FDG uptake under various conditions. Coronal and axial sections are shown. White lines in the coronal sections indicate position of axial sections. (A) Not fasted, not warmed, no anesthesia. (B) Fasted, warmed, no anesthesia. (C) Fasted, warmed, isoflurane anesthesia. Red arrow indicates tumor; brown arrow indicates brown fat; white arrow indicates paraspinal muscle; yellow arrow indicates myocardium.

In addition to affecting insulin secretion, anesthetic drugs also have specific effects on the glucose use of various tissues. Brown adipose tissue has a dense sympathetic innervation and its metabolic activity is regulated by β_3 and α_2 receptors. Activation of β_3 receptors increases and activation of α_2 receptors decreases perfusion and metabolic activity. Norepinephrine binds to both types of receptors, but its effect on β_3 receptors is predominant and norepinephrine markedly stimulates metabolic activity (*31*). Accordingly, ketamine, which increases norepinephrine plasma levels, has been shown to stimulate metabolic activity and ^{18}F-FDG uptake of brown adipose tissue (*32*). However, our data indicate that during anesthesia with a combination of ketamine and xylazine the effects of α_2 receptor stimulation by xylazine are predominant and lead to a marked decrease in ^{18}F-FDG uptake.

Isoflurane anesthesia also decreased ^{18}F-FDG uptake by brown adipose tissue, which is consistent with its inhibiting effect on thermogenesis by brown adipose tissue (*33*). As observed in a previous study for BALB/c mice, isoflurane markedly (up to 5.4-fold) increased ^{18}F-FDG uptake by the myocardium (*12*). The mechanisms underlying the high myocardial ^{18}F-FDG uptake during isoflurane anesthesia are currently unknown, but ^{18}F-FDG uptake could be significantly decreased by fasting of the animals and using isoflurane only during ^{18}F-FDG injection.

CONCLUSION

On the basis of our data we propose the following protocol for imaging tumor xenografts with ^{18}F-FDG. Mice should be fasted the night before the ^{18}F-FDG PET scan and warmed on a heating pad before and after ^{18}F-FDG injection. This approach markedly reduces ^{18}F-FDG uptake by brown adipose tissue and skeletal muscle, which otherwise significantly interferes with the visualization of tumor tissue. ^{18}F-FDG should be administered under anesthesia to further decrease skeletal muscle uptake. Keeping the mice under isoflurane anesthesia for the whole uptake period not only minimizes ^{18}F-FDG uptake by skeletal muscle and brown fat but also decreases blood clearance resulting in higher activity concentrations in liver and kidneys. Whereas isoflurane anesthesia demonstrates a favorable effect on ^{18}F-FDG biodistribution, ketamine/xylazine anesthesia should be used with caution as it induces marked hyperglycemia. Standardization of animal handling and anesthesia will be essential to ensure that reproducible and comparable data are obtained from ^{18}F-FDG PET scans of mice performed at different institutions.

TABLE 3
Tumor-to-Organ Ratios for Groups of Mice (n = 3–6) Imaged Under 3 Different Conditions

Tumor	Not fasted, not warmed, no anesthesia	Fasted, warmed, no anesthesia	Fasted, warmed, isoflurane anesthesia
A431			
Muscle	0.25 ± 0.07	1.97 ± 0.71*	2.08 ± 0.49*
Kidney	0.95 ± 0.49	1.83 ± 1.12	0.69 ± 0.13
Liver	1.37 ± 0.41	3.57 ± 1.47	2.21 ± 0.80
Brown fat	0.14 ± 0.04	2.44 ± 1.23*	2.17 ± 0.68*
U251			
Muscle	0.30 ± 0.07	1.90 ± 0.39*	1.83 ± 0.42*
Kidney	1.08 ± 0.27	1.86 ± 0.49*	0.94 ± 0.28
Liver	2.02 ± 0.48	4.61 ± 2.23*	1.45 ± 0.38
Brown fat	0.12 ± 0.02	2.58 ± 0.47*	2.21 ± 0.53*

*P < 0.05 for comparison with reference condition (Bonferroni post hoc test).

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