RESEARCH ARTICLE

Anesthesia and Preconditioning Induced Changes in Mouse Brain [18F] FDG Uptake and Kinetics

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Abstract

Purpose: 2-Deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) has been widely used for imaging brain metabolism. Tracer injection in anesthetized animals is a prerequisite for performing dynamic positron emission tomography (PET) scanning. Since preconditioning, as well as anesthesia, has been described to potentially influence brain [18F] FDG levels, this study evaluated how these variables globally and regionally affect both [18F] FDG uptake and kinetics in murine brain.

Procedures: Sixty-minute dynamic [18F] FDG PET scans were performed in adult male C57BL/6 mice anesthetized with isoflurane [control (in 100 % O_2), in medical air, in 100 % O_2 + insulin pre-treatment, and in 100 % O_2 after 18 h fasting], ketamine/xylazine, sevoflurane, and chloral hydrate. An additional group was scanned after awake uptake. Blood glucose levels were determined, and data was analyzed by comparing percent injected dose per cc tissue (%ID/cc) and glucose influx rate and metabolic rate (MRGlu) calculated by Patlak plot.

Results: Ketamine/xylazine and chloral hydrate anesthesia induced a lower whole-brain uptake of [18F] FDG (2.86 ± 0.67 %ID/cc, $p < 0.001$; 4.25 ± 0.28 %ID/cc, $p = 0.0179$, respectively) compared to isoflurane anesthesia (5.04 ± 0.19 %ID/cc). In addition, protocols affected differently distribution of [18F] FDG uptake in brain regions. Ketamine/xylazine reduced [18F] FDG influx rate in murine brain (0.0135 ± 0.0009 *vs* 0.0247 ± 0.0014 ml/g/min; $p < 0.005$) and chloral hydrate increased MRGlu (66.72 ± 3.75 *vs* 41.55 ± 3.06 µmol/min/100 ml; $p < 0.01$) compared to isoflurane. Insulin-pretreated animals showed a higher influx rate (0.0477 ± 0.0101 ml/min/g; $p < 0.05$) but a reduced MRGlu (21.92 ± 3.12 µmol/min/100 ml; $p < 0.01$). Blood glucose levels were negatively correlated to [18F] FDG uptake and influx rate, but positively correlated to MRGlu.

Conclusions: Choice of anesthesia and pre-conditioning affect not only [18F] FDG uptake but also kinetics and regional distribution in the mouse brain. Both anesthesia and pre-conditioning should be carefully considered in the interpretation of [18F] FDG studies due to its great influence on the uptake and distribution of the tracer along the brain regions.

Key words: Isoflurane, Ketamine, Sevoflurane, Chloral hydrate, Glucose

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Introduction

Positron emission tomography (PET) using radiolabeled tracers allows non-invasive, quantitative assessment of biologic, and physiologic processes. Main advantages of PET

include the possibility of longitudinal measurements reducing the number of animals and ease of translation between rodents, primates, and humans [1–4]. Small animal PET requires anesthesia due to long acquisition times, particularly with dynamic studies [1, 2]. Therefore, animals are usually subjected to anesthesia [2–5], most commonly using isoflurane inhalation. However, anesthesia is known to have significant effects on central nervous, cardiovascular, and respiratory systems, as well as on glycemia regulation [1].

2-Deoxy-2-[18F]fluoro-D-glucose [18F] FDG is the most frequently used tracer for imaging brain metabolism in both human and animal studies [1, 3, 4, 6]. Several studies using (14C) deoxyglucose autoradiography [3, 4, 7, 8] or static [18F] FDG PET [2–5, 9] have reported anesthesia effects on local cerebral glucose utilization in rodents. The most widely used quantification measure in [18F] FDG PET studies is semi-quantitative standardized uptake values (SUV) [2, 4]. However, variability in [18F] FDG uptake has been reported in mice [2–4, 6, 9], and several factors can affect uptake quantification in humans [10, 11] and rodents [2–4, 9]. Factors influencing tracer distribution in the rodent brain include effects of various anesthetics, fasting duration, route of [18F] FDG administration, blood glucose level [2–6, 9, 12, 13], and body temperature [9]. However, effects of anesthesia conditions on [18F] FDG brain kinetics and regional distribution are not completely understood.

Pharmacokinetic modeling provides a precise quantitative value, which accounts for altered tracer delivery. Kinetic modeling typically requires invasive catheterization to obtain a plasma blood input function [1, 2, 14], which is not recommended for longitudinal studies in small animals due to required blood volumes. Alternatively, an image-derived input function (IDIF) can be generated from a dynamic scan of the heart [15, 16] or inferior cava vein [17–19].

The aim of this study was to evaluate the effect of different preconditioning and anesthesia protocols routinely use in imaging and neuroscience protocols on [18F] FDG uptake and to test whether kinetic modeling can overcome any of those effects. In addition, we performed regional uptake analysis to gain information about structure-dependent effects of anesthesia and preconditioning.

Materials and Methods

Animals

Adult male C57Bl/6 mice ($n = 38$; Charles River Laboratories, Sulzfeld, Germany) were housed in individually ventilated BCU cages (Allentown, NJ, USA) under standardized environmental conditions (light-dark cycle of 14 h/10 h, temperature 22 ± 1 °C, humidity 50 ± 5 %). Aside from fasting periods, animals had free access to standard laboratory diet (Altromin, Lage, Germany) and to autoclaved water. All experiments are in accordance with the guidelines of the European Directive 2013/63/EU as well as German national laws and are approved by the local institutional

animal care and use committee. All efforts were made to minimize suffering and number of animals used.

We investigated effect of different pre-conditioning on [18F] FDG uptake and kinetics in the following groups: control group (isoflurane in 100 % O_2; $n = 8$); pre-treatment with insulin (6 mU/g, i.p.; humaninsulin normal 100, Lilly Pharma, Bad Homburg, Germany) + glucose (1 g/kg, i.p.; Berlin-Chemie AG, Berlin, Germany) 30 min before start of the scan ($n = 8$); isoflurane in 100 % O_2 after 18 h fasting in their home cage (n = 5); and isoflurane in medical air ($n = 5$) (Table 1).

In addition, we evaluated the effect of anesthesia in the following groups: control group (isoflurane in 100 % O_2; $n = 8$); sevoflurane in 100 % O_2 ($n = 6$); ketamine/xylazine anesthesia (120 mg/kg/20 mg/kg, i.p.; WDT, Garbsen, Germany; n = 4); and chloral hydrate anesthesia (400 mg/kg, i.p.; Sigma-Aldrich Darmstadt, Germany; $n = 6$). Furthermore, we added a group in which the animals were awake for [18F] FDG uptake and kept under isoflurane anesthesia only for a 10-min static PET scan (50–60 min after tracer injection; $n = 7$) (Table 1).

Small Animal PET Imaging

PET imaging was performed as described previously [20]. Briefly, blood glucose concentration was measured from a micropuncture of a peripheral vein just before [18F] FDG injection and at the conclusion of the PET scan using a glucose meter (Contour XT, Bayer Vital, Leverkusen, Germany). Then, animals were placed in a dedicated undocked small animal PET/CT scanner (Inveon, Knoxville, TN, USA). Body temperature was kept at 35–37 °C during the experiment and breathing frequency and heart rate were monitored (BioVet, m2m Imaging). Isoflurane and sevoflurane anesthesia were regulated in order to maintain a breathing frequency between 60 and 80/min (i.e., 1.5–2.5 %). Animals were injected with 8.19 ± 0.17 MBq of [18F] FDG directly after starting the PET scan, except in the awake uptake group. A second glucose measure was performed directly after the end of the PET scan. External source transmission scans were used for attenuation correction (paired 185-MBq Co-57 sources, 10-min acquisition).

List-mode data were histogrammed to 32 frames of 5×2, 4×5, 3×10, 8×30, 5×60, 4×300, and 3×600 s. A standard data correction protocol was applied (normalization and attenuation, scatter, and decay correction). Images were reconstructed using an ordered subset expectation maximization fast maximum *a posteriori* algorithm (OSEM3D/fastMAP, 2 iterations OSEM in 16 subsets, 18 iterations MAP, $\beta = 0.01$, Inveon Acquisition Workplace, Siemens). The reconstruction resulted in voxel sizes of $0.78 \times 0.78 \times 0.80$ mm.

Image Analysis

Image analysis was performed using Pmod 3.7 software (Pmod Technologies, Zürich, Switzerland). [18F] FDG uptake was evaluated at 50–60 min post-injection (last frame). Resulting PET images were co-registered to individual CT images, and

Table 1. Sample size of each experimental group: control group (left), pre-conditioning groups (middle), and anesthesia groups (right)

Experimental group	Pre-conditioning				Anesthesia			
	Control Isoflurane	Iso + insulin	Iso + fasting	Iso + medical air	Awake	Sevoflurane	Ketamine/xylazine	Chloral hydrate
Group size	8	8	5	5	7	6	4	6

CT was then fused to a MRI T2 template [21]. The PET image was then co-registered to the MRI template, and a VOI template [21] was applied. Mean uptake normalized to the injected dose (%ID/cc) of each VOI was calculated. To compare regional brain [18F] FDG uptake between groups, SUV_R (uptake in the region of interest divided by the uptake of the whole brain) were calculated for cortex, hippocampus, and cerebellum as defined by the mouse brain atlas in Pmod [21].

For the kinetic analysis, a VOI ($2 \times 2 \times 4$ mm) was drawn in the inferior cava vein as the left ventricle exhibited significant spill-in of activity from the myocardium, particularly in late frames (Suppl. Fig. 1a–b, see Electronic Supplementary Material (ESM)). Time activity curve (TAC) derived from the vena cava VOI was used as the input function [18]. TACs derived from brain regional VOIs were loaded in the Pmod Pkin tool together with the input function and fitted to the Patlak model [22]. The glucose influx rate (K_i) was calculated from the slope of the Patlak plot. The metabolic rate (MRGlu) was calculated as:

$$MRGlu = Ki \cdot \left(\frac{BG}{LC} \right)$$

where BG is the average blood glucose concentration, and LC is the lumped constant (0.67, as estimated for mice [19]). Because glucose uptake in the brain tended to plateau at 20–60 min after injection (Suppl. Fig. 1c, see ESM), only the first 15 min of the acquisition was considered for Patlak modeling.

Statistical Analysis

Data were analyzed using statistical software (Graphpad Prism 7, La Jolla, CA, USA). Animals anesthetized with isoflurane in 100 % O_2 were considered the standard control group. Glucose levels, tracer uptake, and kinetic parameters were compared using one-way analysis of variance (ANOVA) with Dunnett's *post hoc* test for multiple comparisons. Effect of the average blood glucose levels on the uptake and kinetic [18F] FDG data was investigated by Pearson's correlation analysis. *P* value below 0.05 was considered statistically significant. Data are presented as mean ± standard deviation (SD).

Results

Glucose Levels

Groups anesthetized with isoflurane, using either oxygen or medical air, and fasted animals showed similar average blood glucose levels (10.7 ± 0.7, 10.2 ± 1.2, and 12.5 ± 1.0 mmol/l, respectively) (Fig. 1a). Conversely, pre-treatment with insulin produced a distinct decrease in the blood glucose concentration compared to the control group (4.2 ± 0.6 mmol/l; $p < 0.001$).

Ketamine/xylazine induced a marked hyperglycemia (26.9 ± 0.9 mmol/l; $p < 0.001$) compared to isoflurane (Fig. 1b). Chloral hydrate raised glucose levels to a lesser extent compared to isoflurane anesthesia (15.8 ± 0.8 mmol/l; $p = 0.023$). Sevoflurane-anesthetized and awake-uptake animals did not show differences compared to isoflurane anesthesia.
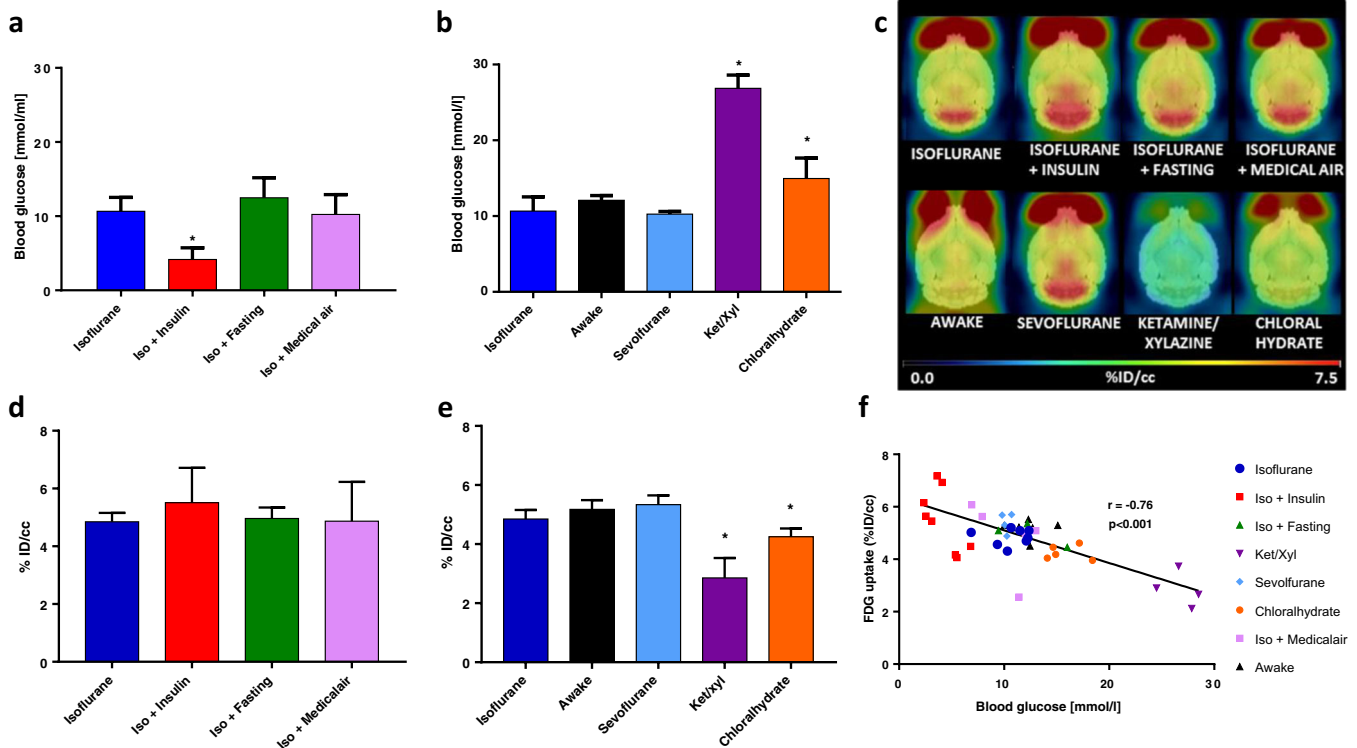
[18F] FDG Uptake

Whole brain [18F] FDG uptake under isoflurane anesthesia was 5.31 ± 0.14 %ID/cc (Fig. 1 through e). No whole brain uptake differences were found when comparing the different pre-conditioning groups to the control animals (Fig. 1d).

Ketamine/xylazine and chloral hydrate anesthesia resulted in a lower uptake of (18F) FDG compared to isoflurane (2.97 ± 0.34 %ID/cc, $p < 0.001$; 4.25 ± 0.28 %ID/cc, $p = 0.0179$, respectively; Fig. 1e). Furthermore, awake whole brain [18F] FDG uptake did not differ from uptake under isoflurane anesthesia.

Pearson regression analysis demonstrated an inverse correlation between whole brain [18F] FDG uptake and blood glucose level, across pre-conditioning and anesthesia protocols ($r = -0.76$; $p < 0.001$) (Fig. 1f).

Regional analysis revealed changes in [18F] FDG distribution to three brain regions (cortex, hippocampus, cerebellum) with changing anesthesia or metabolic preparation (Fig. 2). Insulin pretreatment resulted in lower cortex [18F] FDG SUV_R (0.79 ± 0.03 vs 0.85 ± 0.02; $p < 0.001$) but no difference in cerebellar and hippocampal SUV_R. By contrast, fasting reduced cortex normalized uptake (0.81 ± 0.02; $p = 0.028$) and the use of medical air did not change [18F] FDG SUV_R in those three brain regions.

Compared to isoflurane anesthesia, SUV_R was elevated in cortex under ketamine/xylazine (0.99 ± 0.02; $p < 0.001$) and chloral hydrate (0.99 ± 0.03; $p < 0.001$), as well as awake uptake (1.03 ± 0.03; $p < 0.001$). By contrast, SUV_R in cerebellum was lower with ketamine/xylazine (0.97 ± 0.02; $p < 0.001$), chloral hydrate (0.97 ± 0.02; $p < 0.001$), and no anesthesia (0.98 ± 0.05; $p < 0.001$) compared to isoflurane-anesthesia. [18F] FDG SUV_R under sevoflurane were directly comparable to isoflurane anesthesia in all regions analyzed. In hippocampus, only awake uptake showed

Fig. 1. Blood glucose levels and whole brain [18F] FDG uptake. Mean blood glucose values of **a** pre-conditioning experimental groups and **b** anesthesia experimental groups. **c** Averaged horizontal brain [18F] FDG uptake images between 50 and 60 min after the injection of the tracer fused to a T2-MRI template. Mean [18F] FDG uptake (%ID/cc) in **d** pre-conditioning and **e** anesthesia experimental groups. Every experimental group was compared to the control group (isoflurane anesthesia without pre-conditioning). Data expressed in mean ± SD. Statistical differences assessed by ANOVA and Dunnet's *post hoc* test. *$p < 0.05$. **f** [18F] FDG uptake values of each animal relative to its blood glucose concentration. R and p values calculated by Pearson correlation test.

differences when compared to isoflurane (0.91 ± 0.09 *vs* 1.00 ± 0.02; $p = 0.015$).

[18F] FDG Kinetics

Patlak analysis indicated that pre-treatment with insulin increased whole brain [18F] FDG influx rate compared to control animals (0.0452 ± 0.0213 *vs* 0.0250 ± 0.0041 ml/g/ min; $p = 0.010$) (Fig. 3a and e). No differences were found in slope of fasted or medical air carried isoflurane.

Ketamine/xylazine as anesthetic reduced whole brain [18F] FDG Patlak K_i (0.0135 ± 0.0018 ml/g/min; $p < 0.001$) compared to the isoflurane control group (Fig. 3b, e). Sevoflurane- and chloral hydrate-anesthetized animals showed no differences to the isoflurane control group. [18F] FDG Patlak K_i showed an inverse correlation to the individual blood glucose levels ($r = -0.60$; $p < 0.001$; Fig. 3g).

Insulin pre-treatment reduced significantly MRGlu in the whole brain (24.78 ± 8.35 μmol/min/100 ml; $p = 0.004$) (Fig. 3d, f). Ketamine/xylazine and chloral hydrate anesthesia altered MRGlu, which was higher (51.99 ± 7.09 μmol/ min/100 ml, $p = 0.03$ and 66.72 ± 9.18 μmol/min/100 ml, $p < 0.001$, respectively) compared to isoflurane ($38.28 \pm$

8.57 μmol/min/100 ml) (Fig. 3c, f). As expected, MRGlu was positively correlated to the blood glucose concentration ($r = 0.67$; $p < 0.001$; Fig. 3h).

Discussion

In this study, we present the effects of different pre-conditioning and anesthesia on [18F] FDG uptake and kinetics in the murine brain. Both uptake and kinetics were highly dependent on blood glucose levels. In addition, this study revealed that anesthesia alters [18F] FDG uptake to different extents in different brain regions, which may be obscured by whole brain analysis.

The influence of blood glucose on [18F] FDG brain uptake is well known from human and rodent studies [3, 4, 9, 23, 24]. In our study, [18F] FDG uptake was inversely proportional to the blood glucose increase. Since [18F] FDG and glucose share metabolic route, higher blood glucose concentration competes for the transport and phosphorylation with [18F] FDG leading to a decreased uptake of the tracer [24, 25]. This is an important consideration for interpretation of images from animal models and treatment studies for which blood glucose levels are affected.
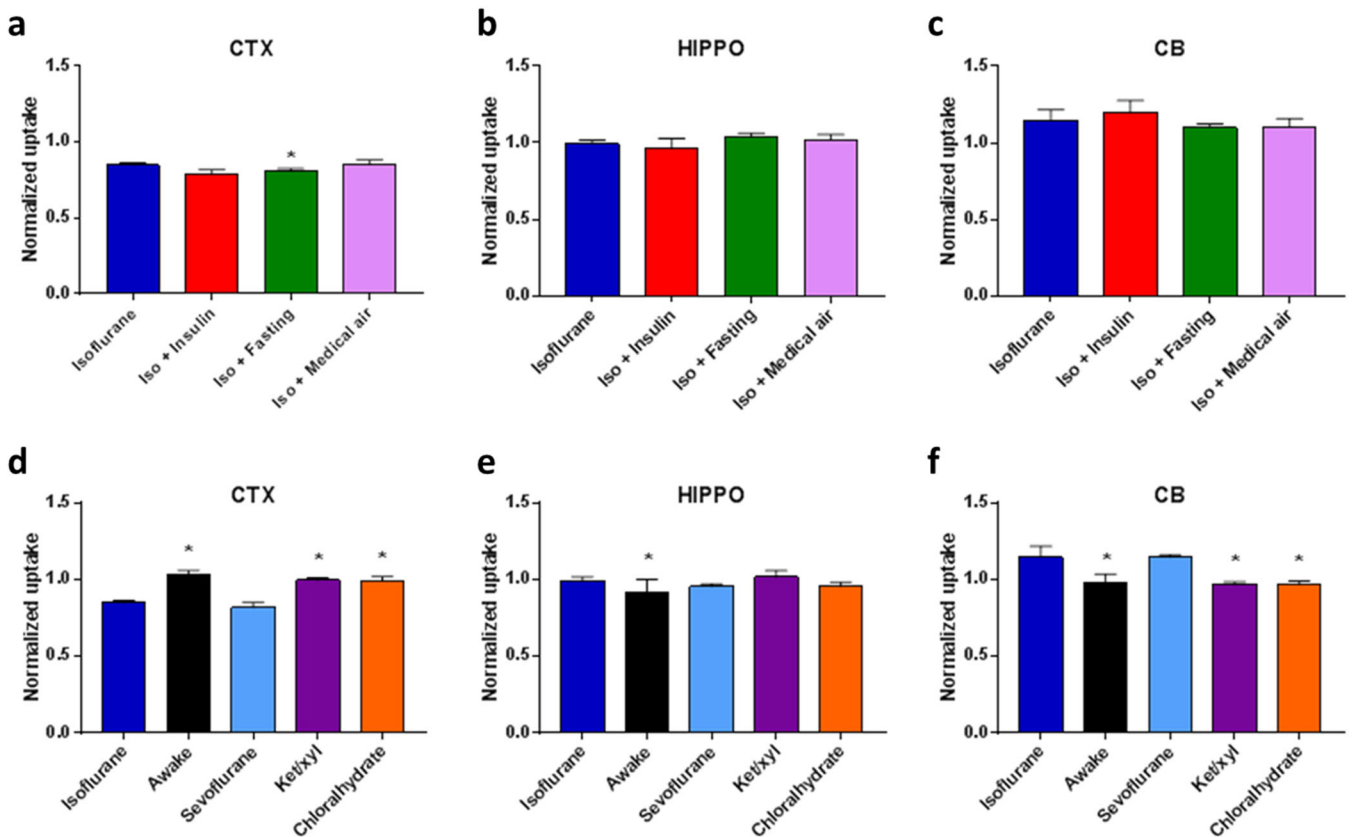
Fig. 2. Regional [^{18}F] FDG SUV$_R$ values (50–60 min) in experimental groups compared to the control group (isoflurane anesthesia). Bar graphs showing [^{18}F] FDG uptake quantification in **a** cortex (CTX), **b** hippocampus (HIPPO), and **c** cerebellum (CB) after pre-conditioning (**a**–**c**) and with different anesthesia protocols: **d** CTX, **e** HIPP, and **f** CB. Data expressed in mean ± SD. Statistical differences *versus* isoflurane-anesthetized control group assessed by ANOVA and Dunnet's *post hoc* test. *$p < 0.05$.

Animals that were awake during the [^{18}F] FDG uptake phase, most closely reflecting the clinical situation, showed similar values of whole brain [^{18}F] FDG uptake to isoflurane-anesthetized mice, supporting the routine use of isoflurane anesthesia in preclinical imaging studies. However, ketamine/xylazine and chloral hydrate exhibited lower [^{18}F] FDG uptake, proportional to the blood glucose level increase. Ketamine/xylazine induced pronounced hyperglycemia due to a known pharmacological effect of xylazine, an alpha-2-adrenergic receptor agonist that activates adrenergic receptors of pancreatic islet cells and blocks insulin release to the blood leading to a hyperglycemic state [26]. We observed that chloral hydrate induces a hyperglycemia, to a lesser degree than ketamine/xylazine anesthesia, a finding in good agreement with earlier studies [3–5, 27, 28]. As expected, these changes in blood glucose concentration affected ^{18}F-FDG uptake.

Contrary to other published studies, which reported lower glycemia in fasted mice [2, 13], we did not observe blood glucose differences after fasting for 18 h. As the animals remained in their home cages in our study, the fasting protocol may have been impeded by coprophagia and/or ingestion of bedding, affecting the blood glucose level [29]. Pre-treatment with insulin and glucose before [^{18}F] FDG

administration induced an expected marked hypoglycemia. However, insulin did not affect the average [^{18}F] FDG uptake in the whole brain. We observed faster washout in animals pre-treated with insulin. Therefore, at the time point the whole brain uptake was calculated (50–60 min), the effect of the increased influx is diluted, resulting in non-significant different uptake compared to the control group.

Importantly, regional analysis revealed specific alterations in response to alternative anesthetic. Isoflurane and sevoflurane anesthetized animals displayed a lower cortex uptake than awake animals when normalizing to whole brain uptake. However, cerebellum uptake ratio was higher with isoflurane and sevoflurane. Isoflurane is known to reduce cortical [^{18}F] FDG uptake dramatically, mainly due to a decreased [^{18}F])FDG phosphorylation [30, 31]. Here, we observed a similar effect with sevoflurane anesthesia. Uptake with ketamine/xylazine and chloral hydrate showed similar distribution of [^{18}F] FDG to awake uptake, with higher cortical and lower cerebellum uptake compared to isoflurane. Surprisingly, insulin pre-treatment induced changes in the regional [^{18}F] FDG distribution, reducing relative uptake in cortex. This result may reflect higher [^{18}F] FDG brain influx in pre-treated animals due to insulin anabolic state, which would accentuate the inter-regional
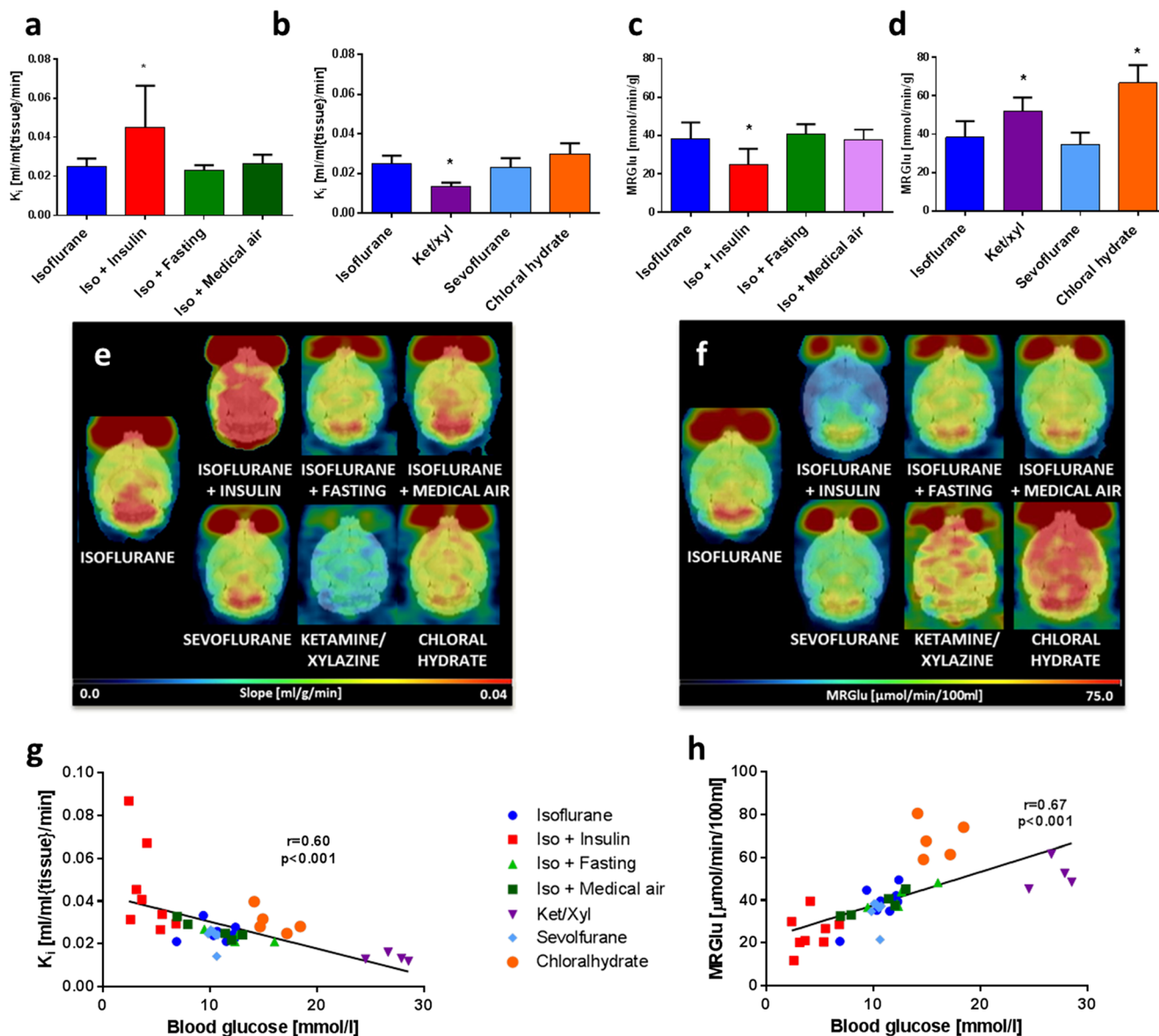
Fig. 3. Averaged whole brain influx rate value (K_i) of **a** pre-conditioning experimental groups and **b** anesthesia experimental group and averaged glucose metabolic rate (MRGlu) in the same groups (**c** and **d**, respectively). Data is expressed in mean ± SD. Statistical differences assessed by ANOVA and Dunnet's *post hoc* test. *$p < 0.05$. Averaged parametric maps for **e** [18F] FDG kinetics parameters (K_i) and **f** MRGl. Dot-plot graph showing correlation between individual blood glucose concentration and **g** [18F] FDG kinetics parameters (Ki) and **h** MRGlu. R and p values calculated by Pearson correlation test.

differences under isoflurane anesthesia. These differences in [18F] FDG regional distribution are important considerations, whereby specific regional information may be accentuated by selecting the appropriate anesthetic.

In addition to affecting whole and region brain uptake at 50–60 min after injection, [18F] FDG kinetics were also influenced by anesthesia and metabolic conditions. Isoflurane control animals showed comparable MRGlu values to previously reported studies [32]. Whole brain [18F] FDG Patlak K_i was only altered by ketamine-xylazine anesthesia, which displayed slower uptake possibly due to hyperglycemia and transporter competition [24, 25]. Conversely, chloral hydrate did not affect

[18F] FDG influx rate, but did increase [18F] FDG MRGlu. Both kinetic parameters calculated in this study, [18F] FDG influx rate and MRGlu, correlated well with blood glucose levels, suggesting that kinetic modeling of [18F] FDG in the brain is not independent from glucose concentration. Interestingly, the correlation between blood glucose values and MRGlu in chloral hydrate anesthetized animals deviated significantly from the regression line. Indeed, the shape of the whole brain TAC was notably different from the other anesthesia groups, whereby the wash out phase in the first 15 min was absent. Chloral hydrate is consider to produce hypnosis and not anesthesia [33] which may explain the lack of

wash-out phase [34] and, by extension, the differences in [¹⁸F]FDG kinetics. By contrast, insulin pretreatment had an opposite effect on [¹⁸F] FDG kinetics, accelerating tracer influx in the first 15 min after injection, probably due to the low blood glucose concentration observed in this group.

Some limitations of this study should be noted. First, the input function was derived from the vena cava. While IDIF may not be as precise as blood sampling input function, it is a more practical approach and frequently used in small animal studies. We used the vena cava rather than the left ventricle cavity due to significant spill-in from the myocardium in late frames. It should be noted that the small dimension of the vena cava renders it susceptible to partial volume effects. Nonetheless, previous work has demonstrated the robustness of the fixed volume vena cava IDIF for Patlak modeling in the myocardium [18], which appears to translate for the brain. Secondly, several atlas-defined murine brain regions are very small, at the limit of small animal PET resolution (~ 1.2-mm FWHM). Accordingly, we selected three of the largest brain structures (cortex, hippocampus, and cerebellum) for our regional analysis, such that partial volume effects were minimized. In addition, we used a fixed lumped constant for all our experimental groups although this constant is known to vary in hypo- and hyperglycemic conditions [35, 36]. However, we decided to standardize all the parameters used for kinetic analysis, including the lumped constant, to reproduce the standard situation in an imaging laboratory.

Conclusion

Choice of anesthesia and pre-conditioning affect not only [¹⁸F] FDG uptake but also kinetics and regional distribution in the mouse brain. Both anesthesia and pre-conditioning should be carefully considered in the interpretation of [¹⁸F] FDG studies due to its great influence on the uptake and distribution of the tracer along the brain regions.

Compliance with Ethical Standards

Conflict of Interest

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