ORIGINAL ARTICLE

Noninvasive quantification of cerebral metabolic rate for glucose in rats using ^{18}F -FDG PET and standard input functionYuki Hori^{1,2}, Naoki Ihara^{1,2}, Noboru Teramoto^{3,4}, Masako Kunimi³, Manabu Honda², Koichi Kato^{1,2} and Takashi Hanakawa^{1,2}

Measurement of arterial input function (AIF) for quantitative positron emission tomography (PET) studies is technically challenging. The present study aimed to develop a method based on a standard arterial input function (SIF) to estimate input function without blood sampling. We performed ^{18}F -fluorodeoxyglucose studies accompanied by continuous blood sampling for measurement of AIF in 11 rats. Standard arterial input function was calculated by averaging AIFs from eight anesthetized rats, after normalization with body mass (BM) and injected dose (ID). Then, the individual input function was estimated using two types of SIF: (1) SIF calibrated by the individual's BM and ID (estimated individual input function, EIF^{NS}) and (2) SIF calibrated by a single blood sampling as proposed previously (EIF¹⁵). No significant differences in area under the curve (AUC) or cerebral metabolic rate for glucose (CMRGlc) were found across the AIF-, EIF^{NS}-, and EIF¹⁵-based methods using repeated measures analysis of variance. In the correlation analysis, AUC or CMRGlc derived from EIF^{NS} was highly correlated with those derived from AIF and EIF¹⁵. Preliminary comparison between AIF and EIF^{NS} in three awake rats supported an idea that the method might be applicable to behaving animals. The present study suggests that EIF^{NS} method might serve as a noninvasive substitute for individual AIF measurement.

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INTRODUCTION

Positron emission tomography (PET) combined with ^{18}F -fluorodeoxyglucose (^{18}F -FDG) has been widely used to examine the regional cerebral metabolic rate for glucose (CMRGlc) in the brain. The technique has contributed to a number of studies investigating the metabolic correlates of neuronal/synaptic/glia activity in living humans as well as in small animals, as reported previously.^{1–6} The quantitative measurement of CMRGlc with PET commonly involves arterial blood sampling to measure the radio-ligand concentration in plasma as a function of time, yielding an arterial input function (AIF). However, determination of the AIF is particularly challenging in small animal, as it is technically difficult to insert a cannula into a small artery and then to draw multiple blood samples without disturbing the animal's physiologic status. It is also difficult to measure the AIF during behavior such as learning and attention tasks without disturbing animals.

Previous studies proposed noninvasive estimation of the AIF in humans. One approach is based on the computation of an image-derived input function (IDIF) using a dynamic PET image of the carotid artery or the left cardiac ventricle.^{7–9} In small animal PET studies, however, the limited spatial resolution of the PET technique complicates the image-derived input function approach with partial volume effects and spillover activity from surrounding tissue.

Additionally, the brain and the heart may not be simultaneously covered during a single PET scan. An alternative approach involves a population-based standard arterial input function (SIF). This method relies on the observation that the shape of the AIF is reasonably comparable among different subjects for a given radio-ligand administration protocol. Several investigators have already validated the SIF approach for human use.^{10–13} Furthermore, a study reported the application of the SIF approach to CMRGlc measurement in small animals with one or two arterial blood samples for scaling the SIF, thereby allowing for accurate estimation of the AIF in each subject's level.¹⁴ It is thus indicated that the SIF technique with a few arterial blood samples may serve as a simplified quantification method of CMRGlc in small animals. Nevertheless, it is still technically challenging to obtain arterial blood samples even a few times for such experimental set-ups as measurement of CMRGlc while performing tasks.^{15,16} It would be ideal to develop a completely noninvasive method for computing accurate estimation of the AIF in small animals without blood sampling. Such a technical advent will enable the quantification of CMRGlc during active tasks such as skill learning and attention tasks in small animals.

The aim of this study was to develop a method for SIF-based estimation of the AIF and then to test the feasibility of the

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noninvasive method for quantifying CMRGlc in rats. First, the SIF was calculated by averaging AIFs from eight anesthetized rats. Second, an individual's AIF was estimated by modifying the SIF with each individual's body weight and injected dose (ID). Third, we compared the area under the curve (AUC) of the input functions and the CMRGlc values among the newly proposed SIF-based method, the conventional SIF-based method, and the AIF. Finally, to test the impact of anesthesia on the proposed SIF-based method, AIFs were measured in awake and restrained rats and were compared with estimated AIFs, using the SIF derived from the anesthetized rat group.

MATERIALS AND METHODS

Animal Preparation

Eleven 9-week-old male Long-Evans rats (315 ± 9 g body weight (mean ± s.d.; range 292 to 365 g)) were used in this study (Institute for Animal Reproduction, Kasumigaura, Japan). All animal experiments were performed under approval of the Animal Care and Use Committee, National Institute of Neuroscience, National Center of Neurology and Psychiatry, and were performed in accordance with guidelines for the care and use of laboratory animals. Experiments were reported according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

The rats were anesthetized with a mixture of midazolam (0.5 mg/kg body weight) and xylazine (2.0 mg/kg body weight) intramuscularly as a preanesthesia. Thereafter, under anesthesia by inhalation of 2% isoflurane, polyethylene tubes (PE 50, inner diameter of 0.54 mm) were inserted into the femoral artery and vein for an arteriovenous (AV) shunt. The length of the tubes was 55 and 35 cm for the left femoral artery and vein, respectively, and 10 cm of the ends of the two lines was connected to a thicker polyethylene tube (PE 100, inner diameter of 0.86 mm) to form an AV shunt. This AV-shunt method has already been validated in a recent study.¹⁷ A 26 G venous catheter was also inserted into a tail vein for the administration of ¹⁸F-FDG. Rats were fasted for at least 5 hours before the start of the experiment. Eight out of the eleven rats were used to establish a noninvasive method for quantifying CMRGlc and to test its feasibility in anesthetized rats. The remaining three rats were used to test the impact of anesthesia on AIF and to preliminarily examine whether the SIF-based method developed in anesthetized rats can be applied to awake rats.

Data Acquisition

A PET scanner for small animals was used (Clairvivo, Shimadzu Corporation, Kyoto, Japan). The specifications and physical performance of this scanner were reported in a previous article.¹⁸ The rats were anesthetized by inhalation of 2% isoflurane through a mask, and they were positioned on supine with their brains centred in the field-of-view of the PET scanner. A part of the PE 50 tube inserted into the femoral artery was used for continuous measurement of the AIF using a Gd₂SiO₅:Ce (GSO) detector (GSO input function monitor system, Molecular Imaging Laboratory, Osaka, Japan).¹⁹

In the eight anesthetized rats, a PET scan was performed over 90 minutes in a list mode, after ¹⁸F-FDG (39 ± 12 MBq; 0.5 mL injection volume) administration to the tail vein as short-term infusion over 30 seconds. The AIF was also measured over 90 minutes by the GSO detector. After the PET scan, glucose concentration was measured in a blood sample collected from the tail vein, using a blood glucose meter (Blood Glucose Monitoring System, Fora Care Inc., CA, USA). The three awake rats were fixed into a dedicated animal restrainer with a bite bar and a neck holder, and AIFs were measured with GSO detector during an awake condition, after ¹⁸F-FDG (31 ± 7.6 MBq; 0.5 mL injection volume) administration to the tail vein. However, PET scanning was not carried out for these three awake rats since the animal restrainer did not fit into the PET scanner; therefore, CMRGlc data were not available for the awake rat group.

Data Processing

The dynamic PET images were reconstructed using a two-dimensional filtered-back-projection algorithm from the rebinned sinogram, obtained using the Fourier Rebinning.²⁰ The matrix size in the reconstructed images was 128 × 128 × 213. The voxel size was 0.8 × 0.8 × 0.7 mm³. The dynamic images consisted of 12 frames of 5 seconds, 18 frames of 30 seconds, 10 frames of 60 seconds, 2 frames of 300 seconds, and 6 frames of 600 seconds. Corrections were implemented in the rebinning or reconstruction

process for the detector normalization, the random coincidence, the dead time, the radioactive decay, the attenuation, and the scatter. The AIFs were obtained from the activity concentration data measured by the GSO detector, corrected for the physical decay of ¹⁸F, cross calibration between the GSO detector and PET scanner, and calibration between the activity in whole blood and in plasma count.²¹

Data Analysis

The SIF was investigated using a leave-one-out cross validation (LOOCV) procedure. Specifically, an SIF was computed for each of eight rats by averaging the normalized AIF of the remaining seven rats as described below.¹⁰

$$SIF_i(t) = \frac{\sum_{j=1, j \neq i}^n ((BM_j/ID_j) \times AIF_j(t))}{n-1}, \quad (1)$$

where t is the elapsed time from the ¹⁸F-FDG injection, i is the animal identity number, BM_j and ID_j are the body mass and the injection dose in animal j , respectively, and n is the number of animals. We normalized the AIF using BM and ID before averaging the AIF because the concentration of radioactivity in plasma is obtained by dividing the ID by the distribution volume of ¹⁸F-FDG and then the distribution volume is proportional to BM.^{10,22}

Using SIF, ID, and BM, we estimated an individual input function without using information from the blood sampling (estimated individual input function, EIF^{NS}):

$$EIF_i^{NS}(t) = \left(\frac{ID_i}{BM_i} \right) \times SIF_i(t). \quad (2)$$

We also generated another EIF¹⁵ using a single arterial plasma count of 6 minutes after ¹⁸F-FDG injection for scaling the SIF as proposed previously.¹⁴

Area under the curve in the range from $t=0$ minutes to $t=45$ minutes (middle of the scanning time) after ¹⁸F-FDG injection is usually employed to calculate CMRGlc. We hence tested the validity of the EIF^{NS} based on the SIF as follows: (1) AUC was compared among AIF, EIF^{NS}, and EIF¹⁵ in the range from $t=0$ minutes to $t=45$ minutes after the ¹⁸F-FDG injection and (2) CMRGlc was compared among AIF, EIF^{NS}, and EIF¹⁵. Cerebral metabolic rate for glucose (CMRGlc) was calculated as described below.²³

$$CMRGlc = \frac{C_p \times (K_1 \times k_3)}{LC \times (k_2 + k_3)} = \frac{C_p}{LC} \times K, \quad (3)$$

where K_1 is the rate constant for the unidirectional transport of FDG from plasma to brain, k_2 and k_3 are the rate constants for the return to the plasma compartment and for the phosphorylation of FDG, respectively, LC is the lumped constant (the correction factor for the use of glucose rather than FDG in the brain),²⁴ and C_p is the concentration of glucose in blood. A LC of 0.625 was used¹⁴ and K was estimated using the input function and radioactivity concentration in the whole brain regions of PET dynamic data by Patlak graphical methods.²⁵

All statistical tests were performed using PASW Statistics 18 software (IBM, Armonk, New York, NY, USA). In the eight anesthetized rats, we compared the AUC across AIF, EIF^{NS}, and EIF¹⁵, using repeated measures analysis of variance (RM-ANOVA). Additionally, the AUC was compared across the AIF, EIF¹⁵, and the EIF^{NS} methods, using a correlation analysis across subjects after LOOCV. Regression lines were drawn to evaluate the relationships between a pair of the two different methods. Similarly, we evaluated the CMRGlc derived from the AIF-, EIF^{NS}-, and EIF¹⁵-based methods. Finally, we used Bland-Altman analysis to assess CMRGlc between the EIF^{NS} and AIF or between the EIF^{NS} and EIF¹⁵ across all subjects. In these comparisons, a threshold of $P < 0.05$ was used to infer statistical significance.

To test the impact of anesthesia on the proposed EIF^{NS} method, the AIFs measured from the three awake rats were compared with EIF^{NS} according to the SIF computed from the eight anesthetized rats.

RESULTS

After the normalization with ID and BM, the time-activity curve of the AIF showed a high level of similarity across measurements in different rats (Figure 1). The peak time of averaged AIFs was 30.75 ± 5.15 seconds (mean ± s.d.) and it ranged between 25 and 41 seconds. The SIF used for a particular rat was calculated by averaging the normalized AIFs derived from the remaining seven

rats (LOOCV, see equation 1), and then the EIF^{NS} was calculated using the SIF scaled by ID and body weight (equation 2). The calculation of the SIF was thus iterated eight times. Using the same LOOCV procedure, we also computed the EIF¹⁵ using the individual-specific SIF scaled by a single arterial plasma count.

The AUCs based on AIF, EIF^{NS} and EIF¹⁵ were 103 ± 39 , 102 ± 34 , and 104 ± 46 (MBq seconds/mL) (mean $\pm$ s.d.), respectively (Table 1). No difference in the AUC was found across the AIF-, EIF^{NS}-, and EIF¹⁵-based methods ($F_{[2]} = 0.026$, $P = 0.975$ by RM-ANOVA). We then tested the correlation of the AUC of the EIF^{NS} with that of the AIF, or that of the EIF¹⁵. The results showed that the AUC of the EIF^{NS} was highly correlated with that of the AIF and also that of the EIF¹⁵ (Pearson correlation coefficients of 0.924 and 0.854, respectively). The regression line between the EIF^{NS} and the AIF was expressed as $AUC(AIF) = 1.07 \times AUC(EIF^{NS}) - 3.18$ ($R^2 = 0.88$, $P < 0.01$) (Figure 2). The intercept was not significantly different from zero ($P = 0.855$), and the slope of the line was close to unity ($P < 0.01$). The average difference in the AUC between the EIF^{NS} and the AIF was $-3.38 \pm 11.50\%$ (mean $\pm$ s.d.; range -20% to 13%). These results showed that the AUC showed good agreement between the EIF^{NS} and the AIF. Meanwhile, the average difference in the AUC between the EIF^{NS} and EIF¹⁵ was $-1.62 \pm 12.29\%$ (mean $\pm$ s.d.; range -17% to 21%), and the regression line was expressed as follows: $AUC(EIF^{15}) = 1.03 \times AUC(EIF^{NS}) - 4.06$ ($R^2 = 0.91$, $P < 0.01$). Again, the intercept was not significantly different from zero ($P = 0.782$), and the slope of the line was close to unity ($P < 0.01$). Hence, the AUC of the EIF^{NS} was comparable to the AUC of the EIF¹⁵. The correlation of AUC between the AIF and the EIF^{NS}, along with that between the AIF and the EIF¹⁵, is shown in Figure 3. These regression lines were expressed as $AUC(EIF^{NS}) = 0.82 \times AUC(AIF) + 14.3$ ($R^2 = 0.88$) and $AUC(EIF^{15}) = 0.88 \times AUC(AIF) + 7.16$ ($R^2 = 0.90$). The intercept was not significantly different from zero in either method, and the slope of the regression line was close to unity ($P < 0.01$).

The individual CMRGlc values were calculated using the concentration of glucose in blood and the rate constant K , which was obtained from a Patlak Plot using the input function and PET dynamic data. The average glucose level of all studies was 9.65 ± 0.06 (mmol/L) (mean $\pm$ s.d.). The CMRGlc values obtained by AIF, EIF^{NS}, and EIF¹⁵ were 70 ± 14 , 67 ± 16 , and 67 ± 14 ($\mu\text{mol}/100 \text{ g}$ per minute), respectively (mean $\pm$ s.d.) (Table 1). No significant differences were observed among the CMRGlc of AIF, EIF^{NS}, and EIF¹⁵ by RM-ANOVA ($F_{[2]} = 0.917$, $P = 0.422$). The CMRGlc of the EIF^{NS} was highly correlated with that of the AIF and that of the EIF¹⁵ (Pearson correlation coefficients of 0.956 and 0.911, respectively). Figure 4A shows the correlation of CMRGlc calculated using the EIF^{NS} and the AIF. The CMRGlc computed from the two methods were highly correlated ($\text{CMRGlc}[AIF] = 0.84 \times \text{CMRGlc}[EIF^{NS}] + 13.08$ [$R^2 = 0.91$, $P < 0.01$]). The intercept was not significantly different from zero ($P = 0.122$), and the slope of the line was close to unity ($P < 0.01$). Additionally, we constructed Bland-Altman plots of CMRGlc comparing the EIF^{NS} and the AIF (Figure 4B). The average CMRGlc difference was 2.2 ± 4.8 ($\mu\text{mol}/100 \text{ g}$ per min) (mean $\pm$ s.d.), and the percent difference was within 4.0% ($3.3 \pm 7.2\%$ (mean $\pm$ s.d.)). Figure 4C shows the correlation of CMRGlc between the two methods using the EIF^{NS} and the EIF¹⁵. The best-fit regression line can be expressed as $\text{CMRGlc}(EIF^{15}) = 0.80 \times \text{CMRGlc}(EIF^{NS}) + 13.70$ ($R^2 = 0.83$, $P < 0.01$). The CMRGlc given by both analyses were highly correlated, and the intercept was not significantly different from zero ($P = 0.227$). The slope of the line was close to unity ($P < 0.01$). In the Bland-Altman plots of CMRGlc comparing the EIF^{NS} and the EIF¹⁵ (Figure 4D), the average difference in CMRGlc was 0.094 ± 6.6 ($\mu\text{mol}/100 \text{ g}$ per minute) (mean $\pm$ s.d.), and the percent difference was $0.14 \pm 9.8\%$ (mean $\pm$ s.d.).

In the three awake rats, the peak values of AIF were 0.329, 0.335, and 0.360 (MBq/mL), which tended to be higher than those of EIF^{NS} (0.320, 0.200, and 0.198 (MBq/mL), respectively) computed

Figure 1. In the main graph, the black time-activity curves represent the eight individual arterial input functions (AIFs) normalized by injected dose and body weight (AIF), and the red curve represents the mean of the normalized AIF. The insert graph shows the same data focusing around the peak of the AIFs. Although the mean AIF shown here used the whole data set ($n = 8$), the following analysis on the estimation of AIFs uses a leave-one-out cross-validation method, which excluded the individual data to be estimated from the computation of the standard input function (SIF).

Table 1. The AUC and CMRGlc obtained by AIF-, EIF^{NS}-, and EIF¹⁵-based method

	AIF	SIF ^{NS}	SIF ¹⁵	F	P
AUC (MBq seconds/mL)	103 ± 39	102 ± 34	104 ± 46	0.026	0.975
CMRGlc ($\mu\text{mol}/100 \text{ g}/\text{min}$)	70 ± 14	67 ± 16	67 ± 14	0.917	0.422

An area under the curve (AUC) and the cerebral metabolic rate for glucose (CMRGlc) were obtained by the three methods using AIF, EIF^{NS}, and EIF¹⁵. The values represent mean ± 1 standard deviation. F and P values were calculated by repeated measures analysis of variance (RM-ANOVA).

from the SIF derived from the eight anesthetized animals. This estimation error was ascribed to the mean peak AIF difference between the awake (3.99 ± 1.11 (g/mL)) and anesthetized (2.98 ± 0.63 (g/mL)) rats, which most likely reflected difference in cardiovascular states. However, the AUCs of the AIFs (94.1, 54.3, and 57.6 (MBq seconds/mL)) were comparable to those of the EIF^{NS} (96.5, 60.5, and 59.8 (MBq seconds/mL), respectively). Accordingly, the mean AUC differences between the AIFs and the EIF^{NS} were only $5.9 \pm 4.7\%$ (mean $\pm$ s.d.; range, 2.6% to 11%). Moreover, the mean AUC of the ID- and BM-normalized AIFs in the awake rats (755 ± 33 (g seconds/mL)) was comparable to the mean AUC of the normalized AIFs in the anesthetized rats (785 ± 86 (g seconds/mL)).

DISCUSSION

In the present study, we developed a novel method to estimate the AIF without blood sampling, and investigated the feasibility of the method for the estimation of CMRGlc. The conventional SIF technique for small animals still requires a few blood samples to scale the SIF for the computation of the individual's AIF.¹⁴ This conventional SIF technique has served well for a simplified quantification of CMRGlc. However, it is technically difficult and cumbersome to obtain even a few arterial blood samples only for scaling, which has made it virtually impossible to quantify CMRGlc from small animals during many behavioral tasks. Here, we showed that the proposed SIF-based estimation of the AIF without blood sampling was able to provide reasonable estimates of the AIF.

In this study, we used a LOOCV procedure to ensure that the SIF was not biased toward the respective AIF that needs to be estimated. Using the LOOCV procedure, we calculated the SIFs for

Figure 2. (A) Linear correlation between the area under the curve (AUC) of the estimated input function without blood sampling (EIF^{NS}) and that of the invasively measured arterial input function (AIF). The regression line was expressed as follows: $AUC(AIF) = 1.07 \times AUC(EIF^{NS}) - 3.18$ ($R^2 = 0.88$, $P < 0.001$). (B) Linear correlation between the AUC of EIF^{NS} and that of the estimated input function with one blood sampling (EIF¹⁵). The regression line was expressed as follows: $AUC(EIF^{15}) = 1.03 \times AUC(EIF^{NS}) - 4.06$ ($R^2 = 0.91$, $P < 0.01$).

Figure 3. Linear correlation between the area under the curve (AUC) of the invasively measured arterial input function (AIF) and that of the estimated input function without blood sampling (EIF^{NS}) (black circle), and between the AUC of the AIF and that of the estimated input function with one blood sampling (EIF¹⁵) (red circle). The regression line was expressed as follows: $AUC(EIF^{NS}) = 0.82 \times AUC(AIF) + 14.3$ ($R^2 = 0.88$), $AUC(EIF^{15}) = 0.88 \times AUC(AIF) + 7.16$ ($R^2 = 0.90$).

each individual rat, and then calculated the CMRGLc using either the EIF^{NS} or the EIF¹⁵. The CMRGLc obtained by the EIF^{NS} as well as the values obtained by the AIF and the EIF¹⁵ had almost the same values as the previously reported values in anesthetized rats.^{3,24} Furthermore, the SIF derived from the anesthetized rats provided reasonable estimates of AIFs (EIF^{NS}) for awake rats in terms of AUC. This finding supports the contention that the proposed methods would provide reasonable estimates of AUC and CMRGLc not limited to an anesthetized condition. Given this fact, the new noninvasive approach has advantages over the other methods, especially during tasks requiring active engagement of animals (e.g., learning), as the present method enables us to estimate input functions without stressing animals with invasive intervention.

The differences of AUC between AIF and EIF^{NS}, and those between AIF and EIF¹⁵ were $-3.4 \pm 11.5\%$ (range; -20% to 13%) and $-1.6 \pm 12.3\%$ (range; -17% to 21%), respectively. Previously, Meyer et al¹⁴ reported that the difference between AIF and EIF¹⁵ was $0.11 \pm 4.3\%$ (range -6.0% to 5.9%) in which interindividual variation seems to be much lower than that of the present study. However, it should be noted that Meyer et al calculated the AUC in the range from $t = 0$ to $t = 73$ minutes after the ¹⁸F-FDG injection

whereas we used the range from $t = 0$ to $t = 45$ minutes (middle of the scanning time usually employed for CMRGLc measurement). When we used $t = 0$ to $t = 73$ minutes after the ¹⁸F-FDG injection for AUC calculation, the AUC difference between AIF and EIF^{NS}, and that between AIF and EIF¹⁵ was $0.12 \pm 8.0\%$ (range; -15% to 9.6%) and $-0.86 \pm 7.3\%$ (range; -9.7% to 10%), respectively. This procedure has made the interindividual variation in our study got closer to that of the previous study,¹⁴ and also implicated that the AUC variation of EIF^{NS} could be comparable to that of EIF¹⁵. These analyses further support the idea that the present EIF^{NS} approach may be reasonably useful for the estimation of AIF as well as the EIF¹⁵ method.¹⁴

Researchers typically use laboratory animals sampled from a single strain constituting a genetically homogeneous population (e.g., Long-Evans rats as used here) and reared in the same environment, and therefore individual differences are usually very small. In fact, the shape of the AIF for a given radio-ligand administration protocol is quite consistent among different animals of the same species, assuming similar experimental conditions. The differences in the shape of the AIF, if any, mainly result from differences in the ID and the total blood volume of the animal. Because the total blood volume is linearly correlated with body weight in rats,²² we used the body weight for scaling the SIF. As expected after the correction, the shape of the normalized AIFs was similar among different rats; however, the peak times of normalized AIFs were slightly different. This discrepancy was most likely ascribed to differences in injection speeds. In the present study, the radio-ligand was manually injected over 30 seconds, which would result in variability in the injection speeds across animals. It should be noted that the both AUCs and CMRGLc were in good agreement across AIF, EIF^{NS} and EIF¹⁵, despite the manual injection. The difference in the peak time is expected to be decreased by the use of a syringe pump, for example, for standardized injection of ¹⁸F-FDG.^{12,14} The use of a syringe pump would also improve the reproducibility and consistency of the EIF^{NS}; however, obtaining evidence supporting this assumption was beyond the scope of this study. This issue will be formally addressed in future studies.

Another shortcoming of the present study might be that the plasma glucose concentration was measured only after PET scanning, despite possible variations in glucose concentration during PET scanning. A previous ¹⁸F-FDG PET study in rats showed that plasma glucose concentration at ~ 40 minutes after the ¹⁸F-FDG injection was lower by about 10% than the glucose concentration at 10 minutes after the injection.¹⁴ This 10% difference in glucose concentration corresponds to a 10%

Figure 4. (A) Correlation between the whole-brain cerebral metabolic rate for glucose (CMRGlc) computed by the estimated input function without blood sampling (EIF^{NS}) and the CMRGlc measured through the invasive measurement of arterial input function (AIF). (B) The Bland-Altman plots of CMRGlc comparing EIF^{NS} and AIF. Solid and broken lines represent the mean difference between the two measures and the mean $\pm$ 2 standard deviations, respectively. (C) Correlation between the whole-brain CMRGlc of the EIF^{NS} and that of the estimated input function with one blood sampling (EIF¹⁵). (D) The Bland-Altman plots of CMRGlc comparing the EIF^{NS} and the EIF¹⁵.

difference in CMRGlc (equation 3). Thus, it might have been better to measure plasma glucose at both the beginning and end of PET scanning, and then to use the average value for more accurate quantification of CMRGlc. However, this shortcoming would not considerably affect the conclusion of this study because this study did not aim to provide accurate CMRGlc values but rather to compare estimates of CMRGlc across different methods computing input functions.

Although the present study only investigated the validity of the EIF^{NS} approach for quantitative ¹⁸F-FDG PET in small animals, a similar method may also be applicable to other radio-ligand studies. However, it must be kept in mind that ¹⁸F-FDG PET seems to be best suited for the present EIF^{NS} approach. The error in CMRGlc estimation by Patlak plot depends on the AUC of an individually scaled EIF compared with the AUC of the true AIF. In other ligands with high extraction and/or reversible kinetics, the actual shape of the AIF will be of importance and the applicability of the SIF approach without blood sampling needs careful validation.

The proposed EIF^{NS} approach seems suitable for quantitative ¹⁸F-FDG PET studies in behaving small animals. In this regard, we need to consider the possibility that animal surgery to form the AV shunt and/or anesthesia might have substantially altered physiologic status and therefore affected the CMRGlc quantification. In the present study, we calculated the SIF by averaging the BM- and ID-normalized AIF, which is obtained by online measurement using an AV-shunt method. This AV-shunt method has the advantage of carrying no risk of missing the initial peak owing to high time resolution. Moreover, animals do not substantially lose blood during measurement; therefore, we should be able to

obtain an accurate shape of the input function compared with manual blood sampling. Conversely, surgery under anesthesia is required to form the AV shunt, and this procedure might be more invasive than manual blood sampling. However, previous studies have reported that the values for PaO₂, PaCO₂, oxygen saturation, pH, hematocrit value, and blood pressure were stable in the physiologic range during this type of experiment^{21,24} (although we did not confirm these findings here). In fact, the AIF obtained from the AV-shunt method agreed well with the AIF obtained from manual sampling without the AV-shunt technique.²¹ Additionally, we showed that the AUCs of BM- and ID-normalized AIFs obtained from awake rats were in good agreement with those of normalized AIFs from anesthetized rats even in a preliminary manner based on a few of samples. Hence, it is likely that surgery to form the AV shunt under anesthesia would only minimally affect AUC and CMRGlc values. If we want to apply the proposed EIF^{NS} method for CMRGlc measurement in behaving animals, then we also need to consider the effect of anesthesia during not only surgery but also PET scanning, because anesthesia reduces the CMRGlc in the brain, especially in the gray matter.^{1,24} In the present study, the rats were anesthetized with continuous inhalation of 2% isoflurane during the PET scan. The anesthesia level was not very deep, but the influence of anesthesia cannot be completely excluded. However, we suggest that the effect of anesthesia for measurement of CMRGlc during the behavioral task would be minimized if ¹⁸F-FDG was injected into the individual during behavior under awake conditions and then the PET scan was performed under anesthesia after the stabilization of glucose uptake. This assumption will be validated in future studies.

The standard uptake value is a widely used, simple indicator for providing a CMRGlc-like value without measuring input functions. However, we consider that CMRGlc measurements are still advantageous over SUV methods. First, as described previously, an SUV is affected by several experimental factors. Especially, it has been shown that the time between the tracer injection and the beginning of PET scanning impinges an extremely strong effect on the SUV.^{26,27} Despite this, the interval between the injection and the scanning is not standardized; therefore, it is difficult to compare SUV values across studies. In contrast, the CMRGlc is an absolute value and it has been deemed legitimate to compare CMRGlc values across conditions (such as normal and pathologic) and studies. Second, the proposed method has a potential to provide more accurate quantitative values compared with the SUV values, and we consider that the use of the input function will remain meaningful to maintain possibilities to perform a kinetic analysis. Especially when a method for the delay and/or dispersion correction of SIF has been developed, the proposed SIF-based method will enable to estimate more accurate CMRGlc values and even each constant rate such as k_1 , k_2 , and k_3 . However, note that future studies are needed to test the level of quantification at which SIF-based computation of CMRGlc measurement can actually achieve.

The proposed method is yet to employ a correction for the shape of AIF such as delay and dispersion. An important consideration in this regard is that if we applied the current method to awake and behaving animals, the cardiovascular effects of task performance may cause the changes of cerebral blood flow and/or volume, thereby changing the shape of AIF. In fact, the peak values of AIFs were higher in the awake rats than those of AIF probably because of physiologic changes including a heart rate, blood pressure, and so forth. In consistent, the peak values of EIF^{NS} based on the SIF (anaesthetized measurement) tended to be lower than those of AIF in the awake rats. Nevertheless, the AUCs of the AIFs obtained from the awake rats were comparable to those obtained from the anesthetized rats. Furthermore, the SIF derived from the anesthetized rats provided reasonable estimates (EIF^{NS}) of AUC in awake rats. This is most likely because that the precise shape of AIF (delay and dispersion) does not substantially affect the AUC. Therefore, we are quite confident that the present method without an AIF-shape correction will provide reasonable estimates of AUC and CMRGlc during behaving animals, especially in the future after obtaining a formal SIF computed from a greater number of awake rats than now. However, when we are going to apply the SIF approach to other ligands with high extraction and/or reversible kinetics, the actual shape of AIF will be of importance; in such cases we will need to correct the shape of AIF based on SIF plus some physiologic measures (such as heart rate and blood pressure).

Finally, we speculate that the present methods may be applicable to estimating CMRGlc of disease model animals, considering the robustness of CMRGlc measurements. Of course, however, this assumption needs careful validation in the future. In the meantime, we recommend the computation of SIF in a homogenous group to avoid unexpected AIF changes caused by pathologic conditions. In the future, it would be ideal for the research field to share SIFs computed from different groups of healthy animals (e.g., age group) and also of disease model animals. Such an approach would promote the development of the community in providing a biomarker of disease.

CONCLUSION

The present study described a new method for the noninvasive estimation of the AIF using the SIF scaled by ID and BM, carrying a potential that might substitute for conventional invasive, individual AIF measurement. We propose that this new method can provide a reasonable solution, particularly for quantitative ¹⁸F-FDG PET studies in behaving animals.

AUTHOR CONTRIBUTIONS

YH conceived and designed the experiments; contributed to acquisition, analysis, and interpretation of data; drafting the article; final approval of the version to be published. NI, NT, and MK contributed to acquisition of data and final approval of the version to be published. MH contributed to interpretation of data and final approval of the version to be published. KK contributed to interpretation of data, revising the drafting article, and final approval of the version to be published. TH conceived and designed the experiments, contributed to interpretation of data, revising the drafting article, and final approval of the version to be published.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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