ORIGINAL ARTICLE

Rapid quantitative CBF and CMRO₂ measurements from a single PET scan with sequential administration of dual ¹⁵O-labeled tracers

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Positron emission tomography (PET) with ¹⁵O tracers provides essential information in patients with cerebral vascular disorders, such as cerebral blood flow (CBF), oxygen extraction fraction (OEF), and metabolic rate of oxygen (CMRO₂). However, most of the techniques require an additional C¹⁵O scan for compensating cerebral blood volume (CBV). We aimed to establish a technique to calculate all functional images only from a single dynamic PET scan, without losing accuracy or statistical certainties. The technique was an extension of previous dual-tracer autoradiography (DARG) approach, but based on the basis function method (DBFM), thus estimating all functional parametric images from a single session of dynamic scan acquired during the sequential administration of H₂¹⁵O and C¹⁵O₂. Validity was tested on six monkeys by comparing global OEF by PET with those by arteriovenous blood sampling, and tested feasibility on young healthy subjects. The mean DBFM-derived global OEF was 0.57 ± 0.06 in monkeys, in an agreement with that by the arteriovenous method (0.54 ± 0.06). Image quality was similar and no significant differences were seen from DARG; 3.57% ± 6.44% and 3.84% ± 3.42% for CBF, and 2.79% ± 11.2% and 6.68% ± 10.5% for CMRO₂. A simulation study demonstrated similar error propagation between DBFM and DARG. The DBFM method enables accurate assessment of CBF and CMRO₂ without additional CBV scan within significantly shortened examination period, in clinical settings.

Keywords: acute stroke; brain imaging; cerebral blood flow; kinetic modeling; positron emission tomography

INTRODUCTION

Quantitative cerebral blood flow (CBF), oxygen extraction fraction (OEF), and metabolic rate of oxygen (CMRO₂) images can be assessed using positron emission tomography (PET) and ¹⁵O-labeled radiotracers. These parametric images are essential for understanding the pathophysiologic status of cerebral vascular disorders, and this technique has been promoted as a clinical diagnostic tool in some countries. Parametric images have been measured via PET by administering multiple ¹⁵O-labeled tracers,² such as in the steady-state method³,⁴ or the three-step autoradiography method.⁵,⁶ The validity of the technique has been demonstrated with three-step autoradiography on healthy volunteers at rest.⁷ An order of 1-hour period, however, is typically required to complete the whole study, because three independent scans are required in addition to >10-minute intervals between scans to allow for decay of the residual radioactivity of the preceding tracer. Thus, applicability is limited in clinical settings, particularly for patients with acute stroke.⁸

We recently developed a novel PET method of dual-tracer autoradiography (DARG)⁹ for quantitative assessment of CBF, CMRO₂, OEF, and cerebral blood volume (CBV), based on sequential administration of dual tracers during a single PET scan, with additional CBV data obtained from a C¹⁵O scan to compensate for radioactivity from the vascular space. The method allows for shortened examination time compared with previous three-step approaches,⁹ and was shown to provide quantitative OEF values that were in good agreement with those assessed by the arteriovenous oxygen difference in normal monkeys over a wide physiological range, suggesting the validity of quantitative functional values obtained by this method. Of the importance is that the noise property in the calculated functional images by this method is same as that by the three-step autoradiography. A limitation of this method is however attributed to the need for additional C¹⁵O scan. An assumption of the fixed fractionations of arterial and venous vasculature components, as has been done in most of other methods could also cause systematic errors in pathological conditions such as the ischemia, which most likely...
cause dilatation of vasculature and/or the arteriovenous malformation.

In the present study, we developed a formula that eliminates the need for the CBV information, which has been required in the previous DARG approach. This computational refinement for the dual-tracer approach has been done using the basis function method (DBFM). Attention was made so as to minimize the systematic errors attributed to the assumption of fixing the arterial- and venous-fractionations. The technique would also be advantageous for significantly shortening the duration of the total clinical examination. The validity of the present method, in terms of quantitative accuracy and quality of generated images, was tested using the data obtained from anesthetized monkeys and young normal volunteers.

THEORY

The present formula was developed to compute CBF, CBRO2, and CBV simultaneously, thus eliminating the need for additional scan for CBV assessment. The distributions of tracer in the vascular space (VAr (mL/g) for water and VAc (mL/g) for oxygen components) were estimated from dynamic image data acquired during sequential administration of H215O and 15O2. The kinetics for both 15O2 and H215O are expressed using the single-tissue compartment model1 as:

\[
C_i(t) = E \cdot f \cdot A_o(t) \otimes \delta(t) + f \cdot A_w(t) \otimes \delta(t) + V_0^w \cdot A_w(t) + V_A^w \cdot A_o(t)
\]

where Ci(t) (Bq/mL) is the radioactivity concentration in a voxel in a given tissue region, A_o(t) (Bq/mL) and A_w(t) (Bq/mL) are the arterial input functions of 15O-oxygen (15O2) and 15O-water (H215O) contents, respectively, f (mL per minute per gram) is the CBF, E is the OEF, p (mL/g) is the blood/brain partition coefficient for water, and \( \otimes \) indicates the convolution integral. The first and second terms on the right side represent the tissue radioactivity of oxygen and water, respectively. The last two terms signify the radioactivity of 15O2 and H215O in blood vessels. In this study, p was fixed at 0.8 mL/g.10

The first two terms on the right side in equation (1) have nonlinear relationship with f, and we formulated two basis functions11 to calculate parametric images from the dynamic data. The corresponding basis functions were as follows:

\[
F_1(f, t) = A_w \cdot e^{-\frac{t}{f}}
\]

\[
F_2(f, t) = A_o \cdot e^{-\frac{t}{f}}
\]

Equation (1) can then be transformed for each basis function into a linear equation in \( E, V_0^w \), and \( V_A^w \):

\[
C_i(t) = F_1 + E \cdot F_2 + V_0^w \cdot A_w + V_A^w \cdot A_o
\]

For the physiologically reasonable range of f, that is, 0 < f < 2.0 mL per minute per gram, 200 discrete values for f were given. For a given value of f, three values of \( E, V_0^w \), and \( V_A^w \) were obtained using the standard linear least squares optimization technique. The optimized f value was determined from the 200 discrete values, so that the residual sum of squares between left- and right-hand terms in equation (3) became minimum, thus a unique set of optimized parameters for f, \( E, V_0^w \), and \( V_A^w \) could be obtained. Metabolic rate of oxygen is then calculated from the obtained f, E, and the arterial oxygen concentration. The present formula can be applied to either of the two procedures: H215O injection (or C15O2 inhalation) followed by 15O2 inhalation (the H215O–15O2 protocol), or 15O2 inhalation followed by H215O injection (or C15O2 inhalation) (the 15O2–H215O protocol).

MATERIALS AND METHODS

The validity of the present method was first evaluated using the data obtained from a series of PET scanning on anesthetized monkeys, in which the global OEF values obtained using this approach were compared with those derived using the catheter-based method for measuring the arteriovenous difference (A-V difference) of oxygen contents. Second, image consistency was evaluated by comparing the quantitative values of regional CBF and CBRO2 for young normal volunteers derived by the present DBFM and those by the previously proposed DARG methods. Third, the error sensitivity of the present method was evaluated by a simulation study, and was referred to the results for the DARG technique.

Subjects

The subjects consisted of two groups, namely, six normal monkeys of macaca fascicularis under anesthesia and seven young normal volunteers. All monkeys were males with a mean body weight of 5.2 ± 0.8 kg and ages ranging from 3 to 4 years. Animals were maintained and handled in accordance with the Human Care and Use of Laboratory Animals guidelines (Rockville, National Institute of Health/Office for Protection from Research Risks, 1996). The study was approved by the local Committee for Laboratory Animal Welfare, National Cardiovascular Center, Osaka, Japan. The protocol also followed the Guidelines for Animal Experimentation of the National Cerebral and Cardiovascular Center, Osaka, Japan.

All normal human subjects were males with a mean age of 25.3 ± 2.4 years and mean body weight of 64.2 ± 6.8 kg. None had symptoms at the time of PET examination, or histories of cerebral or other relevant diseases. All subjects gave written informed consent, approved by the ethics committee of the National Cerebral and Cardiovascular Center, Osaka, Japan.

Positron Emission Tomography Experiments (Animal)

Details regarding the primate animal study have been previously reported.9 Briefly, anesthesia was induced with ketamine (10 mg/kg, intramuscularly) and maintained during the experiment using intravenous propofol (4 mg/kg/h) and vecuronium (0.05 mg/kg/h). Animals were intubated and their respiration was controlled by an anesthetic ventilator (Cato, Drager, Germany). The PET scanner used was the ECAT HR (Siemens-CTI, Knoxville, TN, USA), installed in the animal PET laboratory of the National Cerebral and Cardiovascular Research Center.

Positron emission tomographic imaging was performed in 2D mode. After a 900-second transmission scan, a dynamic scan was started following the inhalation of C15O. After 10 minutes, a 6-minute dynamic PET scan was performed during sequential administration of 15O (2,200 MBq) and H215O (370 MBq) for 3 minutes each. After 10 minutes, another order of scan, namely, 15O2–H215O was randomized across subjects. Arterial blood was withdrawn continuously from the femoral artery through a catheter (0.6-mm inner diameter) using a syringe pump (Harvard Apparatus, Holliston, MA, USA, model 55-2309) with a withdrawal speed of 0.45 mL/min (2.7 mL in total) and the blood radioactivity concentration was measured with a continuous input function monitor system made of GSO scintillation crystals.12 Arterial and sinus blood samples of 0.2 mL each were drawn simultaneously during each scan. The sinus blood was sampled through a 3-F catheter, which was introduced via the femoral vein to the cerebral sinus using a high-resolution digital X-ray imaging system (GE Medical System, Waukesha, WI, USA). To avoid mixing with venous blood draining from extracranial tissues, the tip of the catheter was carefully placed at the angle of the cerebral sigmoid and transverse sinuses, and its position was confirmed at the conclusion of each PET protocol. Their oxygen contents were measured to obtain the global OEF (gOEF = A_o/A_w) of each region.

Out of the six animals, A–V sampling was performed during the PET scan with the 15O2–H215O protocol at normocapnia (PaCO2 ≈ 40 mm Hg) and also while the respiratory rate was sequentially adjusted to achieve hypocapnia (PaCO2 < 33 mm Hg), mild hypocapnia (45 < PaCO2 < 50 mm Hg), and deep hypocapnia (PaCO2 > 50 mm Hg). At least 30 minutes were allotted to reach a steady-state PaCO2 after which the 15O2–H215O PET scan was initiated.

Positron Emission Tomography Experiments (Young Normal Volunteer)

Young normal volunteers were studied at the Radiology Department of the National Cerebral and Cardiovascular Research Center. Young, healthy

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volunteer subjects were scanned with an ECAT 47 scanner (Siemens-CTI). The scanning was carried out in 2D mode. After a transmission scan, a static scan was started at 2 minutes after the end of a 4-minute inhalation of 3,000 MBq of C15O. After a pause of 10 minutes to permit radioactive decay, two sets of dynamic scans of 540 and 510 seconds were carried out, first during sequential inhalation of C15O2 (3,000 MBq) and 15O2 (4,500 MBq) and second an inhalation of H2 (4,500 MBq) followed by intravenous H3O1 (1,100 MBq), respectively. Since the 15O label in C15O is rapidly transferred to the water pool in the lung capillary bed,14 the C15O2 inhalation is considered essentially identical to the intravenous administration of H3O. Thus, C15O2–15O2 protocol in this article.

Arterial blood was continuously drawn from the brachial artery using a catheter (0.5-mm inner diameter) and syringe pump (Harvard Apparatus, model 901) at a speed of 2.0 mL/min during the PET scan. The total blood withdrawal was ~30 mL. The blood radioactivity concentration was measured using the GSO input function monitor system.15 Data Processing

Dynamic sinogram data were corrected for dead time in each frame and for detector normalization. Tomographic images were reconstructed using the filtered back projection method with 4- and 7-mm Gaussian filtering for mono- and multi-energy subjects, respectively. Attenuation correction was applied using transmission data. Scatter correction was also applied by means of the deconvolution scatter function technique.15 Reconstructed images, with a matrix size of 128 × 128 × 47 and a voxel size of 1.1 mm × 1.1 mm × 3.4 mm for monkeys and 1.8 mm × 1.8 mm × 3.4 mm for normal human subjects, were transferred to a LINUX computer for further processing using in-house programs9.

Measured arterial blood time–activity curves (TACs) were normalized to become consistent PET images, and they were also corrected for dispersion (τ = 3 and 14 seconds for monkeys and humans, respectively).16 After correcting for delay,8,17 the blood curves were separated into 15O (Ao) and H2O (A0) contents as described previously,18,19 in which the recirculation water, that is, the arterial H2O concentration was estimated using manually sampled at nine points and plasma separated activity concentration in the monkey data (details are presented in18) and for human data according to a physiological model validated previously with fixing rate constant values as: k = 0.13 per minute (production rate of recirculating water), ∆t = 20 seconds (delayed appearance time of recirculating water), k0 = 0.38 per minute (forward diffusion rate of recirculating water to body interstitial space) and p0 = 1.38 (k/k0, where k is a backward diffusion rate of recirculating water).18 Cerebral blood flow, OEF, and CMRO2 images as well as those for V0 and k0 were calculated according to the DBFM formula described above, using reconstructed images and the obtained input functions, and the hemoglobin concentration and saturation of oxygen in the arterial blood. Additionally applying CBV data from C15O scan data, CBF, OEF, and CMRO2 images were also generated using the DARG formula.9 With DBFM, blood volume was estimated as V0, and the obtained images were converted to CBV images as: CBV = R1(R2/F0) × V0, where R1 (F0 = 0.85) is the peripheral-to-central hematocrit ratio and F0 (≡ 0.835) is the effective venous fraction. Images without applying physical decay correction were applied to both DBFM and DARG calculations.

Data Analysis

Regions of interest (ROIs) were drawn on CBF images obtained from experiment on monkeys to cover the whole brain. These ROIs were then transferred to the OEF and CMRO2 functional images obtained using the DBFM and DARG methods. Quantitative CBF, OEF, and CMRO2 values generated from DBFM were then compared with those from DARG. Also, OEF values obtained from DBFM were compared with those using the A–V sampling technique (OEFa,v) using Bland–Altman plots. In young normal volunteers, circular ROIs of 6-mm diameter were placed bilaterally on the temporal, frontal, parietal, occipital, and cerebellar, brain stem, caudate, lentiform, thalamus, and central semioval regions, in which attention was made to avoid the region with large CBV such as the sinus region. Values for CBF, and CMRO2 in these ROIs were summarized for the cortical gray matter, cerebellum, and white matter regions, and were compared between DBFM and DARG using Bland–Altman plots. The N-index, which denotes the noise level of parametric images,21 was obtained from the standard deviation of an image’s spatial values, which was derived by subtracting two statistically independent and physiologically equivalent images. This calculation was carried out for CBF, OEF, and CMRO2 from young normal volunteers using even- and odd-numbered frames, and the obtained N-index values were compared between the DARG and DBFM formulae.

All data are presented as mean values ± 1 s.d. Pearson’s correlation analysis and linear regression analysis were used to evaluate relationships between the two CBF values. P < 0.05 was considered statistically significant.

Simulation

Error propagation was evaluated for three error sources, namely: effects of the imperfect delay adjustment,17 by shifting time in an input function from −4 to 4 seconds, where a positive error represents an overcorrection of delay time; errors in dispersion correction in the input function,16 by shifting the time constant from −4 to 4 seconds, where a negative error represents undercorrection, as described previously;8,19,22 and errors in the assumed blood/tissue partition coefficient (p),15 by varying p from 0.7 to 0.9 mL/g.10

The input function for this simulation study was defined based on typical arterial TACs obtained from a human study with water (A0) and also with oxygen (Ao)19 and by adding the Av and Ao with a time lag of 3 minutes between the administrations of H2O–15O2 and 15O–H2O. Applying the kinetic formulation of equation (1), tissue TACs were generated for a ‘normal’ (CBF = 0.50 mL per gram per minute, OEF = 0.4, and CBV = 0.04 mL/g), ‘ischemic’ (CBF = 0.30 mL per gram per minute, OEF = 0.6, and CBV = 0.06 mL/g), ‘hyperperfusion’ (CBF = 0.70 mL per gram per minute, OEF = 0.3, and CBV = 0.04 mL/g), and ‘diachisis’ (CBF = 0.20 mL per gram per minute, OEF = 0.4, and CBV = 0.04 mL/g) conditions.9 Values of CBF, OEF, and CMRO2 were then calculated using the true input function and these TACs, by assuming p = 0.8 mL/g. Errors in calculated functional values were then plotted as a function of the percentage differences of the assumed values for delay, dispersion, and the partition coefficient.

Additional simulation was carried out to evaluate errors in the estimation of recirculating H2O in the arterial blood. Arterial input functions were generated by changing the rate constant (k) that corresponds to the whole body oxygen metabolism, and the delay (∆t) by ±10%, from the fixed value (i.e., k = 0.13 per minute and ∆t = 20 seconds), and regional tissue TACs were calculated according to the equation (1), by assuming a ‘normal’ condition. Cerebral blood flow and CMRO2 values were calculated according to the procedure mentioned above, and the %errors were estimated.

RESULTS

The present DBFM as well as DARG programs successfully calculated functional images of CBF, OEF, CMRO2, and CBV for PET data on both monkeys and young normal volunteers. The computation time for parametric images was ~30 seconds using a standard PC installed with GNU/Linux (fc16.x86_64 64 bit, CUPIntel Core i7 3.07 GHz, Memory: 16 GB).

Quantitative values for whole brain in monkeys were 0.32 ± 0.11 and 0.27 ± 0.09 mL per gram per minute in CBF for DARG and DBFM, respectively, 0.56 ± 0.06 and 0.57 ± 0.06 in OEF for DARG and DBFM, respectively, and 0.029 ± 0.004 and 0.026 ± 0.004 mL per gram per minute in CMRO2 for DARG and DBFM, respectively. The OEF value by the A–V method was 0.54 ± 0.06. The paired t-test did not show any significant differences in these values between both DARG and DBFM for either order, that is, H2O–15O2 and 15O–H2O (P > 0.05, n = 6). Also, there were no significant differences in OEF between the PET and the A–V methods, for either order (P > 0.05, n = 6). During normocapnia, the PCO2, PO2, SO2, and hemoglobin values were 38.3 ± 1.4, 119 ± 12 mm Hg, 97.3% ± 1.2%, and 13.6 ± 1.0 g/dL, respectively. All these values were considered within the normal range.

Figure 1 shows the Bland–Altman plot of OEF as estimated by the DBFM and the A–V method obtained using PCO2 variation. The regression line obtained was: OEF = OEFa,v + 0.01 (r = 0.96, P < 0.001, n = 12). The intercept was not significantly different from zero (P > 0.05), and the slope of the line was close to unity.

Figure 2 shows the Bland–Altman plots of regional CBF and CMRO2 values as estimated by DARG and DBFM for young normal
A comparison of image quality, as defined as the N-index divided by the mean value of each of CBF, OEF, and CMRO₂ is given in Figure 5. Values were slightly but significantly greater with DBFM compared with DARG, except CBF assessed by the ¹⁵O₂–H₂O protocol, which did not show significant difference between DBFM and DARG.

Results of the simulation study for the ‘normal’ condition are shown in Figure 6. Error sensitivity to errors in delay time, the dispersion time constant, and the partition coefficient value were different, but showed the same tendency between the H₂O (C¹⁵O₂)–¹⁵O₂ and ¹⁵O₂–H₂O protocols for both DARG and DBFM. The magnitude of errors in the calculated functional parameters are within a range of < 5% for ± 2 seconds errors in the delay, for ± 2 seconds errors in the dispersion, and for 0.75 to 0.85 mL/g errors in the partition coefficient. The error propagation from each error source to the functional parameters are almost the same in the ‘hyperperfusion’ condition, but significant magnification was seen in the ‘ischemic’ condition, and was 1.1 to 1.2 times for CBF, 1.6 to 2.0 for OEF, and 1.4 to 1.6 for CMRO₂. For the ‘diagnosis’ condition, the magnification factors were only 0.1 to 0.5 times for all CBF, OEF, and CMRO₂ parameters. Regarding the errors in the recirculating H₂O estimation, a change in the oxygen production rate (k) by ± 10% resulted in errors of ± 2.5% and 2.1% in CBF and CMRO₂. A change of the delay by ± 10% resulted in errors of 3.0% and 1.2%, respectively.

**DISCUSSION**

This study demonstrated that the DBFM method developed in this study provided quantitative functional images of CBF, CMRO₂, and OEF from a single, short-duration dynamic PET scan with sequential administration protocols of H₂O (C¹⁵O₂) and ¹⁵O₂ and ¹⁰O₂ and H₂O within a short-time interval. Oxygen extraction fraction values obtained by the DBFM method agreed well with those derived by the A–V oxygen difference in the experiment utilizing six normal monkeys (Figure 1). Cerebral blood flow and CMRO₂ calculated by DBFM also agreed with those by the previously proposed DARG method in young normal volunteers (Figure 2). No significant difference was seen between the DBFM and DARG methods in all functional parameters, except for CMRO₂ values assessed with the ¹⁵O₂–H₂O protocol, indicating significantly smaller values with DBFM than DARG. The magnitude of the difference was ~ 6.68% ± 10.5% in average, which is within an acceptable range for practical use. The simulation also demonstrated that the DBFM and DARG methods were similar in terms of the sensitivity to three known error sources of the delay and dispersion, and the uncertainty in the assumed partition coefficient. A previous study⁹ demonstrated that calculated functional values were identical between DARG and the three-step autoradiography of Mintun et al when the administration interval was longer than 3 minutes in DARG. Dual-tracer basis function method should therefore be able to provide functional values comparable to those of the three-step autoradiography. It should however be noted that the image quality (or the statistical noise) of CBF, OEF, and CMRO₂ images was degraded in DBFM than in DARG as quantitatively evaluated by N-Index (Figure 5). This is attributed that four parameters have to be determined in DBFM while only two in DARG. However, difference of N-index values was only 10% to 20% in average, and not visible in the calculated parametric images shown in Figure 3.

An important advantage of DBFM over DARG is that the former does not require independent scan of CBF using C¹⁵O₁₇O inhalation. This allows shortening the entire scan period. A single dynamic scan for 6 minutes on animal experiments and 9 minutes on young volunteers presented in the present study are significantly shorter than previous protocols. Although additional time is needed for transmission scan and other technical procedures, the entire study period of < 15 minutes is probably practically

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**Figure 1.** Bland–Altman plots of oxygen extraction fraction (OEF) comparing arteriovenous difference (gOEF(A–V)) and dual-tracer basis function method (DBFM) (gOEF(PET)). Solid and broken lines show mean difference and its respective 2 s.d., respectively. Mean ± s.d. values are − 0.03 ± 0.038. The regression analysis exhibited a significant positive correlation with a slope close to unity (y = 0.99 x + 0.01, r = 0.96, number of plots = 12). DBFM was performed with an administration order of O₂–H₂O. PET, positron emission tomography.
possible and feasible. The DBFM technique is similar to Ohta et al.\textsuperscript{24-27} in terms of estimating three parameters of CBF, CMRO\textsubscript{2}, and CBV from a single session of the scan. The essential difference is that the DBFM stands for the sequential administration of two tracers of $^{15}$O and H\textsubscript{2}$^{15}$O (left: A, C) and $^{15}$O-H\textsubscript{2}$^{15}$O (right: B, D) protocols comparing dual-tracer autoradiography (DARG) and dual-tracer basis function (DBFM) regional values in young normal volunteers. Solid and broken lines show mean difference and its respective 2 s.d., respectively. Mean ± s.d. values are 0.024 ± 0.030 ml per minute per gram for CBF by H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O, 0.021 ± 0.019 ml per minute per gram for CBF by \textsuperscript{15}O-H\textsubscript{2}$^{15}$O, -0.000685 ± 0.00536 ml per minute per gram for CMRO\textsubscript{2} by H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O, and -0.00339 ± 0.00426 ml per minute per gram for CMRO\textsubscript{2} by $^{15}$O-H\textsubscript{2}$^{15}$O. Significant difference was observed in CMRO\textsubscript{2} by $^{15}$O-H\textsubscript{2}$^{15}$O in paired t-test (D), not in others. The regression analysis exhibited a significant positive correlation with a slope close to unity (For CBF: $y = 1.07x - 0.015$ mL per minute per gram ($r = 0.99$, P < 0.001) and $y = 1.04x - 0.003$ mL per minute per gram ($r = 0.99$, P < 0.001) by C$^{15}$O\textsubscript{2}-15O and $^{15}$O-H\textsubscript{2}$^{15}$O protocols, respectively, and for CMRO\textsubscript{2}: $y = 0.93x - 0.0022$ mL per minute per gram ($r = 0.93$, P < 0.001) and $y = 0.92x - 0.0007$ mL per minute per gram ($r = 0.95$, P < 0.001) by C$^{15}$O\textsubscript{2}-15O and $^{15}$O-H\textsubscript{2}$^{15}$O protocols, respectively).

Figure 2. Bland–Altman plots of cerebral blood flow (CBF) (upper: A, B) and metabolic rate of oxygen (CMRO\textsubscript{2}) (lower: C, D) for H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O (left: A, C) and $^{15}$O-H\textsubscript{2}$^{15}$O (right: B, D) protocols comparing dual-tracer autoradiography (DARG) and dual-tracer basis function (DBFM) regional values in young normal volunteers. Solid and broken lines show mean difference and its respective 2 s.d., respectively. Mean ± s.d. values are 0.024 ± 0.030 ml per minute per gram for CBF by H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O, 0.021 ± 0.019 ml per minute per gram for CBF by \textsuperscript{15}O-H\textsubscript{2}$^{15}$O, -0.000685 ± 0.00536 ml per minute per gram for CMRO\textsubscript{2} by H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O, and -0.00339 ± 0.00426 ml per minute per gram for CMRO\textsubscript{2} by $^{15}$O-H\textsubscript{2}$^{15}$O. Significant difference was observed in CMRO\textsubscript{2} by $^{15}$O-H\textsubscript{2}$^{15}$O in paired t-test (D), not in others. The regression analysis exhibited a significant positive correlation with a slope close to unity (For CBF: $y = 1.07x - 0.015$ mL per minute per gram ($r = 0.99$, P < 0.001) and $y = 1.04x - 0.003$ mL per minute per gram ($r = 0.99$, P < 0.001) by C$^{15}$O\textsubscript{2}-15O and $^{15}$O-H\textsubscript{2}$^{15}$O protocols, respectively, and for CMRO\textsubscript{2}: $y = 0.93x - 0.0022$ mL per minute per gram ($r = 0.93$, P < 0.001) and $y = 0.92x - 0.0007$ mL per minute per gram ($r = 0.95$, P < 0.001) by C$^{15}$O\textsubscript{2}-15O and $^{15}$O-H\textsubscript{2}$^{15}$O protocols, respectively).

Figure 2. Bland–Altman plots of cerebral blood flow (CBF) (upper: A, B) and metabolic rate of oxygen (CMRO\textsubscript{2}) (lower: C, D) for H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O (left: A, C) and $^{15}$O-H\textsubscript{2}$^{15}$O (right: B, D) protocols comparing dual-tracer autoradiography (DARG) and dual-tracer basis function (DBFM) regional values in young normal volunteers. Solid and broken lines show mean difference and its respective 2 s.d., respectively. Mean ± s.d. values are 0.024 ± 0.030 ml per minute per gram for CBF by H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O, 0.021 ± 0.019 ml per minute per gram for CBF by \textsuperscript{15}O-H\textsubscript{2}$^{15}$O, -0.000685 ± 0.00536 ml per minute per gram for CMRO\textsubscript{2} by H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O, and -0.00339 ± 0.00426 ml per minute per gram for CMRO\textsubscript{2} by $^{15}$O-H\textsubscript{2}$^{15}$O. Significant difference was observed in CMRO\textsubscript{2} by $^{15}$O-H\textsubscript{2}$^{15}$O in paired t-test (D), not in others. The regression analysis exhibited a significant positive correlation with a slope close to unity (For CBF: $y = 1.07x - 0.015$ mL per minute per gram ($r = 0.99$, P < 0.001) and $y = 1.04x - 0.003$ mL per minute per gram ($r = 0.99$, P < 0.001) by C$^{15}$O\textsubscript{2}-15O and $^{15}$O-H\textsubscript{2}$^{15}$O protocols, respectively, and for CMRO\textsubscript{2}: $y = 0.93x - 0.0022$ mL per minute per gram ($r = 0.93$, P < 0.001) and $y = 0.92x - 0.0007$ mL per minute per gram ($r = 0.95$, P < 0.001) by C$^{15}$O\textsubscript{2}-15O and $^{15}$O-H\textsubscript{2}$^{15}$O protocols, respectively).

Ischemia could cause vasodilatation, with a possible change in relative fractionation of arterial-to-venous volumes. The AVM is often characterized by increased arterial blood volume, which likely cause changes in the arterial-to-venous volume fractionations and also in the peripheral-to-central hematocrit ratios. Cerebral blood volume-equivalent information of V\textsubscript{A} and V\textsubscript{P}, has been determined in DBFM, thus likely avoiding the systematic errors attributed to the possible alterations in those assumptions. Similar values of CBF and CMRO\textsubscript{2} between the C$^{15}$O-based CBV correction in DARG and by DBFM methods in young healthy volunteers, on the other hand, empirically supports the validity of such assumptions in normal controls.

By definition, V\textsubscript{A} and V\textsubscript{P} estimated from the DBFM are different from CBF, and also different from each other. The former contains both the arterial and venous volume, but more weighted with the arterial part. The latter contains only the arterial blood volume. Further studies should be carried out to evaluate the significance of V\textsubscript{A} and V\textsubscript{P} parameters, particularly in patient populations.

Interval of the two administration for the two tracers of H\textsubscript{2}$^{15}$O (C$^{15}$O\textsubscript{2})-15O and $^{15}$O-H\textsubscript{2}$^{15}$O in DBFM was 3 minutes in animal study, and 6 minutes in young normal volunteer studies. The longer interval in the volunteer study was due to the limitation in radio-synthesis procedures. Use of C$^{15}$O\textsubscript{2} rather than H\textsubscript{2}$^{15}$O would have an advantage in clinical studies, because the venous cannulation for H\textsubscript{2}$^{15}$O saline infusion can be avoided, thus making...
Table 1. CBF, OEF, and CMRO₂ values in normal human subjects (n = 7) in cortical gray matter, deep gray matter, cerebellum, and white matter regions calculated using DARG and DBFM

|                  | DARG                      | DBFM                      |
|------------------|---------------------------|----------------------------|
|                  | H₂¹⁵O–¹⁵O₂                  | H₂¹⁵O–¹⁵O₂                  |
|                  | ¹⁵O₂–H₂¹⁵O (C₁⁵O₂)          | ¹⁵O₂–H₂¹⁵O (C₁⁵O₂)          |
| CBF (mL per gram per minute) |                  |                            |
| Cortical gray    | 0.530 ± 0.028              | 0.536 ± 0.026              |
| Deep gray        | 0.522 ± 0.033              | 0.522 ± 0.037              |
| Cerebellum       | 0.539 ± 0.040              | 0.573 ± 0.048              |
| White matter     | 0.278 ± 0.042              | 0.275 ± 0.044              |
| OEF              |                           |                            |
| Cortical gray    | 0.39 ± 0.05                | 0.39 ± 0.06                |
| Deep gray        | 0.43 ± 0.05                | 0.38 ± 0.05                |
| Cerebellum       | 0.39 ± 0.04                | 0.40 ± 0.05                |
| White            | 0.38 ± 0.03                | 0.38 ± 0.06                |
| CMRO₂ (mL per gram per minute) |                  |                            |
| Cortical gray    | 0.0415 ± 0.0045            | 0.0393 ± 0.0043            |
| Deep gray        | 0.0478 ± 0.0070            | 0.0450 ± 0.0075            |
| Cerebellum       | 0.0406 ± 0.0039            | 0.0447 ± 0.0039            |
| White            | 0.0207 ± 0.0041            | 0.0201 ± 0.0040            |
| CBV (g/mL)       |                           |                            |
| Cortical gray    | 0.0444 ± 0.0088            | 0.0410 ± 0.0078            |
| Deep gray        | 0.0496 ± 0.0102            | 0.0444 ± 0.0113            |
| Cerebellum       | 0.0644 ± 0.0038            | 0.0303 ± 0.0102*           |
| White            | 0.0188 ± 0.0044            | 0.0224 ± 0.0080*           |

CBF, cerebral blood flow; CBV, cerebral blood volume; CMRO₂, metabolic rate of oxygen; DARG, dual-tracer autoradiography; DBFM, dual-tracer basis function method; OEF, oxygen extraction fraction.

n = 7; values are presented as mean ± s.d.; *significant difference (P < 0.05) between DARG and DBFM; CBF by the DBFM method was calculated from VO₂ images as \( \text{CBF} = \frac{R_{\text{relt}}}{1 - E_{\text{relt}}} \cdot \text{VO₂} \).
per gram, with the step value of 0.1 mL per minute per gram in the present computation, which have been confirmed to interpret the human data presented in this work. For the set of solutions in the present formula, we found no other local minimums in the residual sum of squares, for the physiologically acceptable range for CBF (0 to 2 mL per minute per gram), OEF (0 to 1) and CBV (0 to 1 mL/g).

Optimization of administration doses and their ratios of two tracers of $^{15}$O$_2$ and H$_2^{15}$O (C$_{15}$O$_2$) would be a subject of further investigation. Several factors should be taken into account, including the contribution of the residual tracer from the first administration into the second tracer contribution, random counting rate, and dead-time count losses. These are likely dependent on the physical performance of a PET device, and therefore need to be investigated for PET device-specific manner. The present study, however, demonstrated that the almost equal administration dose could yield stable results.

In conclusion, quantitative CBF, OEF, and CMRO$_2$ could be calculated using the DBFM method from a single PET scan.

**Figure 3.** Representative view of cerebral blood flow (CBF), oxygen extraction fraction (OEF), and metabolic rate of oxygen (CMRO$_2$) images for a normal subject using dual-tracer autoradiography (DARG) and dual-tracer basis function (DBFM) techniques with H$_2$O–O$_2$ and O$_2$–H$_2$O modes. Axial images are sectioned at (A) parietal level, (B) basal ganglia level, and (C) cerebellar level.

**Figure 4.** Representative view of cerebral blood volume (CBV) (upper and middle) and $V_{M}^{o}$ (lower) images in a normal subject, derived by CO scan (upper) and dual-tracer basis function (DBFM) (O$_2$–H$_2$O) (middle and lower) methods.

**Figure 5.** Comparison of noise levels between dual-tracer autoradiography (DARG) and dual-tracer basis function (DBFM) for cerebral blood flow (CBF), oxygen extraction fraction (OEF), and metabolic rate of oxygen (CMRO$_2$) images by means of $N$-index. White and black circles correspond to values from the H$_2^{15}$O (C$_{15}$O$_2$)–O$_2$ and $^{15}$O$_2$–H$_2^{15}$O protocols, respectively. The paired $t$-test shows significant differences in all functional parameters for both protocols between DARG and DBFM except CBF by the $^{15}$O$_2$–H$_2^{15}$O protocol.
acquired during sequential administration of dual tracers with $\text{H}_2^{15}\text{O} (\text{C}^{15}\text{O}_2)$–$^{15}\text{O}_2$ and $^{15}\text{O}_2–\text{H}_2^{15}\text{O}$ protocols. Although further studies are needed, ability of eliminating additional $\text{C}^{15}\text{O}$ scan for $\text{CBV}$ assessment may contribute to improve the accuracy and applicability of the $\text{CBF}$, $\text{OEF}$, and $\text{CMRO}_2$ assessment in clinical settings.

**DISCLOSURE/CONFLICT OF INTEREST**
The authors declare no conflict of interest.

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**REFERENCES**
1. Frackowiak RS, Jones T, Lenzi GL, Heather JD. Regional cerebral oxygen utilization and blood flow in normal man using oxygen-15 and positron emission tomography. *Acta Neurol Scand* 1980; 62: 336–344.
2. Mintun MA, Raichle ME, Martin WR, Herscovitch P. Brain oxygen utilization measured with O-15 radiotracers and positron emission tomography. *J Nucl Med* 1984; 25: 177–187.
3. Lammertsma AA, Heather JD, Jones T, Frackowiak RS, Lenzi GL. A statistical study of the steady state technique for measuring regional cerebral blood flow and oxygen utilisation using $^{15}\text{O}$. *J Comput Assist Tomogr* 1982; 6: 566–573.
4. Subramanyam R, Alpert NM, Hoop B Jr., Brownell GL, Taveras JM. A model for regional cerebral oxygen distribution during continuous inhalation of $^{15}\text{O}_2$, $^{15}\text{O}_2$, and C$^{15}\text{O}_2$. *J Nucl Med* 1978; 19: 48–53.
5. Mintun MA, Lundstrom BN, Snyder AZ, Vlassenko AG, Shulman GL, Raichle ME. Blood flow and oxygen delivery to human brain during functional activity: theoretical modeling and experimental data. *Proc Natl Acad Sci USA* 2001; 98: 6859–6864.
6. Hatazawa J, Fujita H, Kanno I, Satoh T, Iida H, Miura S et al. Regional cerebral blood flow, blood volume, oxygen extraction fraction, and oxygen utilization rate in...
normal volunteers measured by the autodigraphic technique and the single
breath inhalation method. Ann Nucl Med 1995; 9: 15–21.
7 Hattori N, Bergsneider M, Wu HM, Glenn TC, Vespa PM, Hovda DA et al. Accuracy of a method that uses short inhalation of 18O2 for measuring cerebral oxygen extraction fraction with PET in healthy humans. J Nucl Med 2004; 45: 765–770.
8 Shimosegawa E, Hatazawa J, Ibaraki M, Toyoshima H, Suzuki A. Metabolic penumbra of acute brain infarction: a correlation with infarct growth. Ann Neurol 2005; 57: 495–504.
9 Kudomi N, Hayashi T, Teramoto N, Watabe H, Kawachi N, Ohta Y et al. Rapid quantitative measurement of CMRO2 and CBF by dual administration of 15O-labeled oxygen and water during a single PET scan—a validation study and error analysis in anesthetized monkeys. J Cereb Blood Flow Metab 2005; 25: 1209–1224.
10 Iida H, Kann I, Miura S. Rapid measurement of cerebral blood flow with positron emission tomography. Ciba Foundation Symp 1991; 163: 23–37, discussion 37–42.
11 Koeppe RA, Holden JE, Ip WR. Performance comparison of parameter estimation techniques for the quantification of local cerebral blood flow by dynamic positron computed tomography. J Cereb Blood Flow Metab 1985; 5: 224–234.
12 Kudomi N, Choi E, Watabe H, Kim KM, Shidahara M, Ogawa M et al. Development of a GSO Detector Assembly for a Continuous Blood Sampling System. IEEE TNS 2003; 50: 70–73.
13 Baron JC, Steining M, Tanaka T, Cavallejo E, Soussaline F, Collard P. Quantitative measurement of CBF, oxygen extraction fraction (OEF) and CMRO2 with the 15O continuous inhalation technique: experimental evidence and normal values in man. J Cereb Blood Flow Metab 1981; 1: 55–56.
14 West JB, Dollery CT. Uptake of oxygen-15-labeled CO2 compared with carbon-13-labeled CO2 in the lung. J Appl Physiol 1962; 17: 9–13.
15 Shao L, Karp JS. Cross-plane scattering correction-point source deconvolution in PET. IEEE Trans Med Imaging 1991; 10: 234–239.
16 Iida H, Kann I, Miura S, Murakami M, Takahashi K, Uemura K. Error analysis of a quantitative cerebral blood flow measurement using 15O autoradiography and positron emission tomography, with respect to the dispersion of the input function. J Cereb Blood Flow Metab 1986; 6: 536–545.
17 Iida H, Higano S, Tomura N, Shishido F, Kann I, Miura S et al. Evaluation of regional differences of tracer appearance time in cerebral tissues using 15O water and dynamic positron emission tomography. J Cereb Blood Flow Metab 1988; 8: 285–288.
18 Kudomi N, Hayashi T, Watabe H, Teramoto N, Piao R, Ose T et al. A physiologic model for recirculation water correction in CMRO2 assessment with 15O inhalation PET. J Cereb Blood Flow Metab 2009; 29: 355–364.
19 Kudomi N, Watabe H, Hayashi T, Iida H. Separation of input function for rapid measurement of quantitative CMRO2 and CBF in a single PET scan with a dual tracer administration method. Phys Med Biol 2007; 52: 1893–1908.
20 Phelps ME, Huang SC, Hoffman EJ, Kuhl DE. Validation of tomographic measurement of cerebral blood volume with C-11-labeled carboxyhemoglobin. J Nucl Med 1979; 20: 328–334.
21 Kudomi N, Watabe H, Hayashi T, Oka H, Miyake Y, Iida H. Optimization of transmission scan duration for 15O PET study with sequential dual tracer administration using N-index. Ann Nucl Med 2010; 24: 413–420.
22 Iida H, Law I, Pakkenberg B, Krapau-Hansen A, Eberl S, Holm S et al. Quantification of regional cerebral blood flow corrected for partial volume effect using O-15 water and PET. I. Theory, error analysis, and stereologic comparison. J Cereb Blood Flow Metab 2000; 20: 1237–1251.
23 Shidahara M, Watabe H, Kim KM, Oka H, Sago M, Hayashi T et al. Evaluation of a commercial PET tomograph-based system for the quantitative assessment of rCBF, rOEF and rCMRO2 by using sequential administration of 15O-labeled compounds. Ann Nucl Med 2002; 16: 317–327.
24 Ohta S, Meyer E, Thompson CJ, Gjedde A. Oxygen consumption of the living human brain measured after a single inhalation of positron emitting oxygen. J Cereb Blood Flow Metab 1992; 12: 179–192.
25 Ohta S, Reutens DC, Gjedde A. Brief vibrotactile stimulation does not increase cortical oxygen consumption when measured by single inhalation of positron emitting oxygen. J Cereb Blood Flow Metab 1999; 19: 260–265.
26 Meyer E, Tyler JL, Thompson CJ, Redies C, Diksic M, Hakim AM. Estimation of cerebral oxygen utilization rate by single-bolus 15O2 inhalation and dynamic positron emission tomography. J Cereb Blood Flow Metab 1987; 7: 403–414.
27 Fujita H, Kuwabara H, Reutens DC, Gjedde A. Oxygen consumption of cerebral cortex fails to increase during continued vibrotactile stimulation. J Cereb Blood Flow Metab 1999; 19: 266–271.
28 Inomata T, Fujimara M, Iida H, Kudomi N, Miura I. Neuron reduction of the small cyclotron for production of oxygen-15-labeled gas. Int Congress Series 2004; 1265: 97–100.
29 Miyake Y, Iida H, Hayashida K, Ishida Y. New method for the synthesis of 15O-labeled carbon monoxide and 18O-labeled dioxide for rapid supply in clinical use. Int Congress Series 2004; 1265: 93–96.
30 Iuchi S, Hori T, Moriyama M, Nitta Y, Yamamoto A, Koshino K et al. Verification of a semi-automated MRI-guided technique for non-invasive determination of the arterial input function in 15O-labeled gaseous PET. Nucl Instr Meth Phys Res B 2012 (in press).
31 Lammasrampa AA, Wise RJ, Heather JD, Gibbs JM, Leenaders KL, Frackowiak RS et al. Correction for the presence of intravascular oxygen-15 in the steady-state technique for measuring regional oxygen extraction ratio in brain. 2. Results in normal subjects and brain tumour and stroke patients. J Cereb Blood Flow Metab 1983; 3: 425–431.
32 Kudomi N, Slimani L, Jarvais ES, Kiss J, Lautamaki R, Naum GA et al. Non-invasive estimation of hepatic blood perfusion from H15O PET images using tissue-derived arterial and portal input functions. Eur J Nucl Med Mol Imaging 2008; 35: 1899–1911.
33 Kaisti K, Langsjo JW, Alavo S, Oikonen V, Silpa H, Teras M et al. Effects of sevoflurane, propofol, and adjunct nitrous oxide on regional cerebral blood flow, oxygen consumption, and blood volume in humans. Anesthesiology 2003; 99: 603–613.
34 Altman DI, Lich LL, Powers WJ. Brief inhalation method to measure cerebral oxygen extraction fraction with PET: accuracy determination under pathologic conditions. J Nucl Med 1991; 32: 1738–1741.
35 Frykholm P, Andersson JL, Valtysson J, Silander HC, Hillered L, Persson L et al. A metabolic threshold of irreversible ischemia demonstrated by PET in a middle cerebral artery occlusion-reperfusion primate model. Acta Neuroscand 2002; 108: 18–26.
36 Pappata S, Fiorelli M, Rommel T, Hartmann A, Dettmers C, Yamaguchi T et al. PET study of changes in local brain hemodynamics and oxygen metabolism after unilateral middle cerebral artery occlusion in baboons. J Cereb Blood Flow Metab 1993; 13: 416–424.
37 Schumann P, Touzani O, Young AR, Verard L, Morello R, MacKenzie ET. Effects of indomethacin on cerebral blood flow and oxygen metabolism: a positron emission tomographic investigation in the anesthetized baboon. Neurosci Lett 1996; 220: 137–141.
38 Touzani O, Young AR, Derlon JM, Beaudouin V, Marchal G, Rioux P et al. Sequential studies of severely hypometabolic tissue volumes after permanent middle cerebral artery occlusion. A positron emission tomographic investigation in anesthetized baboons. Stroke 1995; 26: 2112–2119.
39 Young AR, Sette G, Touzani O, Rioux P, Derlon JM, MacKenzie ET et al. Relationships between high oxygen extraction fraction in the acute stage and final infarction in reversible middle cerebral artery occlusion: an investigation in anesthetized baboons with positron emission tomography. J Cereb Blood Flow Metab 1996; 16: 1176–1188.
40 Cselenyi Z, Olsson H, Hallidin C, Gylas B, Farde L. A comparison of recent parametric neuroreceptor mapping approaches based on measurements with the high affinity PET radioligands [123I]FLB 457 and [123I]WAY 100635. NeuroImage 2006; 32: 1695–1708.
41 Schuitmaker A, van Berckel BN, Kropholler MA, Kloot RW, Jonker C, Scheltens P et al. Evaluation of methods for generating parametric (R)-[11C]PK11195 binding images. J Cereb Blood Flow Metab 2007; 27: 1603–1615.