

# Population-Based Input Function for TSP0 Quantification and Kinetic Modeling with [11C]-DPA- 713

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# Population-Based Input Function for TSPO Quantification and Kinetic Modeling with [<sup>11</sup>C]-DPA-713

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**Running title:** PBIF-based [<sup>11</sup>C]DPA-713 Kinetic Modeling

## ABSTRACT

**Introduction:** Quantitative positron emission tomography (PET) studies of neurodegenerative diseases typically require the measurement of arterial input functions (AIF), an invasive and risky procedure. This study aims to assess the reproducibility of [<sup>11</sup>C]DPA-713 PET kinetic analysis using population-based input function (PBIF). The final goal is to possibly eliminate the need for AIF.

**Materials and Methods:** Eighteen subjects including six healthy volunteers (HV) and twelve Parkinson disease (PD) subjects from two [<sup>11</sup>C]-DPA-713 PET studies were included. Each subject underwent 90 minutes of dynamic PET imaging. Five healthy volunteers underwent a test-retest scan within the same day to assess the repeatability of the kinetic parameters. Kinetic modeling was carried out using the Logan total volume of distribution ( $V_T$ ) model. For each data set, kinetic analysis was performed using a patient specific AIF (PSAIF, ground-truth standard), and then repeated using the PBIF. PBIF was generated using the leave-one-out method for each subject from the remaining 17 subjects, and after normalizing the PSAIFs by 3 techniques: (a)  $\text{Weight}_{\text{subject}} \times \text{Dose}_{\text{Injected}}$  (b) Area Under AIF Curve (AUC), and (c)  $\text{Weight}_{\text{subject}} \times \text{AUC}$ . The variability in the total distribution volume ( $V_T$ ) measured with PSAIF, in the test/retest study were determined for selected brain regions (white matter, cerebellum, thalamus, caudate, putamen, pallidum, brainstem, hippocampus and amygdala) using the Bland-Altman analysis, and for each of the 3 normalization techniques. Similarly, for all subjects, the variabilities due to the use of PBIF were assessed.

**Results:** Bland-Altman analysis showed systematic bias between test and retest studies. The corresponding mean bias and 95% limits of agreement (LOA) for the studied brain regions were 30% and  $\pm 70\%$ .

Comparing PBIF- and PSAIF-based  $V_T$  estimate for all subjects and all brain regions, a significant difference between the results generated by the three normalization techniques existed for all brain structures except for the brainstem ( $P$ -value = 0.095). The mean % difference and 95% LOA is -10% and  $\pm 45\%$  for  $\text{Weight}_{\text{subject}} \times \text{Dose}_{\text{Injected}}$ ; +8% and  $\pm 50\%$  for AUC; and +2% and  $\pm 38\%$  for  $\text{Weight}_{\text{subject}} \times \text{AUC}$ . In all cases, normalizing by  $\text{Weight}_{\text{subject}} \times \text{AUC}$  yielded the smallest % bias and variability (% bias =  $\pm 2\%$ ; LOA =  $\pm 38\%$  for all brain regions).

Estimating the reproducibility of PBIF-kinetics to PSAIF based on disease groups (HV/PD) and genotype (MAB/HAB), the average  $V_T$  values for all regions obtained from PBIF is insignificantly higher than PSAIF (%difference = 4.53%,  $P$ -value = 0.73 for HAB; and %difference = 0.73%,  $P$ -value = 0.96 for MAB). PBIF also tends to overestimate

the difference between PD and HV for HAB (%difference = 32.33% versus 13.28%) and underestimate it in MAB (%difference = 6.84% versus 20.92%).

**Conclusions:** PSAIF kinetic results are reproducible with PBIF, with variability in  $V_T$  within that obtained for the test-retest studies. Therefore,  $V_T$  assessed using PBIF-based kinetic modeling is clinically feasible, and can be an alternative to PSAIF.

**Keywords:** population-based input function; kinetic modeling; [ $^{11}\text{C}$ ]DPA-713; normalization

## 1. INTRODUCTION

18-kDa translocator protein (TSPO) receptor has been shown as a potential target for imaging neuroinflammation using PK-11195 PET (1–3). Recently, a putative antagonist of TSPO, [ $^{11}\text{C}$ ]-N,N-diethyl-2[2-(4-methoxyphenyl)-5,7-dimethyl-pyrazolol[1,5- $\alpha$ ]pyrimidin-3-yl]-acetamide ([ $^{11}\text{C}$ ]DPA-713), was developed concurrently with the TSPO agonist, fluoro-ethoxy derivative [ $^{18}\text{F}$ ]DPA-714.(4–6) Both [ $^{11}\text{C}$ ]DPA-713 and [ $^{18}\text{F}$ ]DPA-714 were shown to have higher affinity than the first generation TSPO tracer [ $^{11}\text{C}$ ]-(*R*)-PK11195 (4,7,8). Several studies have now demonstrated the usefulness of [ $^{11}\text{C}$ ]DPA-713 PET in quantifying neuroinflammation in different diseases, including multiple sclerosis (MS), Parkinson’s disease (PD), and Alzheimer disease (AD), both in animal and human studies (4,9–12).

In PET, kinetic modeling is often essential for the accurate quantification of tracer uptake and metabolism in the tissue. This often requires the measurement of the tracer concentration in the arterial blood over time, an invasive and a potentially risky procedure(13), which can also adversely influence subject accrual (14).

An alternative technique such as an image-derived input function (IDIF) (15,16) or population-based input function (PBIF) (17,18) can facilitate the adoption of PET protocols requiring input functions. In brain studies, IDIF is usually deduced from the dynamic images of the carotid arteries, and hence is susceptible to partial volume effect (15–17). Previous studies showed the feasibility of PBIF as a robust alternative to IDIF for some radiopharmaceuticals (17,18). PBIF is generated by averaging the normalized patient-specific arterial input functions (PSAIFs) deduced from a cohort of subjects. Several normalization techniques have been reported in the literature; for example traditional

scaling using blood samples by correlating the measured plasma activity with the AUC (17); correlation of the PBIF with PSAIF venous samples (19); scaling by injected dose and weight (20,21); and non-invasive scaling using individual parameters like weight, body surface area (BSA), and lean body mass (LBM) (17). Many studies have assessed the feasibility of PBIF for kinetic analysis using [<sup>18</sup>F]FDG (18,22–25), yet very few studies involved neuroreceptor PET tracers (17,26), including TSPO brain studies (19,21,27). To the best of our knowledge, no PET kinetic modeling study has been performed with [<sup>11</sup>C]DPA-713 using PBIF.

The aims of this study were to: (1) estimate the test-retest repeatability of the [<sup>11</sup>C]DPA-713 PET imaging in healthy subjects, and (2) assess the reproducibility of kinetic analysis of [<sup>11</sup>C]DPA-713 dynamic PET images of the brain with PBIF compared to PSAIF in healthy and PD subjects (based on the test-retest results). Kinetic parameters resulting from different PSAIF normalization techniques are also compared.

## **2. MATERIALS AND METHODS**

### **A. Subjects**

In total, twelve subjects (9 males and 3 females; age  $56.6 \pm 11.9$  years) were recruited from a Parkinson's Disease (PD) dynamic [<sup>11</sup>C]DPA-713 PET research study. Six additional healthy male subjects (age  $42.6 \pm 11.2$  years) were also included, out of which five healthy subjects underwent test-retest studies to assess the repeatability of DPA kinetics. The inclusion criteria for the PD cohort are PD clinical diagnosis of 3 to 12 years of duration from onset of symptoms, age 30 to 70 years at time of enrollment, Hoehn and Yahr stage 2-3, and absence of a clinical diagnosis of dementia. Exclusion criteria included subjects receiving dopamine receptor blocking agents or treatment with acetylcholinesterase inhibitors; history of another significant neurological or major psychiatric disorder, or autoimmune disorders within the past 5 years. For screening purpose, all patients including healthy volunteers had a blood sample (3 mL) collected for TSPO (rs6971) genotype analysis. Three different genotypes are defined: low affinity binders (LAB), mixed affinity binders (MAB) and high affinity binders (HAB). Patients that are low affinity binders were excluded from participation. Detailed information on all subjects is shown in supplementary Table S1.

## **B. PET Measurements and Reconstruction**

For the PET studies,  $14.2 \pm 1.9$  mCi of [ $^{11}\text{C}$ ]DPA-713 was administered through bolus-intravenous injection, followed by flushing 10-15 ml of saline solution. PET data were acquired simultaneously after injection in list-mode format on a 4-ring Siemens Biograph mCT<sup>TM</sup> for a total of 90 minutes. The PET data were reconstructed into 32 dynamic frames (6×10 s, 4×30 s, 3×60 s, 2×120 s, 5×240 s, 12×300 s) using ordered subset expectation maximization (OSEM) with attenuation, scatter, and randoms corrections. Continuous arterial sampling was performed at 15 second intervals for the first 10 minutes using an automated fraction collector, followed by five additional samples collected at 20, 30, 45, 60, and 90 minutes respectively. Each of the blood samples was weighed and counted using a Wizard<sup>®</sup> automatic gamma counter (Perkin Elmer), and then the activity concentration was calculated. Blood samples drawn at 5, 10, 20, 30, 45, 60, and 90 minutes post-injection were also used to estimate metabolite fractions using the HPLC method of analysis. The blood time activity curves (TACs) were finally corrected for metabolites, yielding a metabolite-corrected, arterial input function.

## **C. Data Analysis and Kinetic Modeling**

Each subject underwent a T1-weighted MRI scan. Inter-frames head motion correction was achieved by rigidly co-registering the individual dynamic PET frames to the last 10 minutes imageset using PMOD (version 3.8; PMOD Technologies Ltd). The resulting dynamic imageset was then rigidly registered to the T1-MR imageset. Brain regions were delineated on the MRI images using FreeSurfer software (28), the corresponding Volumes of Interest (VOIs) were overlaid on the co-registered and motion-corrected dynamic PET images, and finally the corresponding TACs were deduced.

Kinetic modeling was done for each patient using the Logan  $V_T$  model. Kinetic analysis was performed using the PSAIFs, and then repeated using the PBIFs. For each of the selected brain structures (white matter, cerebellum, thalamus, caudate, putamen, pallidum, brainstem, hippocampus and amygdala), the total volume of distribution ( $V_T$ ) were estimated with the blood volume fixed to 5%. These brain regions were selected mainly because they show great affinity for [ $^{11}\text{C}$ ]DPA-713 binding.

#### D. Test-Retest Repeatability and Reliability

Five healthy volunteers underwent a test-retest within the same day to assess the reproducibility of the kinetic parameters in the brain structures. Kinetic analysis was carried out for all the selected brain regions, for both the test and retest datasets, using the Logan  $V_T$  model and the corresponding PSAIF's. The repeatability of  $V_T$  was assessed using the Bland-Altman analysis (29):

$$\% \text{ Relative Diff, } D = \frac{\text{Retest} - \text{Test}}{(\text{Retest} + \text{Test})/2} \times 100 \quad (1)$$

$$\text{Mean Bias} = \frac{\sum_{n=1}^N D}{N} \quad (2)$$

The corresponding confidence interval of the mean bias (CI), 95% limits of agreement (LoA) and the coefficient of repeatability (CR) between test and retest were determined using:

$$\text{LOA} = \text{Mean Bias} \pm 1.96SD \quad (3)$$

$$\text{CI}_i = i \pm (t \times SE) \quad (4)$$

$t$  is the t-value, while  $SE$  is the standard error given by:

$$SE = \begin{cases} SD/\sqrt{N}, & \text{for Bias} \\ \sqrt{3SD^2/N}, & \text{for LOA} \end{cases} \quad (5)$$

where  $N$  = number of subjects, and  $i$  = Mean Bias, LOA

$$\text{CR} = 1.96 \times \sqrt{\frac{\sigma^2}{N-1}} \quad (6)$$

where  $\sigma^2$  is the variance of the relative difference,  $D$ , between the test and retest estimates. This represents the value below which the relative difference between test and retest is expected to lie with a 95% probability (29,30).

#### E. Generation of Population-Based Input Functions

The PBIFs were generated from the metabolic-corrected PSAIFs of all the 18 subjects under review (details in supplementary section S1). The individual PSAIFs were fitted using the “tri-exponential” function and then corrected for metabolites after fitting the later using “Watabe” function (as incorporated in PMOD). The metabolite-corrected

PSAIFs for all subjects were interpolated to the same time grid (with a step of 1 second) and then their peaks were aligned to the 30 second time-point where the majority of the IF peaks occurred. In order to reduce the influence of subject-induced variation on the generated PBIF, each of the metabolite-corrected PSAIFs was normalized separately by three methods: (a)  $\text{Weight}_{\text{subject}} \times \text{Dose}_{\text{Injected}}$  (b) the corresponding AUC, and (c)  $\text{Weight}_{\text{subject}} \times \text{AUC}$ .

For each subject, PBIF was generated by averaging the normalized PSAIF of the other 17 subjects - leave-one-out procedure (17,20,31,32). Individual subject IFs were then generated by appropriately scaling the PBIF with the corresponding factor, i.e. (a)  $\text{Weight}_{\text{subject}} \times \text{Dose}_{\text{Injected}}$  (b) the corresponding AUC, and (c)  $\text{Weight}_{\text{subject}} \times \text{AUC}$ .

AUC scaling was done by tail-fitting the normalized PBIF and the PSAIF using the time-points 30, 45, 60 and 90 minutes. Additionally, three pseudo-time points (37.5, 52.5 and 75 minutes) were created as the average of PSAIF at 30 and 45 minutes, 45 and 60 minutes, and 60 and 90 minutes respectively. This was done in order to find an optimal timepoint which minimizes the difference between the original PSAIF AUC and the scaled PBIF AUC, following a similar approach for TSPO study (27).

The reproducibility of  $V_T$  using PBIF was assessed using Bland-Altman analysis, with PSAIF values as gold reference. For each structure, the % relative difference (Relative Diff),  $D$ , between the parameters was estimated using:

$$\% \text{ Relative Diff, } D = \frac{P_{PBIF} - P_{PSAIF}}{P_{PSAIF}} \times 100 \quad (7)$$

where  $P_{PBIF}$  and  $P_{PSAIF}$  are the kinetic parameters generated by PBIF and PSAIF respectively.

The bias, upper and lower limits of agreements (LoA), and the corresponding 95% confidence intervals (CI) were estimated using equations (2-5).

## **F. Statistical Analysis**

Data were analyzed using SPSS (IBM SPSS statistics for windows, version 26.0) and Real statistics (<http://www.real-statistics.com/>) software. Normality of distribution was tested using the Shapiro-Wilk test. The statistical difference between the three normalization techniques was evaluated using the one-way Analysis of Variance (ANOVA). The pairwise t-test was also performed as a follow-up test to ANOVA in order to reveal which specific pair of the normalization techniques are significantly different, and Bonferroni correction was applied to correct for the potential error due to multiple testing. In all cases, a  $P$ -value  $< 0.05$  was considered to suggest statistical significance.

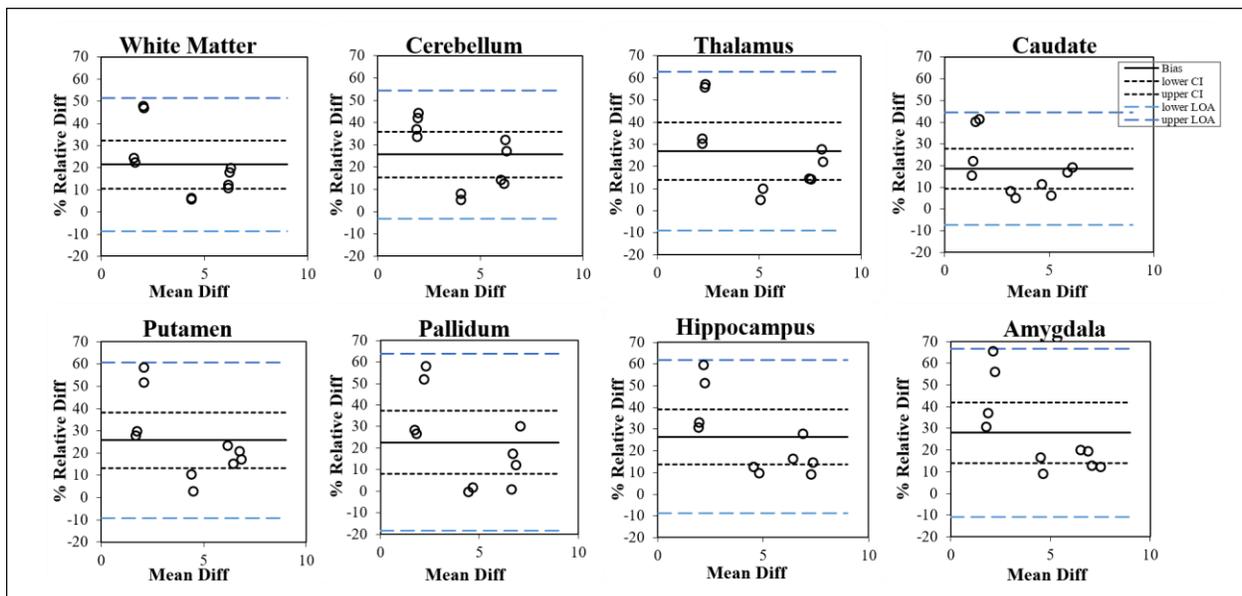
### 3. RESULTS

#### A. Test-Retest Repeatability and Reliability

The repeatability of the  $V_T$  estimates for all selected brain regions of interest in the test-retest studies are shown in Figure 1 and Table 1. For all the brain regions of interest, the mean of the  $V_T$  estimates from all the healthy volunteers is between 3.18 to 4.91 for test estimates, and 3.68 to 5.92 for the retest. The  $V_T$  estimates in the retest studies exhibited positive bias (ranging from 20 to 30%) compared to those deduced from the test studies.

**Table 1** Bland-Altman analysis of the variation in  $V_T$  estimates between the test and retest

Regions	Test Mean	Retest Mean	Mean % bias ± SD	95% CI	95% LoA	ICC
White matter	3.74 ± 2.02	4.43 ± 2.14	21.43 ± 15.08	10.64 to 32.22	-8.74 to 51.60	0.93
Cerebellum	3.61 ± 1.87	4.50 ± 2.15	25.69 ± 14.40	15.38 to 35.99	-3.12 to 54.50	0.88
Thalamus	4.51 ± 2.50	5.61 ± 2.77	26.89 ± 18.01	14.01 to 39.77	-9.12 to 62.90	0.90
Caudate	3.16 ± 1.84	3.68 ± 2.02	18.52 ± 12.94	9.26 to 27.78	-7.36 to 44.40	0.95
Putamen	3.83 ± 2.12	4.71 ± 2.31	25.71 ± 17.47	13.21 to 38.21	-9.24 to 60.65	0.91
Pallidum	4.06 ± 2.24	4.83 ± 2.35	22.70 ± 20.56	7.99 to 37.41	-18.41 to 63.82	0.91
Brainstem	4.91 ± 2.92	5.92 ± 3.15	23.61 ± 15.80	3.99 to 43.23	-7.99 to 55.21	0.94
Hippocampus	4.09 ± 2.29	5.04 ± 2.39	26.44 ± 17.65	13.82 to 39.06	-8.85 to 61.73	0.91
Amygdala	4.04 ± 2.35	4.99 ± 2.42	27.98 ± 19.40	14.11 to 41.86	-10.81 to 66.78	0.92

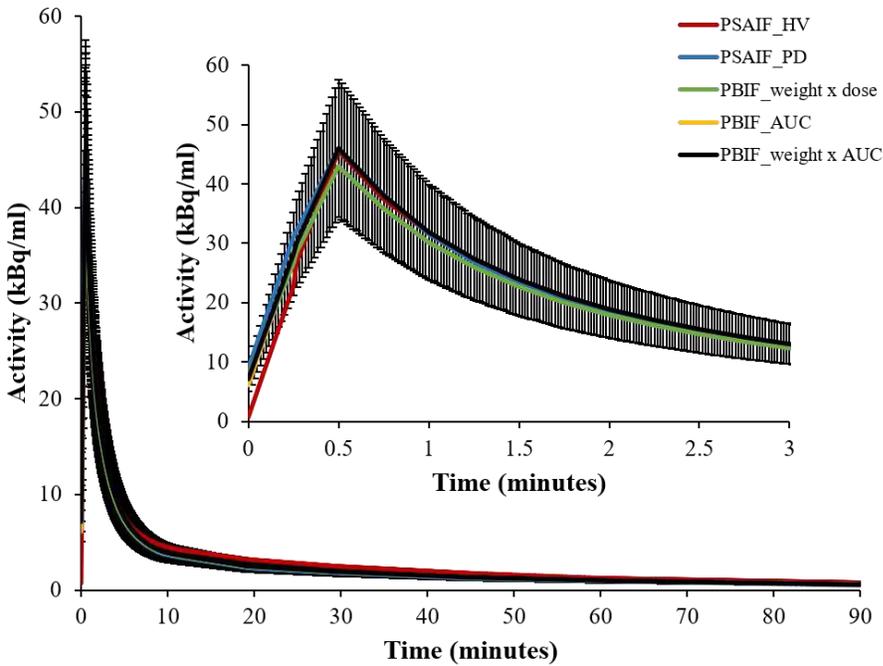


**Figure 1** Bland-Altman plot comparing the test-retest repeatability of  $V_T$  estimates for all selected brain regions of interest. The solid line is the mean % bias between test and retest  $V_T$  estimate, while the dotted and dashed lines represent the %CI and %LOA respectively

A systematic bias is also noticed between the test and the retest results, where all the differences lie above the zero line. The 95% LOA lies within ~3% and ~70% for all regions.

## B. Comparison between PSAIF and PBIF

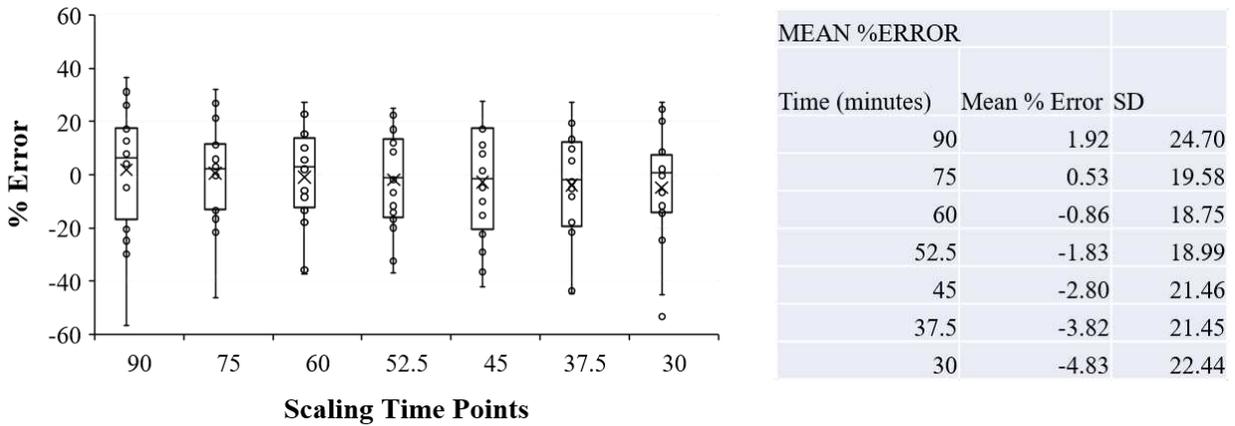
Before generating the PBIF from the pool of subjects, we first examined the shape of the PSAIFs between groups (HV versus PD) and genotype (MAB vs HAB). The results are shown in Figures 2 and Supplementary S2. Visual inspection of the average IF for HV and PD showed no difference between groups (Figure 2). Also, the log-transformation plot showed no difference in the peak or tail for different groups and genotype (Supplementary Figure S2).



**Figure 2** Comparison between the averaged PSAIFs of HV and PD subjects, and the resulting PBIF generated from the three normalization techniques. The inner plot shows the zoomed IF over the first 3 minutes. The standard deviation (SD) is shown for the PBIF generated by normalization with  $\text{Weight}_{\text{subject}} \times \text{AUC}$ .

Therefore, the PSAIFs for all the eighteen subjects involved in this study were pooled together to generate the PBIF using the three normalization techniques used in this study. The individual IFs were generated by appropriately scaling the PBIF with the corresponding normalization factor. AUC scaling was done by tail-fitting the normalized PBIF and

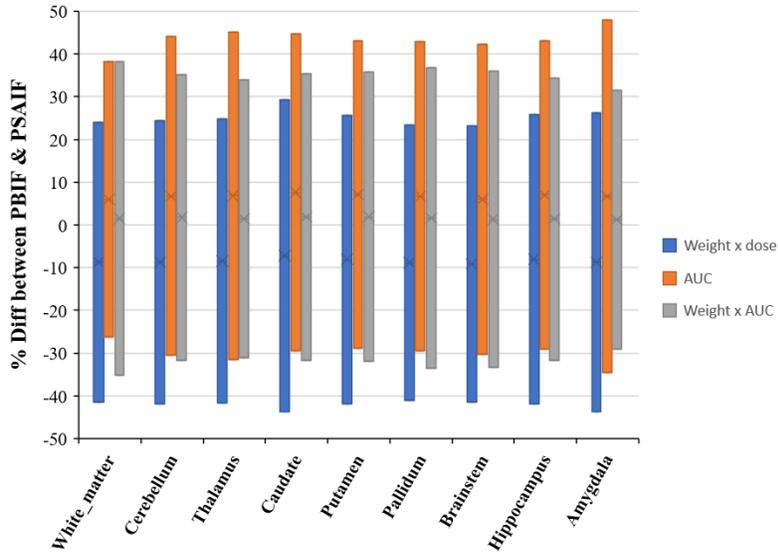
the PSAIF using the timepoints 30, 37.5, 45, 52.5, 60, 75 and 90 minutes. The AUC of the scaled PBIF using the different timepoints and the original PSAIF were then compared by evaluating the %error (result in Figure 3). Although there is no significant difference in the %error between the different time points, the sample at 75 minutes yielded the least %error of 0.53%. Since 75minutes was actually an average between the blood collected at 60 and 90 minutes, the AUC scaling in this study was done by tail-fitting the PBIF and the PSAIF using the last 30 minutes time points (i.e. between 60 and 90 minutes).



**Figure 3** AUC Comparison of the scaled PBIF using the different timepoints and the original PSAIF. Note that no blood was collected at time points 37.5, 52.5 and 75 minutes, they were just an average timepoints of 30 and 45 minutes, 45 and 60 minutes, and 60 and 90 minutes respectively

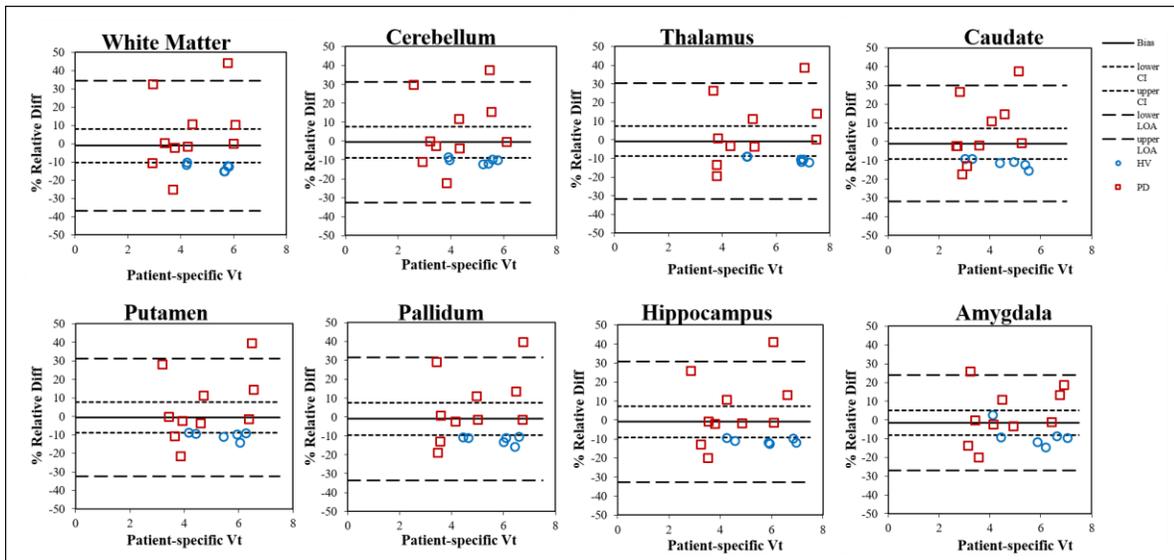
### C. Evaluation of the PBIF and the Normalization Criteria

Figure 4 shows the % difference and the LoAs between the  $V_T$  estimates generated by the PSAIF and PBIF for selected brain regions. The comparison is made using PSAIF and the PBIF generated by the three normalization techniques. The mean % difference is -10% for  $Weight_{subject} \times Dose_{Injected}$ ; +8% for AUC and +2% for  $Weight_{subject} \times AUC$ ; while the LoAs lie within  $\pm 45\%$  for  $Weight_{subject} \times Dose_{Injected}$ ;  $\pm 50\%$  for AUC and  $\pm 38\%$  for  $Weight_{subject} \times AUC$ . The ANOVA analysis shows a significant difference between the results generated by the three normalization techniques for all brain structures except the brainstem ( $P$ -value = 0.095). Although for the same brainstem, the pairwise test shows a significant difference between  $Weight_{subject} \times Dose_{Injected}$  versus AUC ( $P$ -value = 0.034). In all cases, normalizing by  $Weight_{subject} \times AUC$  yielded the smallest % bias and variability (% bias =  $\pm 2\%$ ; LOA =  $\pm 38\%$  for all brain regions).



**Figure 4** The % relative difference in  $V_T$  and the LoAs between PSAIF and PBIF of some specific structures as generated by the three normalization techniques.

The mean bias ( $\pm$ SD) between the PSAIF and PBIF for the  $V_T$  generated by normalization with  $\text{Weight}_{\text{subject}} \times \text{AUC}$  are shown in Figure 5 and Table 2 (for all brain regions).



**Figure 5** Bland-Altman analysis showing the variation in the  $V_T$  between the PSAIF and PBIF (normalization with  $\text{Weight}_{\text{subject}} \times \text{AUC}$ ). The values are shown for the HV (blue circles) and the PD (red circles) groups. The solid line is the mean % bias between PSAIF and PBIF  $V_T$  estimate, while the dotted and dashed lines represent the %CI and %LOA respectively

**Table 2** Bland-Altman analysis of the variation in  $V_T$  estimate between the PSAIF and PBIF (normalization with  $\text{Weight}_{\text{subject}} \times \text{AUC}$ )

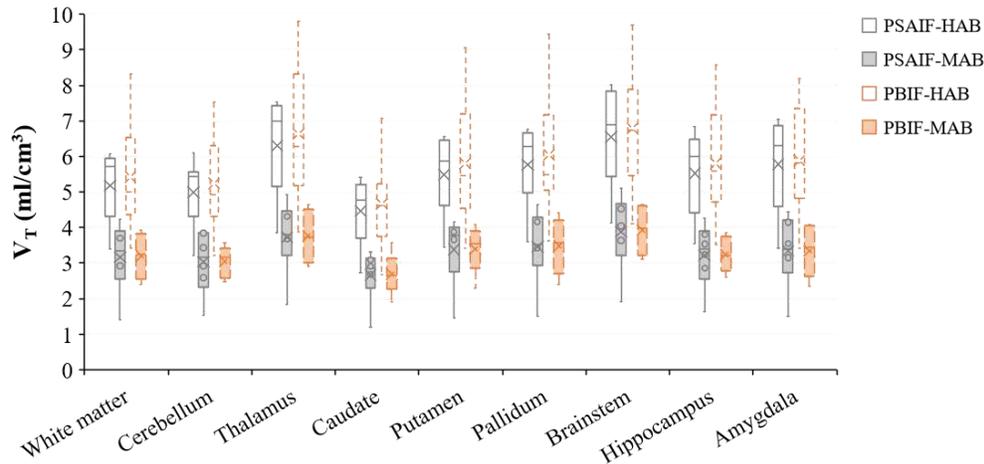
Regions	Mean % bias $\pm$ SD	95% CI	95% LoA
White matter	1.51 $\pm$ 18.74	-6.06 to 9.07	-35.22 to 38.23
Cerebellum	1.73 $\pm$ 17.09	-5.17 to 8.63	-31.75 to 35.22
Thalamus	1.45 $\pm$ 16.57	-5.24 to 8.14	-31.02 to 33.93
Caudate	1.80 $\pm$ 17.14	-5.12 to 8.73	-31.79 to 35.40
Putamen	1.91 $\pm$ 17.27	-5.07 to 8.89	-31.96 to 35.77
Pallidum	1.57 $\pm$ 17.95	-5.67 to 8.82	-33.60 to 36.75
Brainstem	1.28 $\pm$ 17.67	-9.39 to 11.96	-33.35 to 35.92
Hippocampus	1.36 $\pm$ 16.86	-5.44 to 8.18	-31.67 to 34.41
Amygdala	1.21 $\pm$ 15.42	-5.01 to 7.45	-29.01 to 31.45

The mean bias for  $V_T$  lies within  $\pm 2\%$ , with amygdala showing the smallest (1.21%) deviation, and putamen showing the highest (1.91%). Overall, the 95% LoA for all brain regions lies within  $\pm 38\%$ .

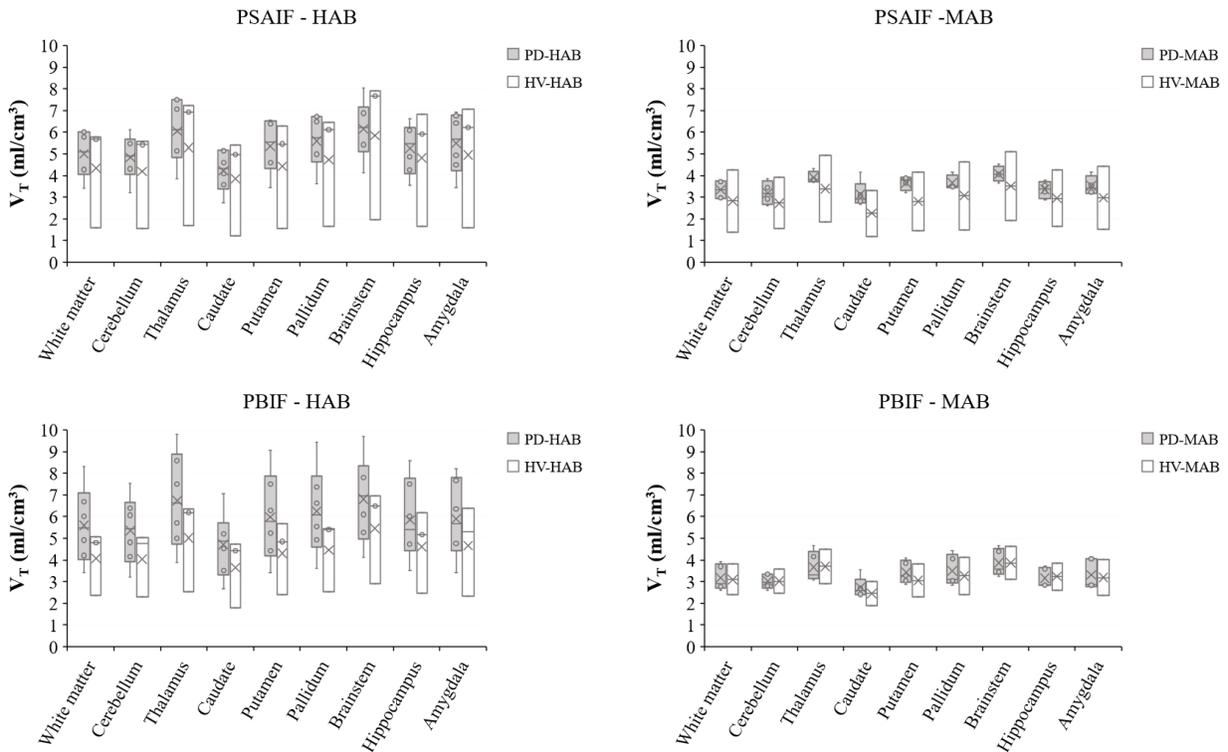
#### D. Agreement of $V_T$ between PSAIF and PBIF (based on disease groups and genotype)

Finally, we estimated how well the PBIF-kinetics replicate the PSAIF-kinetics based on disease groups (HV versus PD) and genotype (MAB versus HAB). Figure 6 shows the Logan  $V_T$  values for all subjects calculated with PSAIF and PBIF for the two genotype groups (HAB and MAB). For all brain regions, the average  $V_T$  values obtained from PBIF is slightly higher than PSAIF, but the difference is not significant for each genotype group (%difference = 4.53%,  $P$ -value = 0.73 for HAB; and %difference = 0.73%,  $P$ -value = 0.96 for MAB). Comparing MAB to HAB, there is a significant reduction in  $V_T$  both with PSAIF and PBIF. PSAIF showed an average reduction of 40% in  $V_T$  across the brain regions, while the average reduction with PBIF is 42%. T-test showed a  $P$ -value  $< 0.01$  for all the brain regions, both for PSAIF and PBIF, and on average, the  $P$ -value for PBIF is about 50% higher than PSAIF.

Figure 7 shows the difference in Logan  $V_T$  values calculated with PSAIF and PBIF between HV and PD subjects, and also HAB and MAB groups. The average  $V_T$  values are insignificantly higher in PD patients compared to HV. PBIF tend to overestimate the difference between PD and HV for HAB (%difference = 32.33%,  $P$ -value = 0.32 with PBIF; %difference = 13.28%,  $P$ -value = 0.64 with PSAIF). However, this difference is underestimated in MAB (%difference = 6.84%,  $P$ -value = 0.77 with PBIF; %difference = 20.92%,  $P$ -value = 0.55 with PSAIF).



**Figure 6** Logan  $V_T$  values for all subjects calculated with PSAIF and PBIF for the two genotype groups (HAB and MAB). Comparing MAB to HAB, there is a significant reduction in  $V_T$  both with PSAIF and PBIF. PSAIF showed an average reduction of 40% in  $V_T$  across the brain regions, while the average reduction with PBIF is 42%



**Figure 7** Logan  $V_T$  values between HV and PD subjects, and also HAB and MAB groups calculated with PSAIF and PBIF

#### 4. DISCUSSION

Several studies have shown the feasibility to image neuroinflammation in multiple sclerosis (MS), Parkinson's disease (PD), and Alzheimer disease (AD) using [ $^{11}\text{C}$ ]DPA-713 PET for quantifying differences between patients and controls (4,9–12). Accurate quantification of tracer uptake and metabolism in the tissue through kinetic modeling often requires blood sampling,(13) or some alternative approach such as simplified reference modeling (33,34), cluster analysis (33,35) or image-derived input function (IDIF) techniques. The apparent limitations of these approaches (15–17,19,21,36) are giving way to the exploration of the population-based input function (PBIF) approach as a more quantitatively reliable and less invasive alternative.

In this study, we have assessed the reproducibility of kinetic analysis of [ $^{11}\text{C}$ ]DPA-713 dynamic PET images using PBIF, compared to PSAIF, in a cohort of subjects with Parkinson Disease and healthy volunteers. The repeatability of the  $V_T$  estimate was also assessed in a cohort of healthy volunteers that underwent a test-retest [ $^{11}\text{C}$ ]DPA-713 dynamic PET within the same day. Kinetic analysis with PSAIF was determined to be reproducible with PBIF if the corresponding LoA are within those of the test-retest study.

The test-retest repeatability of the [ $^{11}\text{C}$ ]DPA-713 uptake exhibited systematic increase in uptake values between test and retest (Figure 1, and supplementary Figure S1). Although the cause of this bias is yet to be fully explored in same day test-retest repeatability studies because most repeatability studies are done days or even weeks apart (37). Few recent studies that performed same day test-retest have reported the same systematic bias, and they suggested that the possible explanation to this systematic bias could be due to hormone-mediated changes in TSPO expression, tonic changes due to scan-related stress/anxiety, or alteration in blood cholesterol due to food intake between the test and retest scans (38,39). While performing test and retest studies under similar conditions on different days could eliminate this bias, other parameters such as alteration in TSPO density due to chronic disease as well as non-disease-related factors may be difficult to control (40).

One potential approach to compensate for the bias between the test and retest studies is by normalization by the corresponding kinetic parameters of the GM as suggested by past studies (40,41) and also shown by this study (Supplementary Figure S1). Without GM normalization, the % relative difference between test and retest  $V_T$  values

lies significantly above the zero line for all structures, indicating that retest values are always higher than test values. But with GM normalization, the % relative difference is symmetric about the zero line. The mean % Diff and the LOA are also significantly reduced, thereby improving repeatability. However, past studies involving gray matter normalization was validated in a clinical population (HIV with associated cognitive deficits) with regional inflammation. We believe there is no sufficient justification of using the GM normalization approach for PD cohorts since neuroinflammation can occur in any brain region, and therefore we elected not to adopt it.

The PBIF was generated from the PSAIF of all 18 subjects after examining the shape of the PSAIFs between groups (HV versus PD) and genotype (MAB vs HAB). This was motivated by Owen *et al.* (42,43), who demonstrated that the second generation TSPO tracers target two binding sites in humans, which leads to three affinity patterns: low-, high-, and mixed-affinity binders (LABs, HABs. and MABs, respectively). Past researches have shown that this variability in binding affinity has a major influence on the kinetic parameters where the values for HABs could be approximately twice that of MABs (43,44). For [<sup>11</sup>C]-DPA-713 dynamic PET studies, Coughlin *et al.* (40) argued that those genotype- as well as other unknown physiological factors have varying degrees of influence on the global TSPO changes in the brain, thereby hindering accurate PET analysis, even among individuals with the same genotype. This was also confirmed in other TSPO studies (37,45,46). Our results (Figures 2 and supplementary Figure S2) however showed no significant difference in PSAIF between the groups, and so, all subjects PSAIFs were included in the generation of the PBIF. This was also in agreement with other studies (27).

Ye *et al.* (47) opined that the bias in kinetic parameter estimation in direct reconstruction with PBIF depends on the normalization and scaling technique used. In this study, we have assessed and compared three normalization approaches: (a)  $\text{Weight}_{\text{subject}} \times \text{Dose}_{\text{Injected}}$ , (b) AUC, and (c)  $\text{Weight}_{\text{subject}} \times \text{AUC}$ . An example of the normalized PSIFs and the resulting PBIF are shown in supplementary Figure S3. The performance of these techniques was evaluated using the percent relative difference between the PSAIF- and PBIF- derived  $V_T$  in selected brain regions (Figure 4). There is a significant difference between the three normalization techniques for all brain structures except the brainstem.

Several normalization techniques have been reported in the literature which include: traditional scaling using blood samples by correlating the measured plasma activity at a given time-point with the AUC;(17) correlation of the PBIF with AIF at any time-point using venous samples (19); by accounting for injected dose and weight (20,21); non-invasive scaling using individual parameters like weight, body surface area (BSA), and lean body mass (LBM) (17). In this study, we have assessed the three aforementioned normalization approaches. Subsequently, a subject IF was deduced by scaling the PBIF by his/her weight and injected dose. In the case of AUC normalization, this was measured after scaling the PBIF by the ratio of the average activity concentration of blood samples acquired over the last 30 minutes of the dynamic scan (i.e. between 60 and 90 minutes) and that of the tail of the PBIF over the same time frames. This setting was used as this best minimizes the error between PSAIF AUC and the scaled PBIF (Figure 3). In this study, the AUC between PSAIF and PBIF was minimized by scaling the PBIF with an arterial blood value at 75minutes, as also recommended by past similar TSPO studies (27,48). Since our goal is to possibly eradicate arterial sampling, a venous sample can potentially be obtained at 75minutes to scale the PBIF for each subject. In fact, it has been shown that venous blood samples may practically be used instead for scaling purpose since arterial and venous blood tend to reach equilibrium at about 30-45 minutes post-injection time (17). Although this was not tested in this work, but similar TSPO studies have also found that PBIF can be appropriately scaled using one blood sample (27,48).

In this study, normalization by  $\text{Weight}_{\text{subject}} \times \text{AUC}$  yielded the smallest % bias ( $\pm 2\%$ ) and variability (LoAs  $\pm 38\%$ ) between PBIF and PSAIF (Figure 4).  $V_T$  measured with PBIF showed good reproducibility (LOA of  $\pm 38\%$ ) but with a positive bias ( $\pm 2\%$ ) (Figure 5 and Table 2). These were also in agreement with the findings of Lavisse *et al.* (19) As a final note, the reproducibility of the PBIF-based  $V_T$  estimates compared with PSAIF-based  $V_T$  fall well within the test-retest results (Table 1), hence showing the feasibility of [ $^{11}\text{C}$ ]-DPA-713 PET kinetic modelling using PBIF.

PBIF was able to reproduce the PSAIF kinetic results because of the similar patterns in average AIF between disease groups and genotypes (Figures 2 and supplementary Figure S2). Although, we expect that PBIF cannot exactly reproduce the peak and shape of the PSAIF (as shown in supplementary Figure S2), but having a similar AUC between PSAIF and PBIF will result in less bias in kinetic parameter estimation. That is why previous studies have recommended that Logan  $V_T$  method is more suitable for PBIF than 2-tissue compartment model because Logan  $V_T$  relies on the AUC of the IF and therefore less sensitive to the shape (20,49).

A major limitation for this study is the relatively small sample size ( $n = 18$ ), even though our findings are in agreement with previous results of smaller ( $n = 9$ ) (19) and larger ( $n = 42$ ) (20) sample sizes. A common factor among these studies is the normalization of the individual input functions to remove variabilities in the PBIF. This might suggest that the efficiency of the PBIF in accurately estimating the kinetic parameters depend less on the sample size used, but more on the normalization. This was also consolidated by Ye *et al.* (47) who opined that the bias in kinetic parameter estimation in direct reconstruction with PBIF was mostly due to inaccuracy in normalization and scaling.

## CONCLUSION

This study demonstrated the feasibility of [ $^{11}\text{C}$ ]-DPA-713 PET kinetic modelling using PBIF with Logan graphical analysis, thus potentially alleviating the need for arterial blood sampling. Moreover, it was shown that the optimal result in terms of kinetic parameter accuracy was obtained when the PSAIFs were normalized with  $\text{Weight}_{\text{subject}} \times \text{AUC}$ . Since the utmost aim is to potentially alleviate the need for arterial blood sampling, the AUC component of the PBIF normalization can be obtained by scaling the normalized PBIF by the ratio of the average activity concentration of blood samples (possibly venous blood) acquired over the last 30 minutes of the dynamic scan (i.e. between 60 and 90 minutes) and that of the tail of the PBIF over the same time frames (as was done in this study). However, more relevant clinical studies need to be conducted to establish a correlation between activity concentration in arterial and venous blood samples at these latter time points of the scan.

## DECLARATIONS

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**Ethical Approval:** All procedures performed in the human studies contained in this work were in accordance with the ethical standards of the institutional review board at the Weill Cornell Medical College, New York, USA.

**Informed Consent:** Informed consent was obtained from all individual participants included in this study.

**Authors' Contributions:** MI Akerele and SA Nehmeh conceived the idea, designed the study and participated in data acquisition. MI Akerele, SA Zein, S Pandya, NA Karakatsanis and SA Nehmeh carried out the data processing and analysis. J Babich and A Nikolopoulou performed the radiotracer production and metabolite analysis. PD Mozley was

involved in the arterial line and procedure. A Gupta participated in the study design and collaboration. SA Gauthier, A Raj and C Henchcliffe are the principal investigators for the DPA studies, they are in charge of funding acquisition and subject accrual. MI Akerele wrote the manuscript. All authors reviewed the manuscript and approved the final version for submission.

**Conflict of Interest:** All authors have no relevant financial or non-financial conflicts of interest to disclose.

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# Figures

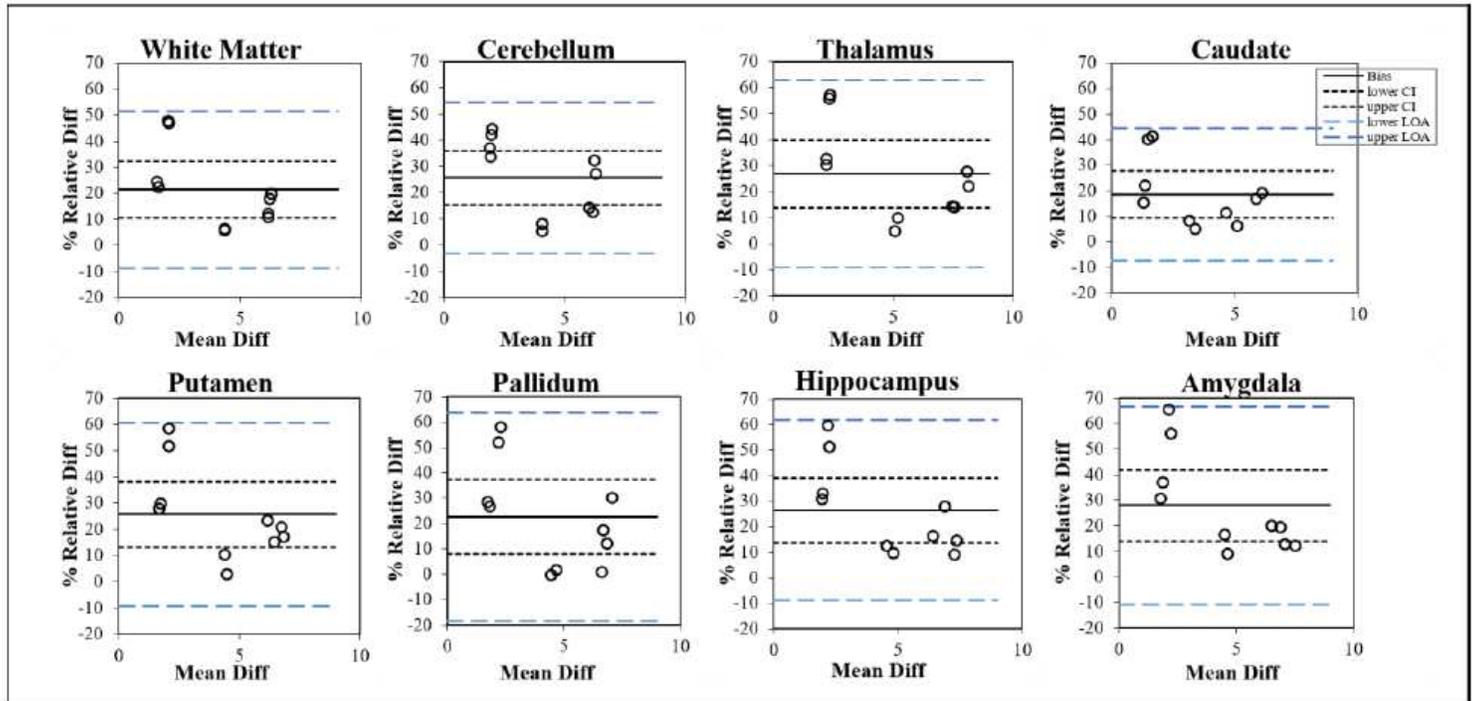


Figure 1

Bland-Altman plot comparing the test-retest repeatability of Vt estimates for all selected brain regions of interest. The solid line is the mean % bias between test and retest VT estimate, while the dotted and dashed lines represent the %CI and %LOA respectively

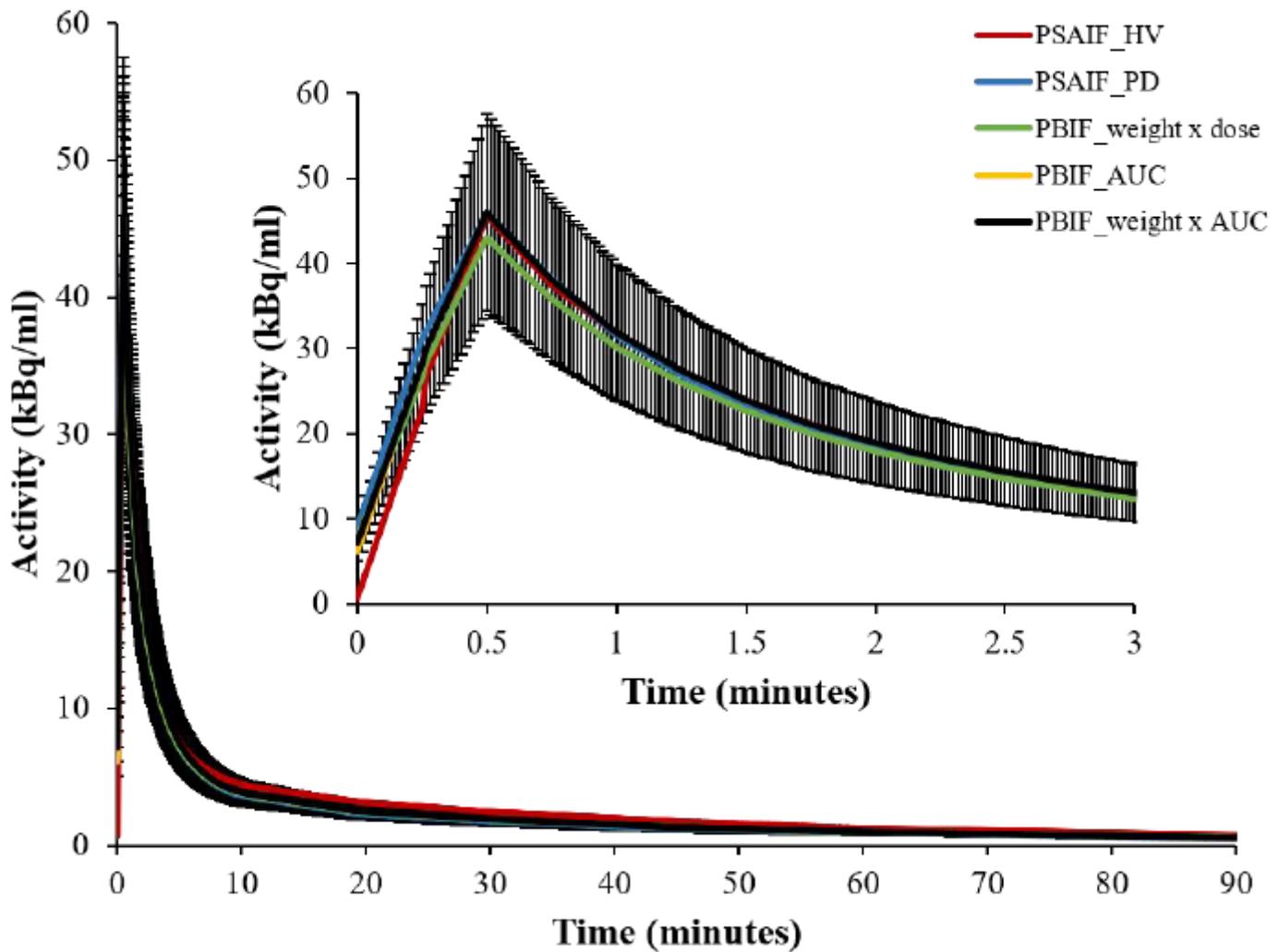
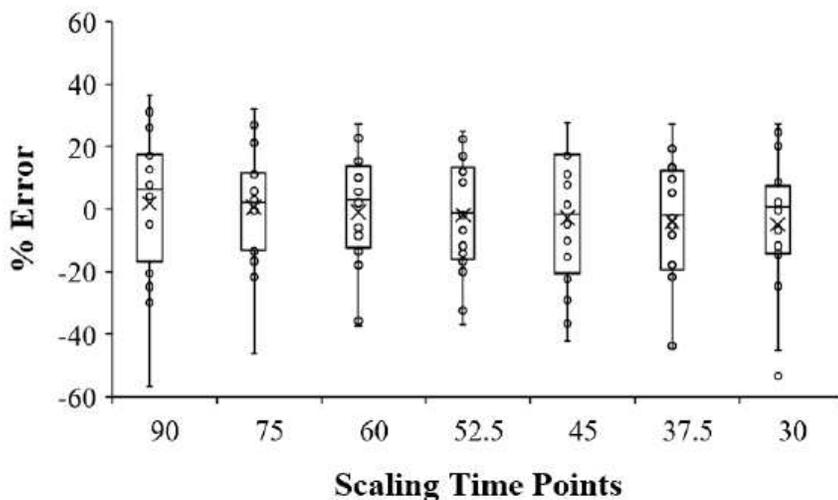


Figure 2

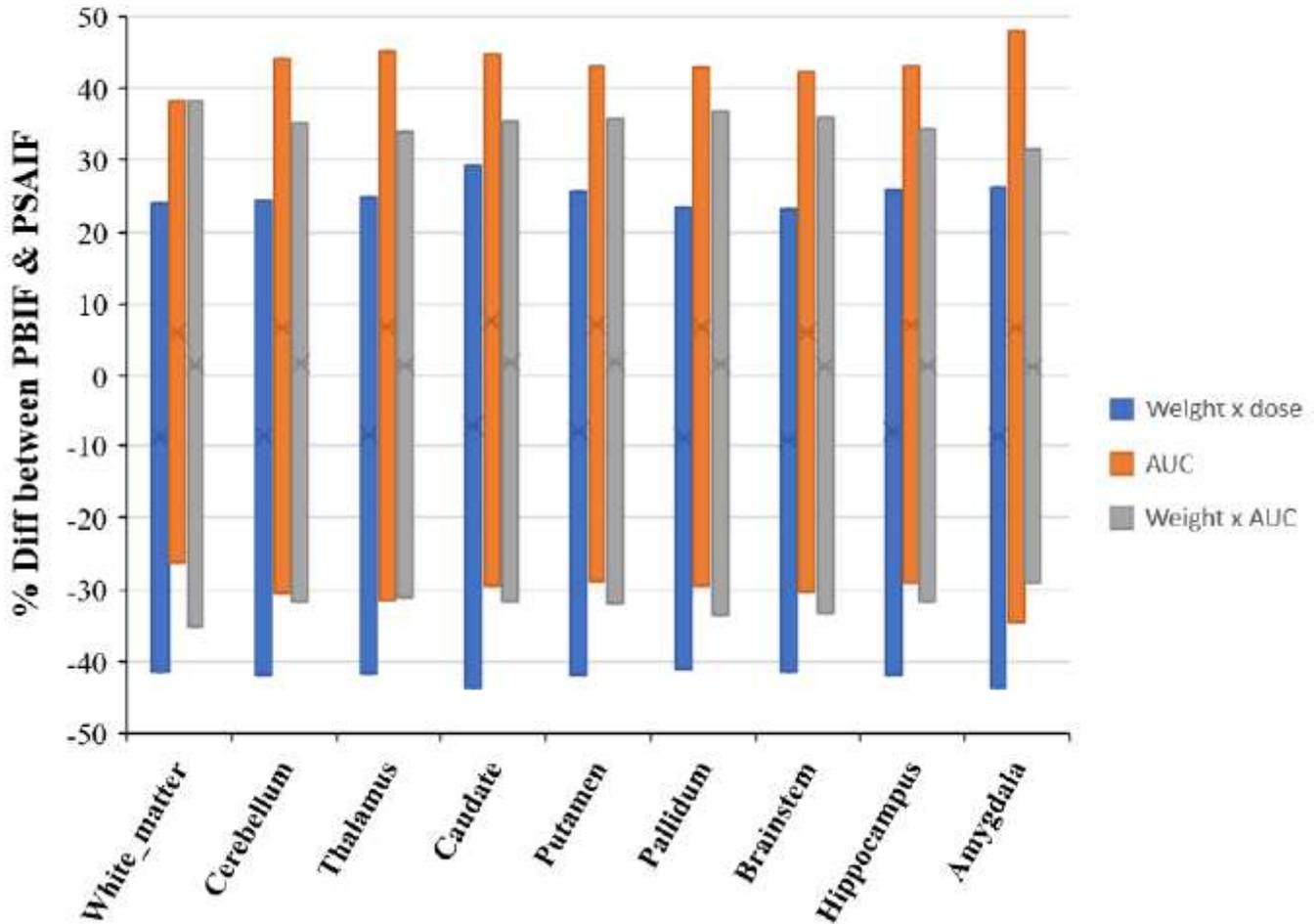
Comparison between the averaged PSAIFs of HV and PD subjects, and the resulting PBIF generated from the three normalization techniques. The inner plot shows the zoomed IF over the first 3 minutes. The standard deviation (SD) is shown for the PBIF generated by normalization with Weights<sub>subject</sub> × AUC.



MEAN %ERROR		
Time (minutes)	Mean % Error	SD
90	1.92	24.70
75	0.53	19.58
60	-0.86	18.75
52.5	-1.83	18.99
45	-2.80	21.46
37.5	-3.82	21.45
30	-4.83	22.44

**Figure 3**

AUC Comparison of the scaled PBIF using the different timepoints and the original PSAIF. Note that no blood was collected at time points 37.5, 52.5 and 75 minutes, they were just an average timepoints of 30 and 45 minutes, 45 and 60 minutes, and 60 and 90 minutes respectively



**Figure 4**

The % relative difference in VT and the LoAs between PSAIF and PBIF of some specific structures as generated by the three normalization techniques.

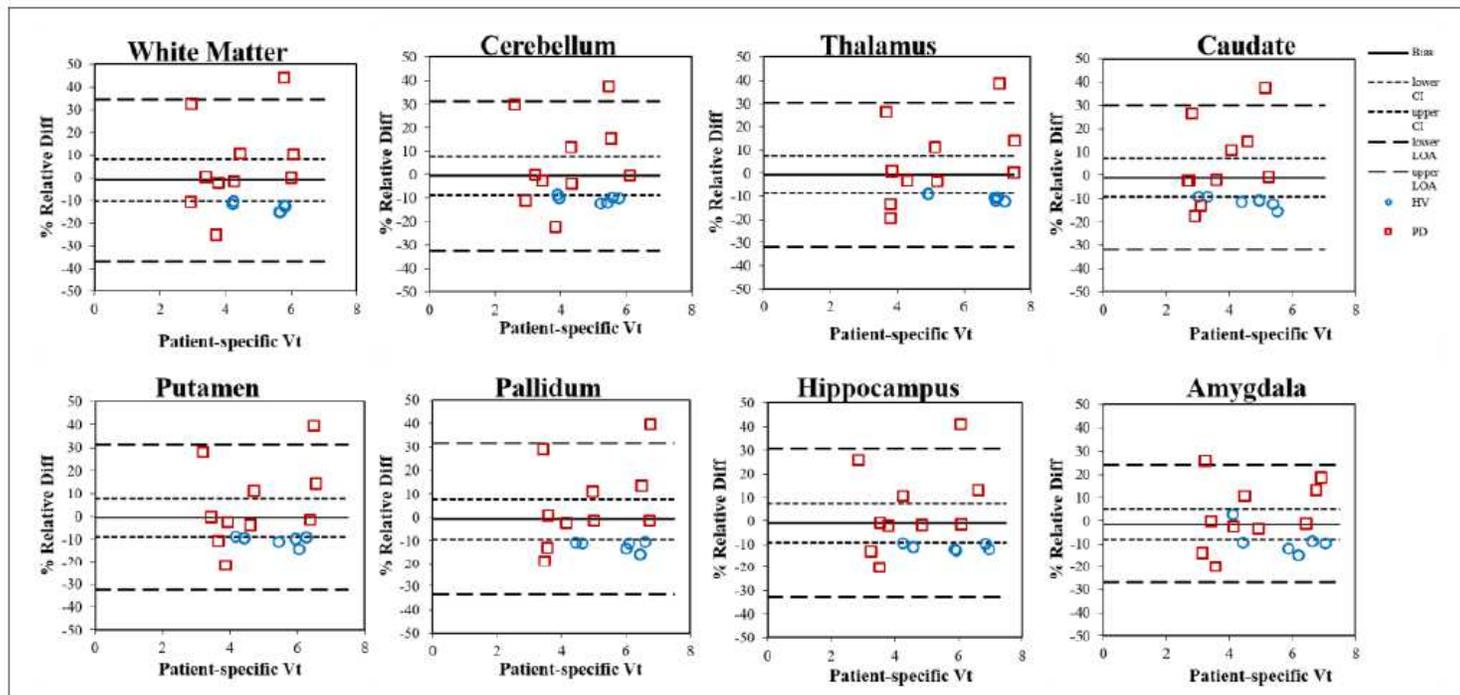


Figure 5

Bland-Altman analysis showing the variation in the VT between the PSAIF and PBIF (normalization with  $Weight_{subject} \times AUC$ ). The values are shown for the HV (blue circles) and the PD (red circles) groups. The solid line is the mean % bias between PSAIF and PBIF VT estimate, while the dotted and dashed lines represent the %CI and %LOA respectively

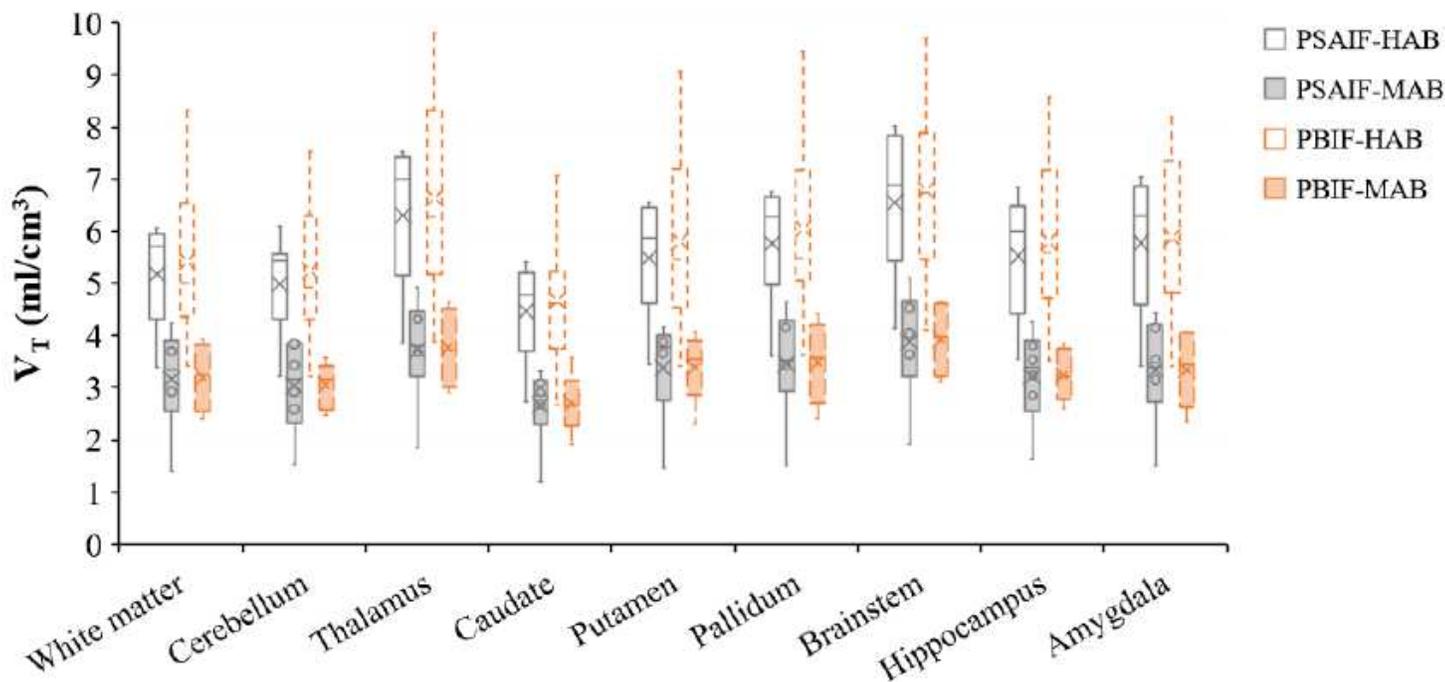
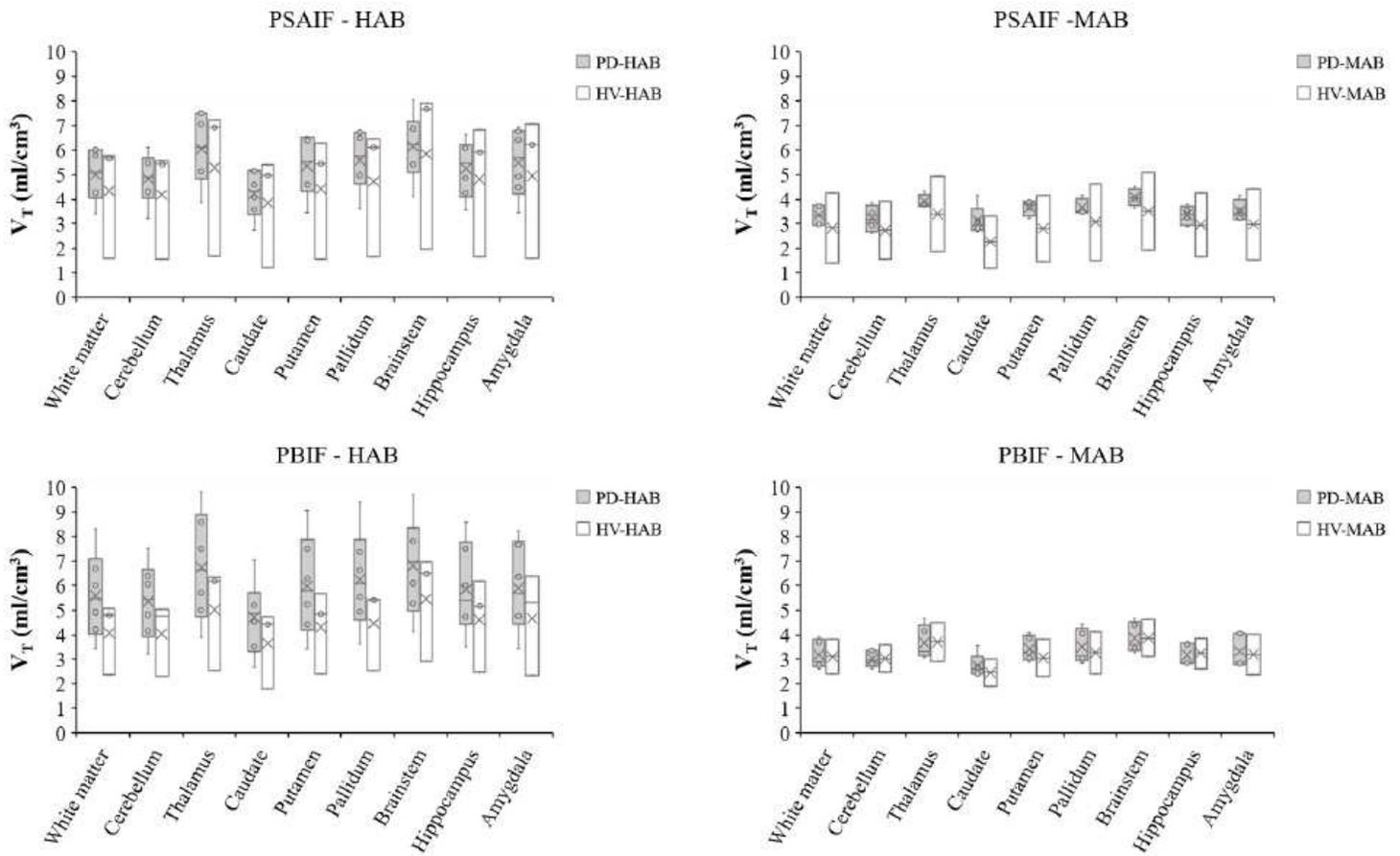


Figure 6

Logan VT values for all subjects calculated with PSAIF and PBIF for the two genotype groups (HAB and MAB). Comparing MAB to HAB, there is a significant reduction in VT both with PSAIF and PBIF. PSAIF showed an average reduction of 40% in VT across the brain regions, while the average reduction with PBIF is 42%



**Figure 7**

Logan VT values between HV and PD subjects, and also HAB and MAB groups calculated with PSAIF and PBIF

## Supplementary Files

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