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[¹¹C]arachidonic acid incorporation measurement in human brain: Optimization for clinical use

Francesca Zanderigo^{1,2,*}, Yeona Kang³, Dileep Kumar¹, Anastasia Nikolopoulou³, P. David Mozley³, Paresh J. Kothari³, Bin He³, David Schlyer³, Stanley I. Rapoport⁴, Maria A. Oquendo^{1,2}, Shankar Vallabhajosula³, J. John Mann^{1,2,5}, and M. Elizabeth Sublette^{1,2}

¹Molecular Imaging and Neuropathology Division, New York State Psychiatric Institute, New York, New York ²Department of Psychiatry, Columbia University, New York, New York ³Department of Radiology, Weill Cornell Medicine, New York, New York ⁴National Institute on Aging, Bethesda, MSC 2292, Maryland ⁵Department of Radiology, Columbia University, New York, New York

Abstract

Arachidonic acid (AA) is involved in signal transduction, neuroinflammation, and production of eico-sanoid metabolites. The AA brain incorporation coefficient (K^*) is quantifiable *in vivo* using [¹¹C]AA positron emission tomography, although repeatability remains undetermined. We evaluated K^* estimates obtained with population-based metabolite correction (PBMC) and image-derived input function (IDIF) in comparison to arterial blood-based estimates, and compared repeatability. Eleven healthy volunteers underwent a [¹¹C]AA scan; five repeated the scan 6 weeks later, simulating a pre- and post-treatment study design. For all scans, arterial blood was sampled to measure [¹¹C] AA plasma radioactivity. Plasma [¹¹C]AA parent fraction was measured in 5 scans. K^* was quantified using both blood data and IDIF, corrected for [¹¹C]AA parent fraction using both PBMC (from published values) and individually measured values (when available). K^* repeatability was calculated in the test-retest subset. K^* estimates based on blood and individual metabolites were highly correlated with estimates using PBMC with arterial input function ($r = 0.943$) or IDIF ($r = 0.918$) in the subset with measured metabolites. In the total dataset, using PBMC, IDIF-based estimates were moderately correlated with arterial input function-based estimates ($r = 0.712$). PBMC and IDIF-based K^* estimates were ~6.4% to ~11.9% higher, on average, than blood-based estimates. Average K^* test-retest absolute percent difference values obtained using blood data or IDIF, assuming PBMC for both, were between 6.7% and 13.9%, comparable to other radiotracers. Our results support the possibility of simplified [¹¹C]AA data acquisition through eliminating arterial blood sampling and metabolite analysis, while retaining comparable repeatability and validity.

Correspondence: Francesca Zanderigo, Molecular Imaging and Neuropathology Division, New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York. zanderi@nyspi.columbia.edu.

*Orcid: Francesca Zanderigo <http://orcid.org/0000-0001-6510-0676>

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Supporting Information: Additional Supporting Information may be found online in the supporting information tab for this article.

Keywords

arachidonic acid; positron emission tomography; brain; repeatability; noninvasive estimation; image-derived input function; population-based metabolite correction

1. Introduction

Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid present in phospholipids of brain cell membranes. Phospholipids participate in membrane remodeling and synthesis, and phospholipase A₂-mediated signal transduction (Axelrod, Burch, & Jelsema, 1988; Fisher & Agranoff, 1987; Stephenson et al. 1994). AA is involved in regulation of signaling enzymes and is a key inflammatory intermediate (Bazan & Rodriguez de Turco, 1980; Rabin et al. 1997); pro-inflammatory cytokines and other stimuli activate phospholipase A₂, which triggers release of AA from the phospholipid membrane, freeing it for metabolic transformation into pro-inflammatory eicosanoids (Sun, Horrocks, & Farooqui, 2007).

Using in vivo quantification of intravenously injected [¹¹C]AA into rodent brain (Rapoport, 1999; Robinson et al. 1992), the AA incorporation rate was found to be a marker of changes in second messenger activity following sensory or pharmacological activation. AA incorporation was reduced by phospholipase A₂ pharmacological inhibition (Grange, Rabin, Bell, & Chang, 1998), decreased in contralateral visual structures after acute unilateral visual deprivation (Wakabayashi, Freed, Bell, & Rapoport, 1994), and increased in response to arecoline (DeGeorge, Noronha, Bell, Robinson, & Rapoport, 1989). Studies in animal models of Alzheimer's and Parkinson's diseases found alterations in phospholipase A₂-mediated cholinergic (Nariai, DeGeorge, Lamour, & Rapoport, 1991) and dopaminergic signaling (Hayakawa et al. 1998).

Quantification of the [¹¹C]AA incorporation rate into human brain membrane phospholipids using positron emission tomography (PET) (Giovacchini et al. 2002; Rapoport, 1999; Robinson et al. 1992) has been used to study AA brain incorporation with respect to effects of healthy aging (Giovacchini et al. 2004), signal transduction during visual stimulation (Esposito et al. 2007), dopaminergic neurotransmission (Thambisetty et al. 2012), and presence of Alzheimer's disease (Esposito et al. 2008). The latter study found that AA uptake was elevated in widespread cortical regions in Alzheimer's patients compared with healthy controls, consistent with the premise that elevated AA turnover is a marker for neuroinflammation. The continuing optimization of [¹¹C]AA methodology could enhance its usefulness for the study of neuroinflammation, as an alternative to current radiotracers for the translocator protein (Tronel et al. 2017).

Quantification of [¹¹C]AA incorporation into human brain could be applied to longitudinal studies, but repeatability of [¹¹C]AA incorporation coefficient and rate measurements has not been characterized. Furthermore, the invasive arterial blood sampling required for [¹¹C]AA quantification, and the complex assay required to measure blood [¹¹C]AA metabolites, limit the feasibility of large-scale studies to investigate potential differences in AA incorporation between healthy controls and patients with brain disorders.

Here we assess for the first time test-retest properties of [^{11}C]AA incorporation coefficient measurements using data from participants imaged twice, 6 weeks apart, to mimic a pre- and post-treatment design or a longitudinal study. Furthermore, we investigate the effects of using a noninvasive estimation of input function instead of arterial blood sampling, and population-based metabolite correction (PBMIC) instead of individual metabolite assays on estimation and repeatability of [^{11}C]AA incorporation coefficient (K^*).

2. Materials and Methods

2.1. Sample

Eleven healthy volunteers gave informed written consent to participate in a protocol approved by the Institutional Review Boards of New York State Psychiatric Institute and Weill Cornell Medical College. Exclusion criteria were: unstable medical condition; neurologic disease, history of seizures or loss of consciousness > 1 min; recent exposure to radiation during research/medical procedure; pregnancy, lactating, recent abortion or unwilling to use birth control; metal implants; drug/alcohol abuse or dependence within 6 months; history of intravenous drug use; use of ecstasy more than twice; current tobacco use; history of suicide attempt, psychosis, anorexia or bulimia nervosa in the past year; history of DSM-IV Axis I diagnosis except for specific phobia, DSM-IV Axis II diagnosis of borderline or antisocial personality disorder, or first degree relatives with major depressive disorder, bipolar disorder, schizophrenia or schizoaffective disorder.

2.2. Radiochemistry

[^{11}C]AA was synthesized as previously described (Channing, 1993). High-performance liquid chromatography analysis of the formulated final product showed $98.8 \pm 1.7\%$ ($n = 16$ injections) radiochemical purity.

2.3. PET acquisition

PET data were acquired at the Citigroup Biomedical Imaging Center (Weill Cornell Medicine, New York, NY, USA) using a Siemens Biograph mCT (Siemens Medical Solutions USA, Malvern, PA). Each participant was imaged once, with 81 2.03 mm-thick slices covering the brain and in-plane pixel size of 1.02 mm (400×400 matrix, zoom52). A registered CT scan was acquired for attenuation correction. [^{11}C]AA was infused intravenously over a 3 min period (624.19 ± 154.29 MBq) with an automated pump (Graseby 3400 Syringe Pump, Graseby 3000, Watford, UK). Listmode data were acquired for 60-min and rebinned into 33 frames: 12×15 s, 4×30 s, 4×60 s, 4×120 s, 4×240 s, 4×300 s, 1×420 s. A Bio-Rad 2100 fraction collector (Bio-Rad Laboratories, Hercules, CA, USA) collected 40 arterial blood samples at a fixed drip rate of 18-to-22 drops per 15 s until 10 min post-infusion. Two additional samples were taken manually at 20 and 60 min post-infusion. Plasma [^{11}C]AA radioactivity concentrations were determined with a Wallac Wizard 1480 automatic gamma counter (now PerkinElmer, Waltham, MA, USA).

Five participants received a second [^{11}C]AA scan after approximately 6 weeks, following the same protocol, with an average [^{11}C]AA infusion of 649.35 ± 143.19 MBq. A total of 16 scans (5 test-retest and 6 baseline) are therefore considered here.

2.4. [^{11}C]AA metabolite analysis

In 5 scans (3 participants at baseline, 1 participant at both test and retest scan) [^{11}C]AA metabolites in blood plasma were measured. We elected not to use liquid chromatography techniques (Giovacchini et al. 2002), due to time constraints, and performed instead a sequential extraction process from plasma, as follows (Giovacchini et al. 2002). Blood samples taken at 2, 4, 10 and 20 min after [^{11}C]AA administration were centrifuged at 3,500 rpm (Centrifuge Model 228, Fisher Scientific) for 10 min. The supernatant was transferred (0.5 ml) to a tube (15 ml) containing a mixture (6.25 ml) of methanol-chloroform-heptane (1.41:1.25:1.0) and potassium carbonate (1.8 ml, pH 10). The resulting mixture was vortexed (10 s) and centrifuged (3,400 rpm for 10 min) to separate phases. One ml of the upper phase was transferred to another vial, and the remainder (~3.2 ml) acidified with concentrated H_2SO_4 (30 μL), and vortexed for 10 s. Heptane (2.0 ml) was added to the acidic solution, which was vortexed (10 s) and centrifuged (3,400 rpm, 10 min) to separate the phases. The organic heptane phase was separated and collected to a tube (5 ml). Radioactivity counts for 1 ml extract, heptane phase and remainder of acidic extraction were then obtained, corrected for background radioactivity and physical decay, and the percentage of free [^{11}C]AA in plasma calculated by dividing the counts of heptane fraction with total plasma counts at each time point. These values were normalized with reference plasma sample, assuming that 100% of the injected dose was [^{11}C]AA. The procedure was validated in reference plasma or plasma obtained from participants' blood samples prior to [^{11}C]AA administration and spiked with [^{11}C]AA.

2.5. Magnetic resonance imaging acquisition

T1-weighted magnetic resonance imaging (MRI) images were acquired for regions of interest (ROIs) identification using a 3 T Signa HDx system (General Electric Medical Systems, Milwaukee, Wisconsin) at the New York State Psychiatric Institute. Ten ROIs were considered: temporal lobe, gray and white matter cerebellum, parietal lobe, occipital lobe, orbital prefrontal cortex, cingulate, thalamus, hippocampus, dorsal caudate.

2.6. Image processing

Raw MRI images were cropped to remove non-brain material using Atropos (<http://www.picsl.upenn.edu/ANTs>) (Avants, Tustison, Wu, Cook, & Gee, 2011), and segmented using statistical parametric mapping software (<http://www.fil.ion.ucl.ac.uk/spm/software/>). An automated algorithm was used to identify ROIs, as previously described (Milak et al. 2010). The FMRIB linear image registration tool (FLIRT) version 5.0 (FMRIB Image Analysis Group, Oxford, UK) was used to correct for head motion during PET scanning. Each participant's mean PET image was coregistered to the corresponding MRI using FLIRT with a mutual information cost function, six degrees of freedom, and trilinear interpolation, optimized as previously described (Milak et al. 2010). Time activity curves (TACs) were generated averaging the activity within a ROI over the scan time course.

2.7. PET outcome measure estimation

The irreversible uptake model proposed for [^{11}C]AA in (Giovacchini et al. 2002) expresses each regional TAC as a linear combination of the [^{11}C]AA total radioactivity in whole blood

($\mu\text{Ci/mL}$), $C_b(t)$, the metabolite-corrected [^{11}C]AA radioactivity in arterial plasma, $C_p(t)$, and the predicted concentration of [^{11}C]Co $_2$ in brain tissue, $C_{\text{Co}_2}(t)$. Thus,

$$C_t(t) = V_b C_b(t) + K^* \int_0^t C_p(s) ds + C_{\text{Co}_2}(t),$$

where V_b is blood volume (ml blood/ml brain) and K^* (L·min $^{-1}$ ·mL $^{-1}$ ·brain) is the [^{11}C]AA unidirectional incorporation rate. TACs were fitted using Matlab R2012b (The Mathworks, MA, USA), and the following modifications were made to the model. First, the delay between TACs and input function was accounted for by aligning the two curves before quantification. Second, given the time constraints required for accurate rapid assay of [^{11}C]Co $_2$, we did not measure its concentration in the blood and therefore were not able to correct the TACs for $C_{\text{Co}_2}(t)$ contribution. This contribution to the TAC was estimated to be in the range 7–9% in (Giovacchini et al. 2002). Third, [^{11}C]AA total radioactivity $C_b(t)$ was measured over time in plasma rather than whole blood. To estimate the impact of this last methodological difference, in our 16 available scans we compared estimates of K^* (free parameter of interest) and blood volume (V_b , the other free parameter in the model (Giovacchini et al. 2002)) obtained using the total [^{11}C]AA radioactivity in plasma to those obtained using the total [^{11}C]AA radioactivity in plasma scaled by the average whole blood-to-plasma ratio (0.77) reported in (Giovacchini et al. 2002). We found that the percentage difference between the two sets of measurements was, $29.86 \pm 0.11\%$ for V_b and $0.0001 \pm 0.0002\%$ for K^* (average across ROIs and scans). The whole blood-to-plasma scaling has a negligible effect on the estimation of K^* as it only applies to the term multiplying V_b in the linear model proposed for the [^{11}C]AA TAC.

K^* values were quantified using 3 different curves for the metabolite-corrected [^{11}C]AA radioactivity over time in arterial plasma: (a) arterial blood plasma concentration with individual metabolite correction, when data were available (resulting values denoted as K^*_{BLOOD}); (b) arterial blood plasma concentration with PBMC, using average parent fraction values published in (Giovacchini et al. 2002) (resulting values denoted as $K^*_{\text{BLOOD-PBMC}}$); (c) image-derived input function (IDIF) (Chen et al. 1998) with PBMC (resulting values designated as $K^*_{\text{IDIF-PBMC}}$).

2.8. Population-based metabolite correction

We applied a PBMC by interpolating the average parent fraction values published in (Giovacchini et al. 2002) over the times of sampling of the arterial blood (for arterial input function) or the times of sampling of the PET tissue data (for the IDIF), and then multiplying the interpolated curve by either the blood-based plasma total radioactivity counts or the IDIF. Average and standard deviation (SD) parent fraction values reported by Giovacchini et al. (Giovacchini et al. 2002) are the following: 0.76 ± 0.11 , 0.51 ± 0.18 , 0.23 ± 0.17 , 0.11 ± 0.10 , and 0.02 ± 0.02 at 5, 10, 20, 30, and 60 min post injection.

2.9. Image-derived input function

We applied the IDIF approach reported in (Chen et al. 1998). Briefly, circular volumes of interest (diameter: 4 mm) were manually placed around the carotid arteries cavernous segment on summed transaxial PET images acquired between 90 and 120 s after start of radiotracer injection, and projected onto each dynamic frame to obtain decay corrected radioactivity curves. To assess the background spillover, circular volumes of interest

(diameter 8 mm) were placed 2 cm away from the arteries in low activity regions. The radioactivity concentration curves in whole blood were estimated from radioactivity curves of the internal carotid arteries and surrounding regions as $IDIF(t) = SO \cdot C_{carotid}(t) + SI \cdot C_{surr}(t)$ (Chen et al. 1998), where $C_{carotid}(t)$ is the radioactivity in the carotid region, $C_{surr}(t)$ is the radioactivity in the volume of interest near the artery, and SO and SI are the spill out (from the artery) and spill in (from the background) factors. The latter correction terms, which are required to account for PET scanner resolution, were determined based on static phantom data using tubing that included the full range of artery sizes. The diameter of the artery for each subject was determined from the associated MRI scan, and the appropriate correction factors were used to adjust the $IDIF$ values to correct for partial volume effects.

2.10. Comparison of invasive versus noninvasive K^* estimation

$K^*_{BLOOD-PBMC}$ and $K^*_{IDIF-PBMC}$ estimates were compared to K^*_{BLOOD} estimates in the 5 scans for which we had individually measured parent fraction values, using Pearson correlation coefficient, and regression analysis slope and intercept (K^*_{BLOOD} as independent variable), and two-tailed paired t-tests, for each ROI. Using the same metrics, $K^*_{IDIF-PBMC}$ estimates were compared to $K^*_{BLOOD-PBMC}$ estimates in all 16 scans available ($K^*_{BLOOD-PBMC}$ as independent variable).

2.11. Repeatability and time stability analyses

For each ROI and test-retest pair, we quantified for $K^*_{BLOOD-PBMC}$ and $K^*_{IDIF-PBMC}$ the

percent difference with respect to test-retest repeatability (PD_R) as $PD_R = 100 \frac{|K^*_T - K^*_{RT}|}{\frac{K^*_T + K^*_{RT}}{2}}$,

where K^*_T and K^*_{RT} are the K^* estimates in the test and retest scan, respectively (the lower the PD_R , the higher the repeatability). For each ROI, PD_R values of $K^*_{BLOOD-PBMC}$ were compared to those of $K^*_{IDIF-PBMC}$, using two-tailed paired t-tests. For all ROIs, and both $K^*_{BLOOD-PBMC}$ and $K^*_{IDIF-PBMC}$, we also calculated the percentage test-retest repeatability

coefficient (PRC) (Obuchowski et al. 2016) as $PRC = 100 \cdot 2.77 \cdot \sqrt{\frac{1}{P} \sum_{j=1}^P \frac{(K^*_T - K^*_{RT})^2}{\left(\frac{K^*_T + K^*_{RT}}{2}\right)^2}}$, with

P the number of test-retest pairs. PRC is an unscaled index of agreement between the test-retest readings that is proportional to the within-subject coefficient of variation (Barnhart & Barboriak, 2009; Vaz, Falkmer, Passmore, Parsons, & Andreou, 2013), takes into account both random and systematic errors (Hopkins, 2000), and represents the value below which the absolute differences between two measurements would lie with 0.95 probability (Beckerman et al. 2001; Bland & Altman, 2003; Lexell & Downham, 2005).

To explore the stability of K^* estimates obtained with data from shorter durations of the scan, the $[^{11}C]AA$ TAC model (Giovacchini et al. 2002) was fit to data with later frames deleted, corresponding to a total scan duration of 60, 53, 48, 43, 38, 33, 29, 25, 21, 17, 15, 13 and 11 min. Using all 16 scans and for each ROI, we computed the percent difference

with respect to scan time (PD_S) as $PD_S = 100 \frac{|K_{t=60\text{min}}^* - K_{t=n\text{min}}^*|}{K_{t=60\text{min}}^*}$, where $K_{t=60\text{min}}^*$ and

$K_{t=n\text{min}}^*$ are the K^* estimates obtained with full duration scan and with the shorter scan time, respectively. K^* estimation for an ROI was considered stable at a given scan duration if the mean of PD_S values for that scan duration across all 16 scans was between 0% and 5%.

Once the minimum scan time that ensured stability of K^* estimates across ROIs was selected as described above, we calculated test-retest repeatability PD_R and PRC values for $K^*_{\text{BLOOD-PBMC}}$ and $K^*_{\text{IDIF-PBMC}}$ estimates obtained using the minimum scan time, and compared them to test-retest repeatability values obtained using full scan duration using two-tailed paired t-tests, ROI by ROI.

3. Results

3.1. Sample

The study sample comprised 11 volunteers (2 males, 9 females) with mean age $\pm$ SD of 33 ± 5.8 years.

3.2. Individual versus population-based metabolite correction

In agreement with (Giovacchini et al. 2002), our metabolite analysis found the percentage of unmetabolized [^{11}C]AA to be $86.1 \pm 5.2\%$ of total plasma radioactivity at 2 min, $76.7 \pm 8.3\%$ at 4 min, $61.0 \pm 6.9\%$ at 10 min, and $47.1 \pm 7.9\%$ at 20 min. For correlations between $K^*_{\text{Blood-Pbmc}}$ and K^*_{BLOOD} estimates, $r = 1.00$ in all 5 scans for which [^{11}C]AA plasma parent fraction was individually measured, and the slope values of the regression analysis indicated very good agreement between the two sets of measurements in 4 out of 5 scans (Figure 1, top). Across ROIs and subjects, regression analysis with K^*_{BLOOD} estimates as the independent variable, and $K^*_{\text{BLOOD-PBMC}}$ estimates as the dependent variable yielded the following: correlation $r = 0.943$, slope = 1.182, intercept = -0.754 . The use of PBMC raised K^* estimates in comparison to using individually measured parent fraction (average across ROIs $\sim 6.9\%$ higher). The difference between values from the two methods was below statistical significance (p values range across ROIs: 0.213–0.263), perhaps due to the small sample size. The same comparison is reported region by region in Table 1.

3.3. Arterial blood plasma versus IDIF

Correlations between $K^*_{\text{IDIF-PBMC}}$ and K^*_{BLOOD} estimates in the five scans for which [^{11}C]AA plasma parent fraction was individually measured were very high ($r > 0.97$) in each scan (Figure 1, middle). Across ROIs and subjects, regression analysis with K^*_{BLOOD} estimates as independent variable, and $K^*_{\text{IDIF-PBMC}}$ estimates as dependent variable yielded the following: correlation $r = 0.918$, slope = 1.055, intercept = 0.313. Using PBMC and IDIF increased K^* estimates in comparison to using individually measured parent fraction and arterial input function ($\sim 10.1\%$ higher on average across ROIs). The difference failed to achieve statistical significance in all considered ROIs (p values range: 0.074–0.253) in this small sample. Direct comparison of $K^*_{\text{BLOOD-PBMC}}$ and $K^*_{\text{IDIF-PBMC}}$ estimates in this

subset yielded correlation $r = 0.992$, slope = 0.909, and intercept = 0.862 (Figure 1, bottom). The same comparisons are reported in each region in Table 1.

Correlations between $K^*_{\text{IDIF-PBMC}}$ and $K^*_{\text{BLOOD-PBMC}}$ estimates, considering all 16 scans, were also very high ($r > 0.94$ in 15 scans; $r = 0.83$ in one scan; Figure 2). Across ROIs and subjects, regression analysis with $K^*_{\text{BLOOD-PBMC}}$ estimates as the independent variable, and $K^*_{\text{IDIF-PBMC}}$ estimates as the dependent variable yielded the following: correlation $r = 0.712$, slope = 0.684, intercept = 2.862. The same comparison is reported in each region in Table 2. Using IDIF increased the K^* estimates compared to using an arterial input function ($\sim 8.3\%$ higher on average across ROIs), when PBMC is used for both, and this difference was statistically significant in 80% of the ROIs, with a trend in occipital lobe and cingulate (p values range: 0.022–0.054).

Underestimation of the blood curve peak and peak width and overestimation of radioactivity in the tail of the curve contributed to decreased IDIF performance in some participants (Supporting Information, Figure S1). This was most likely due to errors in the placement of voxels representing the carotids, and/or noise in the radioactivity curves.

3.4. Test-retest repeatability

Using either IDIF or arterial blood, assuming PBMC for both methods, average PD_R values in the 5 test-retest pairs were all below 14% (6.7–13.9%; Figure 3), and results did not differ on two-tailed paired t-tests in any of the considered ROIs (range of p -values = 0.152–0.813).

Arterial blood and IDIF yielded similar PRC values across ROIs, in the range $\sim 16\%$ to 28%, with differences between PRC values obtained with arterial blood and IDIF values ranging from 0.1% to 5.1%. Using IDIF led to lower PRC values than using arterial blood in all considered ROIs except for orbital prefrontal cortex and dorsal caudate, where PRC values were higher with IDIF, similar to what was seen with PD_R values.

We were not able to assess repeatability of K^* measurements under conditions of individual metabolite correction, as only one test-retest pair had data available for such analysis.

3.5. Time stability

Our exploratory comparison of average PD_S (across all 16 scans for every ROI) with K^* estimates from the full-length scan vs. estimates from shorter scan durations found that PD_S increased as the duration of the scan decreased (Figure 4). For both $K^*_{\text{BLOOD-PBMC}}$ and $K^*_{\text{IDIF-PBMC}}$, differences of 5% or less were seen only in data from 33 min and longer.

Using data from 33 min of scan led to higher test-retest PD_R values than using 60 min for both $K^*_{\text{BLOOD-PBMC}}$ (average PD_R across ROIs was $\sim 17\%$ higher) and $K^*_{\text{IDIF-PBMC}}$ (average PD_R across ROIs was $\sim 29\%$ higher) (Figure 5). The difference reached statistical significance only in one region (dorsal caudate, $p = 0.032$) and only when using IDIF.

Similarly, PRC values obtained comparing test with retest data from 33 min of scan were higher than values obtained using 60 min, in the range 20.8–32.1% for both $K^*_{\text{BLOOD-PBMC}}$ and $K^*_{\text{IDIF-PBMC}}$ across all considered ROIs (Supporting Information, Table S1).

4. Discussion

We present here the first assessment of test-retest repeatability of [^{11}C]AA incorporation coefficient into brain membrane phospholipids in humans. We further demonstrate the potential of using a noninvasive IDIF, instead of arterial blood sampling; PBMC, instead of individual metabolite assays; and shorter scan times, with respect to K^* estimates and repeatability.

The range of average PD_R values obtained for K^* across 10 ROIs (6.7–13.9%), using either method of input function estimation with PBMC, were comparable or superior to test-retest data reported for binding potentials or volumes of distribution (V_T) of other PET radiotracers in use. For example, average reported PD_R values are 20.7% (BP_F), 17.2% (BP_P), and 16.5% (BP_{ND}) for [^{11}C]DASB (Ogden et al. 2007); 9.90% (BP_F) for [^{11}C]CUMI-101 (Milak et al. 2010); from 9.2% to 15.6% (BP_P) for [^{11}C]PE2I (DeLorenzo, Kumar, Zanderigo, Mann, & Parsey, 2009); 16.9% (V_T) for [^{11}C]PBR28 (Collste et al. 2016); and no $>12\%$ (V_T) for [^{11}C]LY2795050 (Naganawa et al. 2015). Moreover, this level of repeatability was attained with 6 weeks occurring between test and retest, in contrast to more standard same-day scan repetition. However, test-retest repeatability was assessed without measurements of individual radiometabolite curves, and the use of PBMC likely increased the test-retest repeatability. The repeatability we observed in both arterial blood-based and IDIF-based measurements of K^* suggests potential usefulness of [^{11}C]AA in treatment and longitudinal studies of neuropsychiatric conditions in which etiology may relate to effects of the AA cascade, such as Alzheimer's disease (Esposito et al. 2008), bipolar disorder (Lee, Rao, Rapoport, & Bazinet, 2007), Parkinson's disease and schizophrenia (Thambisetty et al. 2012).

Given complexity and costs of the assays to determine plasma [^{11}C]AA parent fraction, being able to quantify K^* without blood sampling and metabolite analyses would facilitate larger clinical studies with [^{11}C]AA. When using either arterial blood or IDIF for determination of the input function, K^* estimates based on PBMC were highly correlated with estimates obtained using individually-measured parent fraction curves, both when each scan was assessed individually across ROIs, and when all scans and ROIs were considered together (Figure 1), suggesting that PBMC may be a valid, convenient alternative to individually assaying metabolites.

Overcoming the need for invasive and costly arterial blood sampling during [^{11}C]AA PET would constitute another welcome methodological advance. Using PBMC together with an IDIF led to K^* estimates that highly correlated with either those obtained by sampling arterial blood and using individual parent fraction values (only obtained in a subsample, $n = 5$, Figure 1 and Table 1), or by sampling arterial blood and using PBMC (in the entire dataset, $n = 16$, Figure 2 and Table 2), when considering each scan individually. However, the correlation was more modest ($r = 0.712$) when considering all 16 scans and ROIs together. Furthermore, average IDIF-derived values were higher than arterial-derived values ($\sim 10.1\%$ in the subsample with individual parent fraction values and $\sim 8.3\%$ higher in the total sample, across ROIs), and the difference was statistically significant in the larger sample in 80% of considered ROIs.

According to our results, therefore, simplifying the acquisition of [^{11}C]AA data eliminating arterial blood sampling and metabolite analyses may be possible, at the cost of introducing some bias in K^* estimates. As the bias can be variable across subjects, using an IDIF in addition to a PBMC may increase the variance in subsequent studies with this approach. Such bias complicates comparisons with studies in which individual [^{11}C]AA parent fraction and arterial blood measurements were obtained, but may not constitute a problem in longitudinal studies as long as repeatability is sufficient. In our small sample the difference between PD_R values obtained using IDIF and those obtained using arterial blood, assuming PBMC for both, was below statistical significance in each of the considered ROIs. If replicated in a larger sample, it would confirm that eliminating arterial blood sampling by using an IDIF does not systematically compromise test-retest repeatability of [^{11}C]AA measurements when using a 60-min scan acquisition. However, it remains to be investigated whether use of an IDIF would exhibit a bias in different populations or would be affected by treatment in the case of a treatment study. Furthermore, partial volume effects could limit the use of the suggested IDIF approach if correction for each subject artery diameter is not properly handled.

The exploratory analysis of time stability revealed that by using either arterial- or image-derived input functions with PBMC it was possible to shorten the scan duration to 33 min and still obtain K^* estimates that were, on average, within 5% of estimates obtained from a 60-min scan for all considered ROIs. However, the shorter scans resulted in numerically higher differences in test-retest repeatability (vs. the 60-min scan) when using either arterial blood or IDIF. Such differences were not statistically significant (in this small sample) when using arterial blood, while there was a significant difference in the smallest of the considered ROIs (dorsal caudate) when using IDIF. However, the range of average values obtained for PD_R and PRC using 33 min scans extended up to 15.13% and 30.73% (when using arterial blood), and up to 16.14% and 32.11% (when using IDIF), for PD_R and PRC, respectively. When designing a within-subject investigation or longitudinal study, this effect on K^* measurement repeatability should be weighed against the benefits of reduced patient burden and cost with shorter scan duration. Theoretical (Robinson et al. 1992) and experimental evidence (Chang et al. 1997) indicates that the arachidonic acid incorporation coefficient K^* is not influenced by changes in cerebral blood flow. Furthermore, Giovacchini et al. (Giovacchini et al. 2002; Giovacchini et al. 2004) examined blood flow effects on K^* in humans in two different samples using [^{15}O]water, and found no significant relationship between K^* and cerebral blood flow. However, future studies that aim to further improve clinical feasibility of imaging with this radiotracer by reducing time of scanning while preserving accuracy of the estimates, should investigate whether shorter scan times are associated with increased bias due to blood flow.

Independent of the approach used to estimate K^* , penetration of the [^{11}C]AA radiotracer into brain was relatively low (average total brain uptake was 0.66% 60.14% of injected dose across scans and regions), consistent with a reported uptake of only ~1% of injected dose by Giovacchini et al. (Giovacchini et al. 2002).

5. Limitations

We assessed test-retest repeatability without measurements of individual radiometabolite curves, which is an important part of the variability from scan to scan, and the use of an average curve likely increased the test-retest repeatability.

We did not correct for $[^{11}\text{C}]\text{CO}_2$ contribution in the tissue TACs and thus have not eliminated one possible source of noise, which Giovacchini and colleagues (Giovacchini et al. 2002) estimated as an average gray matter increase in K^* of 8.5% with a 60-min scan. Not correcting for the $[^{11}\text{C}]\text{CO}_2$ component is also likely to have contributed to increasing the reported test-retest repeatability. Although the effect of variations of blood flow on K^* estimates is expected to be minimal (Giovacchini et al. 2002; Giovacchini et al. 2004), this was not tested in our dataset. This study in a small cohort of healthy volunteers would need to be repeated in a larger sample including individuals with documented inflammation in order to validate the test-retest properties of $[^{11}\text{C}]\text{AA}$ in clinical populations.

6. Conclusions

We find the repeatability of measurements of $[^{11}\text{C}]\text{AA}$ incorporation coefficient when using a PBMC to be comparable to that of other radiotracers used in neuroimaging studies, in a 6-week interval between test and retest, indicating that prospective treatment studies may be feasible using $[^{11}\text{C}]\text{AA}$. Our results suggest that simplifying $[^{11}\text{C}]\text{AA}$ data acquisition may be possible by eliminating arterial blood sampling and metabolite analysis, at the cost of introducing some bias in K^* estimation, but without excessively compromising test-retest repeatability. Shortening scans up to 33 min does not appear to significantly affect K^* measurements, but did decrease repeatability. If replicated in a larger sample with and without inflammatory conditions, some or all of these procedural optimizations could reduce expense and subject burden and enhance the utility of $[^{11}\text{C}]\text{AA}$ as an attractive addition to existing radiotracers for studying neuroinflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Scatter plot of $K^*_{\text{BLOOD-PBMC}}$ (top) and $K^*_{\text{IDIF-PBMC}}$ (middle) versus K^*_{BLOOD} estimates in the 5 scans for which $[^{11}\text{C}]\text{AA}$ plasma parent fraction values were individually measured, with results for correlation and regression analysis. $K^*_{\text{BLOOD-PBMC}}$ and $K^*_{\text{IDIF-PBMC}}$ are directly compared in the scatter plot in the bottom. Each data point represents one ROI; all considered ROIs are reported

Figure 2. Scatter plot of $K^*_{IDIF-PBMC}$ versus $K^*_{BLOOD-PBMC}$ estimates in all 16 available scans, with results for correlation and regression analysis. Each data point represents one ROI; all considered ROIs are reported

Figure 3.

Test-retest PD_R values (average + SD across 5 test-retest pairs) for K* in each of the considered ROIs, using either arterial blood (K*_{BLOOD-PBMC}) or IDIF (K*_{IDIF-PBMC}) as input function, assuming for both PBMC. ROIs are reported from left to right according to decreasing average size. TEM, temporal lobe; GMC, gray matter cerebellum; PAR, parietal lobe; OCC, occipital lobe; ORB, orbital prefrontal cortex; WMC, white matter cerebellum; CIN, cingulate; THA, thalamus; HIP, hippocampus; DCA, dorsal caudate

Figure 4.

Percentage differences with respect to scan length (PD_S; average value across all 16 available scans $\pm$ SD) between K^* estimates obtained using data from the entire duration of the scan ($K^*_{t=60 \text{ min}}$) and K^* estimates obtained using data from shorter durations of the scan ($K^*_{t=n \text{ min}}$), in each of the 10 considered ROIs, for both $K^*_{\text{BLOOD-PBMC}}$ (top) and $K^*_{\text{IDIF-PBMC}}$ (bottom). The solid black line indicates PD_S of 5%

Figure 5.

Test-retest PD_R values (average + SD across 5 test-retest pairs) obtained in each of the considered ROIs for $K^*_{BLOOD-pbmc}$ (top) and $K^*_{IDIF-PBMC}$ (bottom) using the entire duration of the scan (60 min) and using data from the first 33 min of scan. TEM, temporal lobe; GMC, gray matter cerebellum; PAR, parietal lobe; OCC, occipital lobe; ORB, orbital prefrontal cortex; WMC, white matter cerebellum; CIN, cingulate; THA, thalamus; HIP, hippocampus; DCA, dorsal caudate

Table 1

Region by region comparison of K^* estimates in the scans with individually measured parent fraction. Results of correlation and regression analysis, region by region, for $K^*_{\text{BLOOD-PBMC}}$ versus K^*_{BLOOD} estimates (top), $K^*_{\text{IDIF-PBMC}}$ versus K^*_{BLOOD} estimates (middle), and $K^*_{\text{IDIF-PBMC}}$ versus $K^*_{\text{BLOOD-PBMC}}$ estimates (bottom) in the subset of 5 scans for which $[^{11}\text{C}]\text{AA}$ plasma parent fraction values were individually measured.

	Correlation (r)	Slope	Intercept
K^*_{BLOOD} vs. $K^*_{\text{BLOOD-PBMC}}$			
GMC	0.938	1.217	-1.065
WMC	0.947	1.245	-1.079
CIN	0.923	1.184	-0.783
DCA	0.932	1.115	-0.298
HIP	0.923	1.180	-0.719
OCC	0.932	1.216	-1.071
ORB	0.951	1.233	-1.102
PAR	0.931	1.161	-0.644
TEM	0.945	1.203	-0.854
THA	0.941	1.184	-0.790
K^*_{BLOOD} vs. $K^*_{\text{IDIF-PBMC}}$			
GMC	0.903	1.030	0.566
WMC	0.918	1.078	0.172
CIN	0.882	0.978	0.838
DCA	0.901	1.005	0.345
HIP	0.869	0.974	0.863
OCC	0.896	1.016	0.734
ORB	0.929	1.117	-0.175
PAR	0.899	0.972	0.965
TEM	0.918	1.047	0.364
THA	0.914	1.050	0.339
$K^*_{\text{BLOOD-PBMC}}$ vs. $K^*_{\text{IDIF-PBMC}}$			
GMC	0.933	0.873	1.256
WMC	0.993	0.887	0.965
CIN	0.994	0.859	1.251
DCA	0.995	0.926	0.466
HIP	0.989	0.868	1.170
OCC	0.994	0.864	1.395
ORB	0.997	0.925	0.684
PAR	0.995	0.862	1.313
TEM	0.996	0.892	0.959
THA	0.995	0.908	0.880

GMC=gray matter cerebellum; WMC=white matter cerebellum; CIN5cingulate; DCA=dorsal caudate; HIP=hippocampus; OCC=occipital lobe; ORB=orbital prefrontal cortex; PAR=parietal lobe; TEM=temporal lobe; THA=thalamus

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Table 2

Region by region comparison of K^* estimates in all available scans. Results of correlation and regression analysis, region by region, for $K^*_{IDIF-PBMC}$ versus $K^*_{BLOOD-PBMC}$ estimates in all 16 available scans.

	Correlation (r)	Slope	Intercept
$K^*_{BLOOD-PBMC}$ vs. $K^*_{IDIF-PBMC}$			
GMC	0.646	0.587	4.000
WMC	0.640	0.578	3.475
CIN	0.624	0.599	3.435
DCA	0.690	0.746	2.113
HIP	0.611	0.683	2.721
OCC	0.672	0.594	3.800
ORB	0.698	0.653	3.147
PAR	0.692	0.569	3.815
TEM	0.692	0.665	2.858
THA	0.658	0.623	3.342

GMC=gray matter cerebellum; WMC=white matter cerebellum; CIN=cingulate; DCA=dorsal caudate; HIP=hippocampus; OCC=occipital lobe; ORB=orbital prefrontal cortex; PAR=parietal lobe; TEM=temporal lobe; THA=thalamus