Supplementary Materials

Materials and Reagents for Radiosynthesis of 5-[^{18}F]Fluoroleucine ([^{18}F]FLE)

No-carrier-added aqueous [^{18}F]fluoride ion was produced *via* the [^{18}O(p,n)^{18}F] nuclear reaction (Siemens Eclipse™ HP 11 MeV Cyclotron). The compounds mesylate precursor (precursor for [^{18}F]FLE) and 5-fluoroleucine (reference standard for [^{18}F]FLE) were custom synthesized from Jubilant Biosys Limited, India. Acetonitrile (99.8 %), Acetonitrile-d3 (≥ 99.8 %), anhydrous potassium carbonate (99.99 %), 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix®222, 98 %), trifluroacetic acid (99 %), and HPLC-grade acetonitrile (≥ 99.9 %) were procured from Sigma-Aldrich, Inc. Furthermore, ethanol 200 proof USP was purchased from Decon Laboratories, Inc. and Gibco™ PBS, pH 7.4 solution was purchased from Thermo Fisher Scientific, Inc. and used as supplied.

All reactions were carried out in closed Thermo Scientific™ conical reacti-vial™ (5 ml capacity). Sep-Pak® light (46 mg) Accell™ plus QMA carbonate (part no. 186004540) and Sep-Pak™ plus short tC18 (part no. WAT036810) were purchased from Waters Technologies Corporation, Inc. Deionized water (18.2 MΩ·cm; ELGA PURELAB Flex 3) was used throughout the process. Radiochemical isolation and purification was conducted by semi-preparative radio-HPLC system (Logi-CHROM ONE from Lablogic Systems, Inc.) comprising of a low pressure gradient quaternary Pump, Manual injection valve (6-port/3-channel), a solvent mixer, a single wavelength UV-Detector (λ=254 nm) and Flow-Count radio-HPLC NaI detection system. Quality control analytical radio-HPLC was performed on a UFLC Shimadzu HPLC system equipped with a Low Pressure Gradient Binary Pump, a dual wavelength UV detector and a positron radiodetector (Posi-RAM 6; Lablogic Systems, Inc.). Radioactivity measurements were made with a CRC-55tW radioisotope dose calibrator (Mirion Technologies (Capintec), Inc.).

Dual-output reversible model

The dual-output reversible compartment model developed in this study represents a methodological advance for small-animal cardiac PET imaging. Standard semi-quantitative standardized uptake values (SUV) or simplified graphical methods (Patlak, Logan plots) lack the sensitivity and specificity required to capture the nuanced kinetic changes in tracer transport and distribution that characterize early-stage metabolic dysfunction. Our 15-parameter model simultaneously recovers the MCIF, corrects for SP and PV contamination, and extracts mechanistically informative kinetic parameters (K_1, V_t, K_i) from a single dynamic acquisition. Incorporating a late-time-point blood sample as a tail-fitting constraint (O_3 cost function) resolved the problem of artifactual MCIF decline observed in initial iterations of the model. This will be critical for enabling longitudinal studies without repeated arterial catheterization.

Model Details

The dual-output model optimized the following composite objective function:

$$O(p) = O_1(p) + O_2(p) + O_3(p) \quad (1)$$

where O_1 minimizes the sum of squared differences between model output and PET-derived time-activity curves (TACs) for both the blood (IDIF) and myocardial regions; O_2 minimizes the squared error between model-predicted and PET-derived peak values; and O_3 minimizes discrepancy between the MCIF and a late-time-point venous blood sample. The parametric blood input function Ca(t) was derived in parallel with myocardial tissue TACs. SP and PV corrections were incorporated through recovery coefficients and SP contamination factors (S_{mb}, S_{bm}, r_b, r_m) [19].

The reversible two-tissue compartment model was implemented as:

$$C_T(t) = K_1/(a_1 - a_2) \times [(k_3 + k_4 - a_1)e^{-a_1 t} + (a_2 - k_3 - k_4)e^{-a_2 t}] \otimes C_a(t) \quad (2)$$

where K_1–k_4 are the kinetic rate constants, $\otimes$ denotes convolution, and a_1, a_2 are derived from the kinetic parameters. Kinetic parameters computed included K_1 (first-pass rate constant, in mL/min/g), $V_t = K_1/k_2$ (total distribution volume), and $K_i = K_1.k_3/(k_2 + k_3)$ (net influx rate constant). Model fitting in Python by minimizing the objective function (Eq 1) was performed using the scipy.optimize module from the SciPy library on a 12-core Linux workstation using parallelized computation across time frames.