Feasibility and dosimetry studies for [^{18}F]NOS as a potential PET radiopharmaceutical for inducible nitric oxide synthase (iNOS) in humans

Supplemental Data

METHODS

Myocardial [^{18}F]NOS kinetics: The distribution volume ratio (DVR) was calculated using the slope of the linear portion of graphical Logan plot with the pectoral muscle as the reference region [18]. The myocardial SUV was calculated at 10 min to minimize the confounding effects of rapid early uptake and late clearance of the radiopharmaceutical on the calculation. The area under the moment curve (AUMC) for [^{18}F]NOS was measured by numerical integration of the myocardial time activity curve expressed in SUV using the trapezoidal rule integration was performed up to 5, 10, and 30 minutes post injection using the following equation.[19]

$$AUMC(T) = \int_0^T MyoTAC(t)dt$$

Mean residence time (MRT; min) was defined in non-compartmental pharmacokinetics as the average total time molecules of a given dose spends in the organ of interest or entire body, and is measured as the integral of the first moment of the myocardial time activity curve divided by the area under the myocardial time activity curve (min) based on the following equation.[20]

$$MRT(T) = \frac{\int_0^T MyoTAC(t)tdt}{AUMC(T)}$$

Because of the rapid uptake and washout of the radiopharmaceutical in myocardium, AUMC and MRT measurements were performed at 5, 10 and 30 min after radiopharmaceutical administration.

Biodistribution and Radiation Dosimetry: Organ activity concentration was measured on the most visible organs as seen on the PET images. For the liver, brain and the spleen, average activity concentration was measured by drawing a 3D elliptical volume of interests (VOIs) inside the largest portion of the organ. For the kidney and heart wall, free hand ROIs were drawn over three adjacent transverse slices of the organ, excluding urine or blood, as seen on the registered CT images. For the gall bladder, stomach and bladder content, a 3D autothresholding VOIs was traced to encompass all the activity accumulation in these organs as seen on the PET images.

The average activity concentration measured in the liver, brain, kidneys, spleen and heart wall was converted to percent injected dose by decay correction to the time of injection and dividing by the amount of injected activity and then multiplying by the standard MIRD organ mass corrected to patient weight relative the MIRD standard male weight. The total activity measured in the bladder, gallbladder and stomach was converted to percent injected dose by decay correcting to the injection time and dividing by the amount of injected activity. Time activity curves combining the percent injected dose for all 16 subjects were then created and fitted by a mono-exponential function, with the exception of the gall bladder which was fitted with a linear function. Activity residence times for each observed organ were calculated from analytical integration of the fit after multiplying by an exponential function to account for radio-active decay. The urinary bladder residence time was calculated using the MIRD bladder voiding model

[20] in OLINDA/EXM [21] with the filling fraction and half-life measured from the cumulative urinary bladder time activity curve. The amount of excreted activity was calculated from integration of the bladder time activity curve minus the modeled bladder residence time. All unmeasured residence times was assigned to the remainder of the body. Radiation doses estimates were then calculated using the OLINDA/EXM (version 1.1) [21] using the standard human male model. OLINDA radiation dosimetry software considers the complete decay scheme of a nuclide and calculates the radiation doses from a source to a specific target organ and calculates the effective dose.

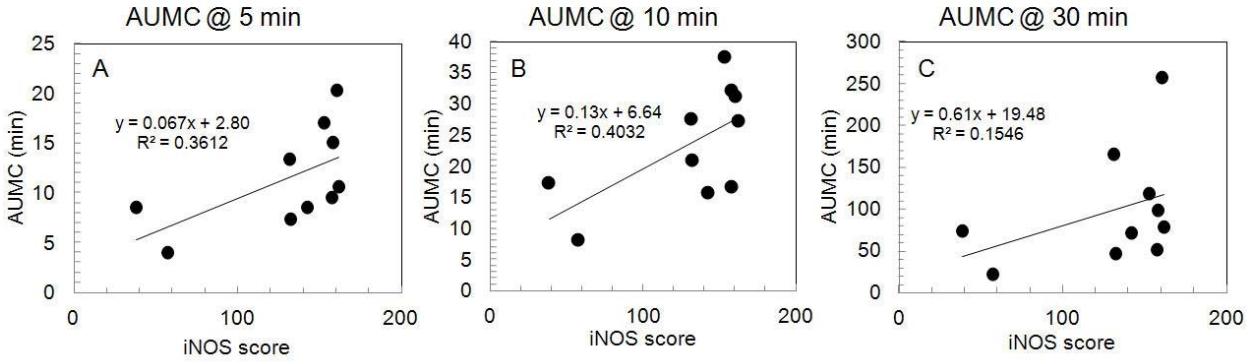
iNOS Protein Measurements: Immunohistochemistry of iNOS in tissue was performed using a modified biotin streptavidin alkaline phosphatase method [5]. After binding to a primary marker antibody, iNOS was visualized by a naphthol phosphate/fast red reaction (DAKO®, Fast Red substrate system K999). The expression of iNOS in cardiac myocytes, endothelial cells and vascular cells was analyzed using the following technique. Stained heart biopsy slides were scanned at 20x resolution using an Aperio ScanScope XT whole slide scanner (Aperio Technologies, Vista, CA). The resulting images were analyzed with ImageScope Software using a spectral deconvolution algorithm. Briefly, an image of the entire biopsy specimen was selected and the 3,3'-diaminobenzidine (DAB) level was measured. DAB is a commonly used chromogen for immunohistochemical staining. In the presence of a peroxidase enzyme, DAB will produce a brown precipitate that is insoluble in alcohol. The slides were dehydrated through alcohols to xylene and mounted with any commercially available mounting media, color channel extracted (corresponding to iNOS positive cells). The number of DAB positive pixels with threshold values corresponding to 0, 1+, 2+, and 3+ staining was then recorded. To correct for different sized

biopsy specimens the number of thresholded positive pixels was then divided by the total number of pixels in the scanned image, resulting in a percentage of cells that was 1+, 2+, or 3+ positive. Furthermore, a score (H) based on the weighted products of these intensities was calculated as follows:

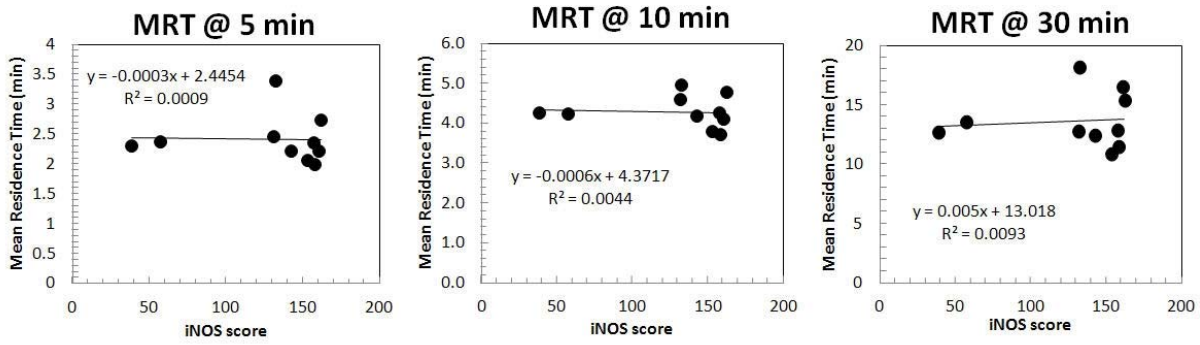
$$H = 100\left(\frac{N_{1+}}{N_{total}}\right) + 200\left(\frac{N_{2+}}{N_{total}}\right) + 300\left(\frac{N_{3+}}{N_{total}}\right)$$

Where N_{1+}, N_{2+}, N_{3+} represent the number of positive pixels at 1+, 2+, and 3+ staining intensities and N_{total} is the total number of pixels. This weighted average positive intensity (H) multiplied by the percentage of positive cells was used as the immunohistochemistry INOS score. This calculation was chosen because it most closely mimics the measured activity on the PET images.

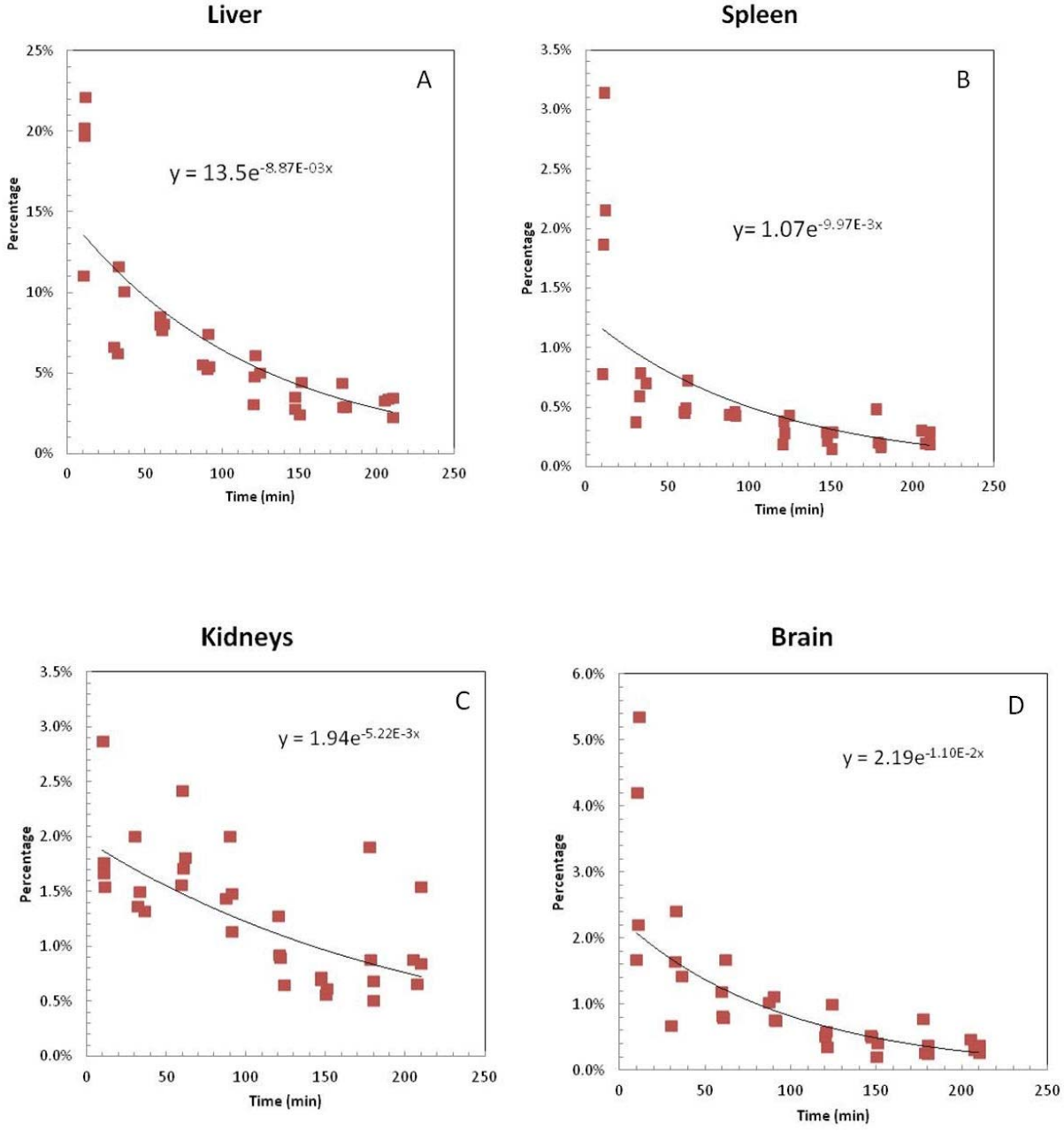
RESULTS

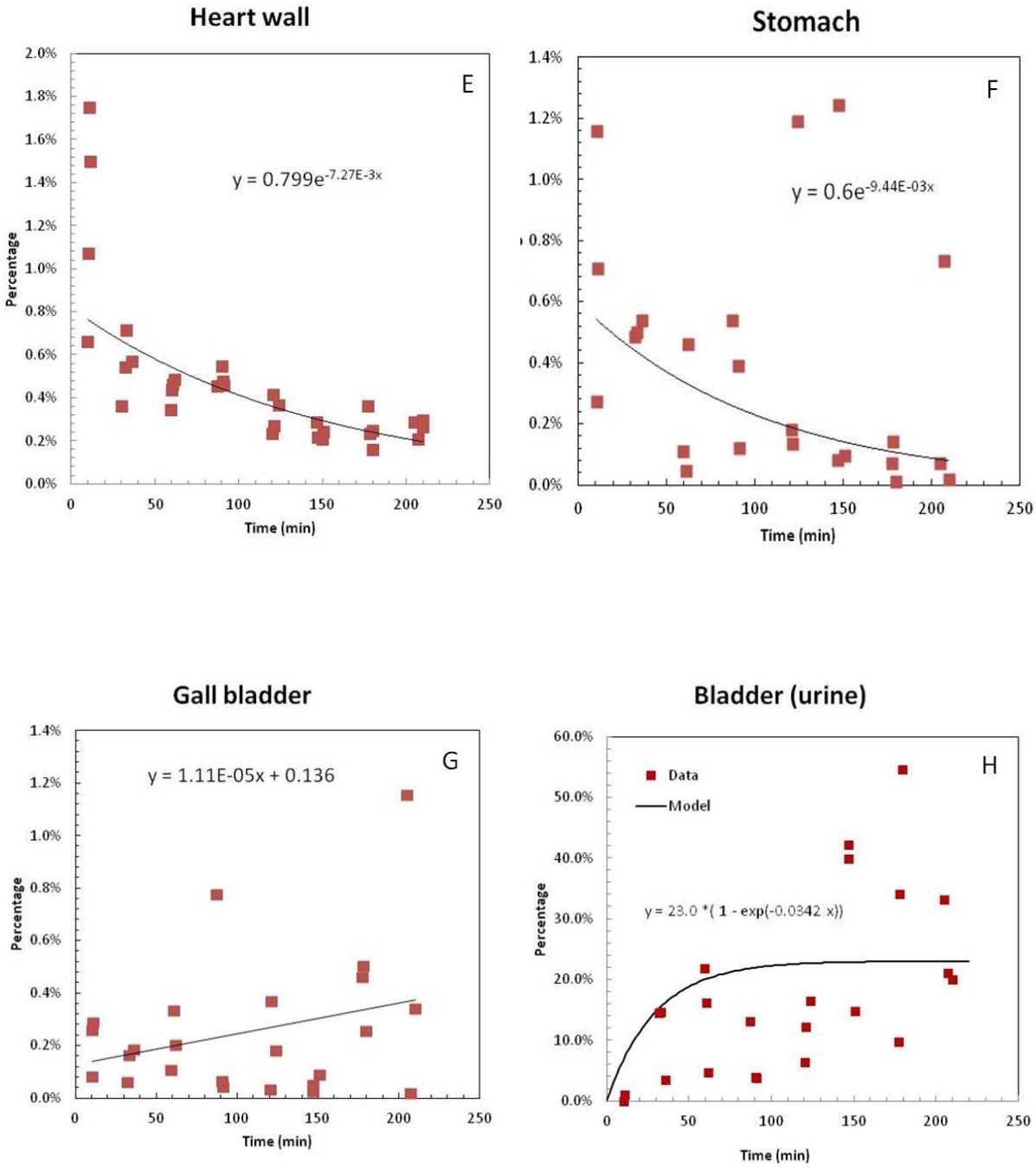


Supplemental Figure 1. Area under the moment time activity curves integrated until 5, 10 and 30 min post injection plotted as a function of iNOS score. Only the correlation coefficient at 10 min was significant, $P < 0.05$

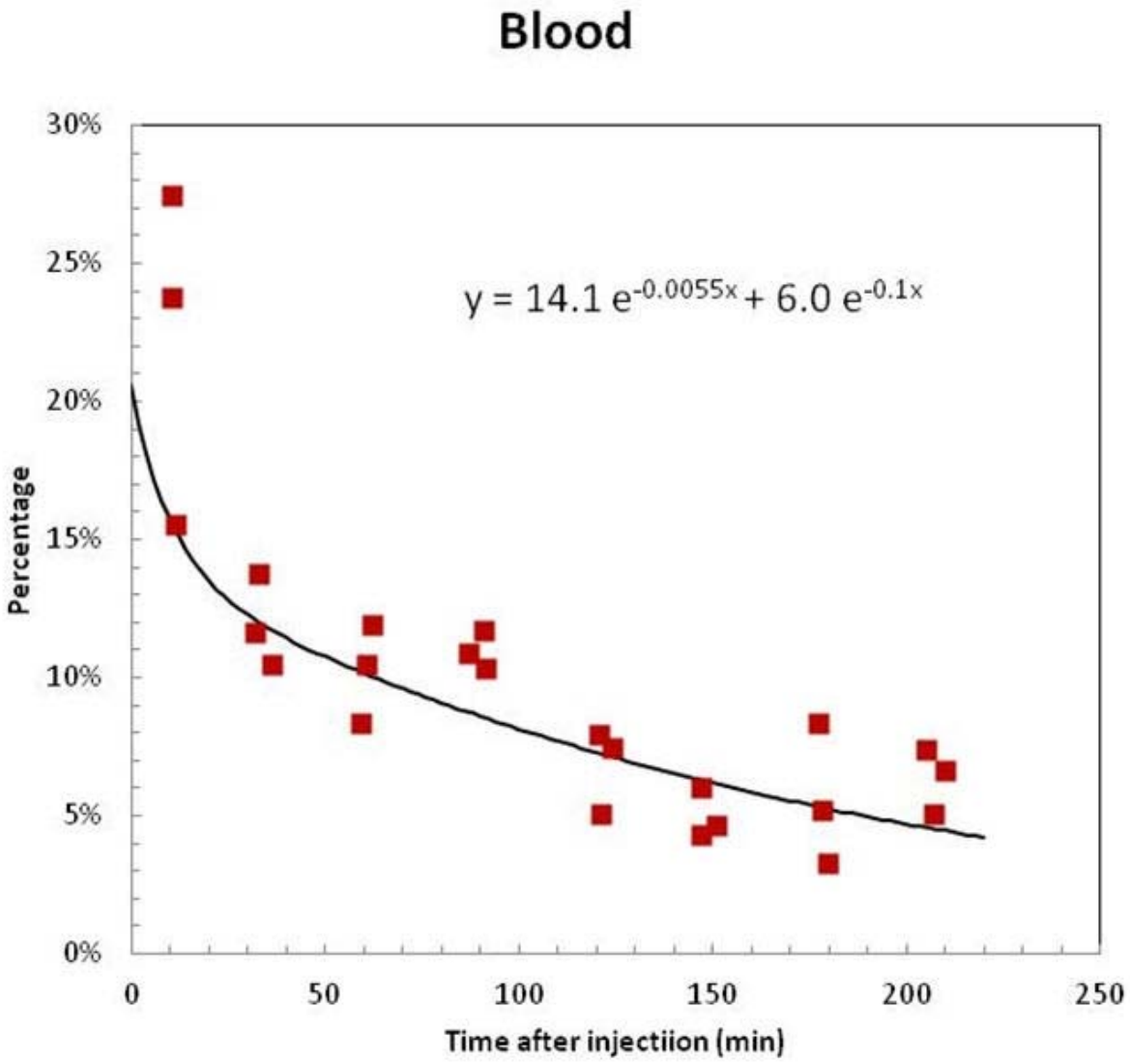


Supplemental Figure 2. Mean Residence Times calculated from the myocardial time activity curves integrated until 5, 10 and 30 min post injection plotted as a function of the iNOS score**.** P = NS for all correlations.


Liver
A
y = 13.5e-8.87E-03x
Percentage
Time (min)
Spleen
B
y= 1.07e-9.97E-3x
Percentage
Time (min)
Kidneys
C
y = 1.94e-5.22E-3x
Percentage
Time (min)
Brain
D
y = 2.19e-1.10E-2x
Percentage
Time (min)



Supplemental Figure 3. Organ time activity curves for the liver (A), spleen (B), kidneys (C), brain (D), heart wall (E), stomach (F), gall bladder (G) and total accumulation in the bladder (H). The fit are shown on the graph along with the fitting parameters.



Supplemental Figure 4. Blood activity concentration extending to 3.5 hr post injection for [^{18}F]NOS fitted by a double exponential clearance function.