Quantification of Glucose Metabolism using Small-Animal Positron Emission Tomography (PET) Imaging

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Outline

- What is quantitative imaging?
- Why small-animal imaging?
- Technical challenges and considerations in small-animal PET imaging
  - Input function measurement
  - Animal handling
  - Administration route
  - Dietary condition
  - Blood glucose level
Quantitative Imaging

Biology and Medicine

Quantitative Imaging

(Radio)chemistry

Imaging and instrumentation
Quantitative Imaging

• “Quantitative imaging” is a specialty area that includes
  - tracer kinetic (pharmacokinetics) modeling,
  - image processing, image alignment, reconstruction, and
  - statistical analysis / estimation, etc

to convert absolute radioactivity measurements into biomedical information.

• It integrates information from various fields and needs to consider factors, including
  - the characteristics of the imaging instruments,
  - the biochemical properties of the labeled compounds, and
  - the biological and medical relevance.
\[ y_i = \sum_j (c_{ij} x_j), \quad \text{for } i = 1, \ldots, m \]
In radioactive decay by positron emission, a proton in the nucleus is transformed into a neutron and a positively charged electron (i.e., positron). The positron and a neutrino are ejected from the nucleus.

Nucleus

 positron scatters in tissue losing energy

511 keV Photon

Annihilation

Yes  Coincidence?

511 keV Photon
# Positron-Emitting Radionuclides

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
<th>$\beta^+$ fraction</th>
<th>Max. Energy</th>
<th>Range</th>
<th>Production</th>
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<tbody>
<tr>
<td>C-11</td>
<td>20.4 mins</td>
<td>0.99</td>
<td>0.96 MeV</td>
<td>0.4 mm</td>
<td>Cyclotron</td>
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<tr>
<td>N-13</td>
<td>9.96 mins</td>
<td>1.00</td>
<td>1.20 MeV</td>
<td>0.7 mm</td>
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<tr>
<td>O-15</td>
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<td>1.00</td>
<td>1.74 MeV</td>
<td>1.1 mm</td>
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<tr>
<td>F-18</td>
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<td>0.63 MeV</td>
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<tr>
<td>Cu-62</td>
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<td>0.98</td>
<td>2.93 MeV</td>
<td>2.7 mm</td>
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<tr>
<td>Cu-64</td>
<td>12.7 hrs</td>
<td>0.19</td>
<td>0.65 MeV</td>
<td>0.3 mm</td>
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<td>Ga-68</td>
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<td>0.88</td>
<td>1.83 MeV</td>
<td>1.2 mm</td>
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<tr>
<td>Br-76</td>
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<td>1.00</td>
<td>1.90 MeV</td>
<td>1.2 mm</td>
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<tr>
<td>Rb-82</td>
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<td>3.15 MeV</td>
<td>2.8 mm</td>
<td>Generator</td>
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<tr>
<td>I-124</td>
<td>4.18 days</td>
<td>0.22</td>
<td>1.50 MeV</td>
<td>0.9 mm</td>
<td>Cyclotron</td>
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</table>
PET-labeled Probes for Molecular Imaging

PET can detect and image these labeled probes \textit{in vivo} at nano- or pico-molar levels.
Quantitative PET Imaging Procedures

Report on tissue biological state

<table>
<thead>
<tr>
<th></th>
<th>MRglc</th>
<th>Kᵢ</th>
<th>SUV</th>
</tr>
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<tbody>
<tr>
<td>brain</td>
<td>nnn</td>
<td>nn</td>
<td>nnnn</td>
</tr>
<tr>
<td>myocardium</td>
<td>aaa</td>
<td>aa</td>
<td>aaaa</td>
</tr>
<tr>
<td>tumor</td>
<td>bbb</td>
<td>bb</td>
<td>bbbb</td>
</tr>
</tbody>
</table>

Biological quantification

Cᵣ  →  Cₑ  →  Cₘ
Tracer Kinetic Modeling and Parameter Estimation

Tracer administration

Body with unknown biology/pathology

Model with parameters

Parameter update algorithm

Simulated kinetics

Measured kinetics
Beginning of “Metabolic” Imaging
The 2-Deoxyglucose (2-DG) Autoradiography Method for Cerebral Metabolic Rate of Glucose (Sokoloff et al, 1977)

$$\text{CMR}_{\text{glc}} = \frac{C_{\text{glc}}}{LC} \cdot \frac{C_i(T) - K_1 e^{-(k_2+k_3)T} \int_0^T C_P(t) e^{(k_2+k_3)t} dt}{\int_0^T C_P(t) dt - e^{-(k_2+k_3)T} \int_0^T C_P(t) e^{(k_2+k_3)t} dt}$$

- $C_{\text{glc}}$: capillary plasma glucose concentration
- $C_P(t)$: time course of capillary plasma 2-DG concentration
- $C_i(t)$: time course of the sum of tissue concentration of 2-DG and 2-DG-6-P
- $K_1, k_2$: First-order rate constants for 2-DG forward and reverse transport
- $k_3, k_4$: First-order rate constants for phosphorylation and dephosphorylation of 2-DG
- $LC$: lumped constant to correct for differences in transport and phosphorylation rates of 2-DG and glucose under steady-state conditions

Early Days of PET Development

PET III Human PET Scanner
(Huang, Hoffman, and Phelps, 1973–1975)

Edward Hoffman, Ph.D.
Sung-Cheng Huang, D.Sc.
Michael Phelps, Ph.D.
Modeling of $^{18}$F-FDG Kinetics


**Plasma**

$\text{Glucose}$

$\text{FDG}$

$\text{FDG}$

$\text{FDG}$

$\text{FDG-6-P}$

**BBB**

$\text{Glucose}$

$\text{Glucose}$

$\text{Glucose}$

$\text{Glucose-6-P}$

**Tissue**

$\text{Tissue}$

$\text{Tissue}$

$\text{Tissue}$

$\text{Tissue}$

$\text{Tissue}$

**FDG in plasma** ($C_P$)

$K_1$

$k_2$

$k_3$

$k_4$

**FDG in tissue** ($C_E$)

**FDG-6-P in tissue** ($C_M$)

$\text{FDG in plasma} (C_P)$

$\text{FDG in tissue} (C_E)$

$\text{FDG-6-P in tissue} (C_M)$

$\text{MRglc} = \frac{C_{\text{glc}}}{LC} \cdot \frac{K_1 k_3}{k_2 + k_3} = \frac{C_{\text{glc}}}{LC} \cdot K_i$
Small-Animal Imaging – Why?

- in vitro ⇆ in vivo
- rodents are small, inexpensive, and easy to bred and maintained
- can investigate the whole animal
- provides us a better understanding of the whole mammalian biology, including physiology, immunology and development
- repeat / longitudinal studies in the same animal
- provides a bridge between animal and human studies
Small-Animal PET and CT Scanners

- microPET (Focus-220)
- microPET (Inveon)
- microCT (microCAT II)
Cardiac Output Measurements in Mice


For anesthetized mice (n = 25):
- Cardiac output (CO): 20.4 ± 3.4 mL/min
  (Cardiac index: 0.73 ± 0.19 mL/min/g)
- Stroke volume (SV): 45.0 ± 6.9 μL

With dobutamine:
- CO increased by ~75%
- SV increased by ~23%

Cardiac Output = \( \frac{\text{Injected dose}}{\text{Area under the LV curve}} \)
Technical Challenges for Small-Animal PET Imaging

• Input function
• Animal handling
• Injection route
• Dietary state
• Plasma glucose level
• Spatial resolution
• Radiation exposure
• Radiopharmaceuticals
• …
Input Function

• Absolute quantification of tissue data obtained from the scanner requires an invasive procedure of arterial catheterization, where a series of blood samples are taken to form the input function for kinetic modeling.

• Arterial catheterization is challenging in mice:
  ▪ Size of blood vessel is small.
  ▪ Blood volume is limited (on average, 6 to 8 mL of blood per 100 g of body weight).
Input Function Alternatives

- Arterialized-venous or venous samples (Phelps et al, 1979)
- Population input function (Takikawa et al, 1993; Meyer et al, 2006)
- Image-derived input function (Gambhir et al, 1988; Chen et al, 1998)
- Factor analysis (Wu et al, 1995; Kim et al, 2006)
- Blind estimation / deconvolution (Di Bella et al, 1999; Riabkov and Di Bella, 2004)
- Compartmental model-based input function estimation (Feng et al, 1997; Wong et al, 2001)
- Reference tissue (Lammertsma et al, 1996)
- Hybrid combination (Ferl et al, 2007; Fang et al, 2008; Wong and Huang, 2008; Su and Shoghi, 2010)
Derivation of Input Function

Input Function
(Blood sampling / image-derived)

Tissue Kinetics
(microPET images)

Impact of Animal Handling on Biodistribution of $^{18}$F-FDG in Tissues


(A) Not fasted, warmed, no anesthesia.
(B) Fasted, not warmed, no anesthesia.
(C) Fasted, warmed, no anesthesia.
(D) Fasted, warmed, no anesthesia, conscious injection.
(E) Reference conditions: not fasted, not warmed, no anesthesia.
(F) microCT: anatomic reference.
(G) Not fasted, warmed, isoflurane.
(H) Fasted, warmed, isoflurane.
(I) Fasted, warmed, ketamine.
Impact of Animal Handling on Biodistribution of $^{18}$F-FDG in Tissues

Animal handling (temperature, dietary condition, injection route, duration and choice/mode of anesthesia) had a dramatic effect on the results of the $^{18}$F-FDG imaging studies.

The time-activity curves for many organs had a somewhat dependence on the route of administration (IV vs. IP), but they were comparable within 60-min post-injection.

However,
- no absolute quantification was performed, although dynamic imaging data were acquired, and
- the results were based on standardized uptake value (SUV) calculated at 60 min post-injection.
Motivations and Aims

• To investigate the impact of dietary condition (nonfasting vs. fasting) and altered blood glucose on $^{18}$F-FDG kinetics, uptake profiles, and kinetic/physiological parameters in normal tissues of intact mice.

• To evaluate the feasibility of using IP $^{18}$F-FDG injection as an alternative to tail-vein bolus injection to study glucose metabolism in mice.
Experimental Methods

- **Male C57BL/6 mice (Anesthesia: 1.5% isoflurane in 100% O₂)**
  - Weight (mean ± SD): 23.8 ± 3.1 g
  - IV group: 6 non-fasting and 5 fasting (Injected dose: 14.2 ± 3.8 MBq)
  - IP group: 6 non-fasting and 6 fasting (Injected dose: 10.5 ± 0.8 MBq)

- **Serial blood samples (~10-15 μL each) were taken from the femoral artery.**
  - IV group: approx. 3, 5, 8, 25, 40, 60, 90, and 120 s, and at 5, 10, 15, 30, 45, and 60 min
  - IP group: 5, 10, 15, 20, 40, 60 min

- **Blood glucose levels were measured throughout the study.**
- **CT scan was performed for attenuation correction.**
- **Filtered-backprojection reconstruction was used:**
  - IV group: 1 x 3 s, 10 x 0.5 s, 1 x 2 s, 1 x 4 s, 6 x 5 s, 1 x 10 s, 2 x 30 s, 2 x 2 min, 1 x 3 min, 2 x 10 min, 2 x 15 min
  - IP group: 12 x 5 min.
Data Analysis

- Images were analyzed using AMIDE*.
- 3D regions of interest (ROIs) were manually drawn over the brain, myocardium, skeletal muscle of forelegs, left ventricle, liver, and renal cortex on the fused PET/CT images.
- For the urinary bladder, ROIs that covered the entire bladder were drawn frame by frame.
- ROIs were applied to each time frame of the PET images, and the average radioactivity concentration was computed to obtain the tissue time-activity curves.
- Because uptake in the tissues was relatively homogeneous within the ROIs and the background radioactivity was low, background subtraction and partial-volume correction were not performed.

Kinetic Analysis

- $^{18}$F-FDG uptake constant ($K_i$) was estimated by Patlak graphical analysis (Patlak et al, 1983):

$$\frac{C_T(t)}{C_p(t)} = K_i \int_0^t \frac{C_p(s)ds}{C_p(t)} + V_e$$

- The metabolic rate of glucose (MRglu) was calculated as:

$$MRglu = \frac{C_{glc}}{LC} \cdot \frac{K_1k_3}{k_2 + k_3} = \frac{C_{glc}}{LC} \cdot K_i$$

- Standardized uptake value (SUV):

$$SUV = \frac{Radioactivity\ concentration\ in\ tissue\ (MBq/mL)}{Injected\ dose\ (MBq)/Weight\ of\ animal\ (g)}$$

Statistical Analysis

• Differences in physiologic parameters among the experimental groups of different injection routes and dietary states are compared by 2-way ANOVA with Bonferroni post hoc test, and by the Mann-Whitney U test whenever appropriate.

• Regression analysis was performed on tissue uptake (SUV), $K_i$ and MRglu in the brain, myocardium, and skeletal muscle to investigate their relationships with blood glucose level, dietary state, $AUC_{plasma}$, plasma clearance rate, and total activity in bladder.

• $P < 0.05$ was considered statistical significance.
• Shape of TACs differed between IV and IP injection.
• Injection route ($F_{1,19} = 0.1, P = 0.53$) and dietary state ($F_{1,19} = 0.78, P = 0.39$) had no significant effect on $AUC_{\text{plasma}}$.
• $^{18}$F-FDG plasma clearance was slightly faster in fasting than in nonfasting mice.
• Significant positive correlations were found between $^{18}$F-FDG plasma clearance rate and total accumulated radioactivity (at 60 min) in urinary bladder in both IV ($R = 0.60, P = 0.025$) and IP ($R = 0.53, P = 0.04$) injections.
Biodistribution of $^{18}$F-FDG in Nonfasting and Fasting Mice after IV and IP Injection
Time-Activity Curves

- **Brain**
- **Myocardium**
- **Skeletal Muscle**
- **Liver**
- **Blood and Plasma**

**Legend:**
- IV (Nonfasted)
- IV (Fasted)
- IP (Nonfasted)
- IP (Fasted)
- Whole blood (IV)
- Plasma (IV)
- Whole blood (IP)
- Plasma (IP)
18F-FDG Uptake and Metabolic Parameters under Different Dietary State and Injection Route

Error bars represent 1 SD. (\(+P < 0.05\), \(#P < 0.01\), \(*P < 0.001\) vs. nonfasted condition using the same injection route).

There were no significant differences in SUV, $K_i$, and MRglu in the brain, myocardium, and skeletal muscle obtained from IV and IP injections, indicating these two routes of administration gave results indistinguishable from one another.
Effects of Blood Glucose Level on Tissue $^{18}$F-FDG Uptakes and Glucose Utilization Rates

Summary

• IP injection is a valid and practical alternative to the tail vein injection commonly used in small-animal $^{18}$F-FDG PET.
• For the brain, the $^{18}$F-FDG uptake constant was inversely related to the plasma glucose level, regardless of which injection route (IV or IP) was used.
• The cerebral MRglu was thus independent of the plasma glucose level (assuming the lumped constant was not a function of plasma glucose level).
• For myocardium, the $^{18}$F-FDG uptake constant (instead of MRglu) was independent of plasma glucose level, but was mainly influenced by the dietary condition of the animal.
• For skeletal muscle, the results were similar to the situation of myocardium with an attenuated sensitivity to the dietary condition.
• If only SUV at 60 min were available, the relationships were not as clearly revealed and one would not be able to make the same conclusion.
• These findings confirmed the value of performing quantitative dynamic imaging studies to provide reliable information that is of high biological relevance.
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