Video Article

Functional Imaging of Brown Fat in Mice with ^{18}F-FDG micro-PET/CT

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Abstract

Brown adipose tissue (BAT) differs from white adipose tissue (WAT) by its discrete location and a brown-red color due to rich vascularization and high density of mitochondria. BAT plays a major role in energy expenditure and non-shivering thermogenesis in newborn mammals as well as the adults [1]. BAT-mediated thermogenesis is highly regulated by the sympathetic nervous system, predominantly via β adrenergic receptor [2, 3]. Recent studies have shown that BAT activities in human adults are negatively correlated with body mass index (BMI) and other diabetic parameters [4-6]. BAT has thus been proposed as a potential target for anti-obesity/anti-diabetes therapy focusing on modulation of energy balance [6-8]. While several cold challenge-based positron emission tomography (PET) methods are established for detecting human BAT [9-13], there is essentially no standardized protocol for imaging and quantification of BAT in small animal models such as mice. Here we describe a robust PET/CT imaging method for functional assessment of BAT in mice. Briefly, adult C57BL/6J mice were cold treated under fasting conditions for a duration of 4 hours before they received one dose of ^{18}F-Fluorodeoxyglucose (FDG). The mice were remained in the cold for one additional hour post FDG injection, and then scanned with a small animal-dedicated micro-PET/CT system. The acquired PET images were co-registered with the CT images for anatomical references and analyzed for FDG uptake in the interscapular BAT area to present BAT activity. This standardized cold-treatment and imaging protocol has been validated through testing BAT activities during pharmacological interventions, for example, the suppressed BAT activation by the treatment of β-adrenoceptor antagonist propranolol [14, 15], or the enhanced BAT activation by β3 agonist BRL37344 [16]. The method described here can be applied to screen for drugs/compounds that modulate BAT activity, or to identify genes/pathways that are involved in BAT development and regulation in various preclinical and basic studies.

Video Link

The video component of this article can be found at https://www.jove.com/video/4060/

Protocol

1. Animal Preparation and Cold Treatment

1. Locate and inspect a 4 °C cold room that has been approved for accommodating laboratory mice.
2. Pre-chill animal cages overnight in the cold room. The cages are assembled without feed and bedding but with a bottle of water.
3. In the morning of the experimental day, place mice one by one into each of the pre-chilled cages at 30 min intervals. Each singly caged mouse should stay in the cold room for nearly 4 hr before it is transported to the imaging lab. Ensure the mice are fasting but with access to water.
4. At 4 hr post cold treatment transport one animal at a time every 30 min to the imaging Lab. This can be achieved by filling a Styrofoam container with ice and placing a pre-chilled housing cage on top of the ice inside the box. Loosely place the cover on the Styrofoam box.

2. Setup Micro-PET/CT Imaging Workflow

In this protocol micro-PET/CT imaging is achieved with the Siemens Inveon Dedicated PET (dPET) System and Inveon Multimodality (MM) System (CT/SPECT) in the docked mode. The animal is placed from the MM entrance, first scanned with the CT for anatomical references, and then slid to the center of the dPET for a static F18 PET acquisition. In order to enable the host computer to carry out these sequential tasks automatically, the following "workflow" is programmed with the Inveon Acquisition Workplace (IAW) software prior to the actual imaging session.

1. **CT Acquisition**: For a whole body CT scan, set current at 500 uA, voltage at 80kV, exposure time at 200 msec, and 240 steps for 240° rotation. For X-ray detector, select resolution at "low system magnification" with 78 mm axial imaging field and single bed mode. Select "real time reconstruction" using the "Common Cone-Beam Reconstruction" method so that the host PC talks with a dedicated real time reconstruction computer (Cobra) to initiate the task.

2. **PET Emission Acquisition**: Set 600 sec (10 min) for "fixed scan time" in the "acquire by time" option. Select F-18 as "study isotope" and use 350-650 keV as "energy level".
3. **PET Emission Histogram**: Set "dynamic frame" as "black" to process data as one frame for the entire duration to achieve static scan. Select "3D" as the histogram type and choose "no scatter correction".
4. **PET Reconstruction**: Use 2D Ordered Subset Expectation Maximization (OSEM2D) as the reconstruction algorithm.

3. Injection of FDG

1. Order a clinical package of ^{18}F-FDG (10 mCi) from a regional vendor for its arrival to the imaging lab ~ 30 min before the scheduled first injection. Follow the institute's safety procedures to receive and survey the package containing radioactive materials (RAM).
2. With the protection provided by an L-block table top shield, aliquot the FDG and make dilutions with sterilized saline. The diluted activity concentration of FDG should be available at 200-300 µCi/100 ul for each injection. Draw the FDG solution into a 1 ml syringe with 26G 1/2 inch needle, and measure the radioactivity of the whole syringe with a dose calibrator.
3. Inject the animal that is just transported from the cold room (see Step 1.4) with 100 µl of FDG solution via the intraperitoneal (i.p.) route. Record the injection time. Measure the residue radioactivity of the syringe again with the dose calibrator.
4. Put the animal back to the cold cage inside a Styrofoam cooler maintained with ice. Incubate the animal in cold (~ 4 °C) for 1 hr for FDG uptake.
5. Calculate the injected FDG activity for each mouse by the following formula:
Injected activity (µCi) = activity in syringe before injection - activity in syringe after injection

4. Micro-PET/CT Imaging

1. The micro-PET/CT imaging starts 1 hr after the FDG injection or 5 hr after the cold treatment. Put the animal into an anesthesia induction chamber with 3% Isoflurane in oxygen.
2. Once anesthesia is induced, the animal is moved onto a micro-CT pellet (animal bed) with its head resting within a cone face mask that continuously delivers Isoflurane (2%) at a flow rate of 2 L/min. An electric heating pad (BioVet, m2m Imaging Corp.) is placed under the animal to help maintain the body temperature.
3. Slide the animal to the entrance of the MM scanner, activate the "laser" icon from the toolbar, and use the touchpad control to move the bed so that the chest of the animal is at the cross of horizontal and vertical laser lines. In the "Laser Align" window select "first scan type" as CT scan, and "PET acquisition included in workflow" as the option.
4. Open the "Scout View" window and acquire a scout view x-ray radiograph. Use IAW to adjust the position of the animal bed so that the center field of view of CT is located in the center of mouse body (liver). Repeat this step if necessary.
5. Start the "workflow" established in Step 2. When options pop up, select an appropriate 3D PET-CT transformation matrix file to be used in CT reconstruction, and choose a recently created normalization file for PET reconstruction with no attenuation correction. The IAW will then start CT and PET scans sequentially as programmed.
6. After the whole workflow is completed, which may take 20-25 min, briefly evaluate the quality of the acquired CT and PET images with the ASIPro VM, a micro-PET analysis software co-installed with IAW. Archive the imaging data to a data storage device or transfer the data through network to a post imaging analysis workstation (see Step 5) for further analysis.
7. Release the animal from the imaging systems and place it to a clean housing cage with normal food and water supply for its recovery at room temperature. The systems are now ready for the next animal in the queue. Note the care and handling of post-imaging animals should follow the institute's regulations regarding "handling of laboratory animals injected with RAM". Also note that used needles/syringes, the absorbent pads, gloves, and all bedding and fecal matter should be considered as radioactive waste, and handled according to the institute's relevant RAM waste disposal regulations.

5. Post-imaging Analysis

1. Open Inveon Research Workplace (IRW) software (Siemens) and manually import both CT and PET data sets. Co-register CT and PET images in "Registration" window using tools with the "General analysis" function, and under the "Review" window make sure a perfect alignment between CT and PET images in all 3 dimensions.
2. From "Region of Interest (ROI) Quantification" window, with the references provided by the co-registered CT images, identify BAT at the interscapular region of the neck, the most predominant cold-inducible BAT in adult mice, and draw volume of interest (VOI) of BAT over the PET data set. Select "Voxel Intensity" as the "Quantification Type" and record the mean radioactivity within the VOI as Bq/ml. A calibration factor which converts counts/sec to Bq/ml has been previously established by scanning a phantom with known radioactivity.
3. FDG uptake in BAT is quantified as percentage injected dose per gram tissue (%ID/g) with decay correction. The injected dose is the result of Step 3.5, however, converted to becquerel (Bq) unit (1 µCi = 37,000 Bq); we assume that 1 ml of tissue equals to 1 g.

Representative Results

An example of micro-PET/CT imaging of mouse BAT is shown in **Figure 1**. While the CT imaging provides anatomical information, the PET imaging encodes the distribution and quantity of ^{18}F-FDG uptake throughout the whole body. These imaging data can be viewed separately (**Figure 1A and 1B**), fused (**Figure 1C**), or demonstrated with a 3D feature such as maximal intensity projection (MIP, 1D). With the help of a 3D imaging tool, a volume of interest (VOI), here the interscapular BAT region (indicated by arrows in **Figure 1**), is drawn over the PET images and the total signals within the VOI can be converted into %ID/g, representing the percentage injected dose (%ID) per gram of tissue. In the mouse demonstrated, FDG uptake in the BAT is 19 %ID/g. In order to verify if this cold-induction and imaging protocol is sensitive enough to detect an altered BAT activity, in either case of up-regulation or down-regulation, we used β adrenoceptor antagonist propranolol to suppress the BAT activation [15], and β3 agonist BRL37344 to enhance BAT induction [16], respectively. These pharmacological interventions were applied during the cold treatment and precisely, at 30 min before the injection of FDG. PET/CT imaging (**Figure 2A**) and the analysis (**Figure 2B**) showed that the

propranolol treatment significantly reduced the FDG uptake in BAT, whereas BRL37344 markedly elevated the uptake, as compared with the vehicle control.

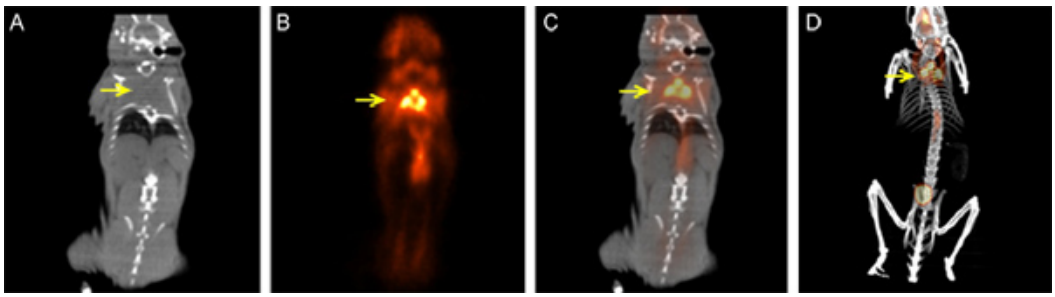

Figure 1. Micro-PET/CT imaging of BAT in a mouse after 5 hr cold-treatment. (A) A coronal section of CT image. (B) A coronal section of PET image co-registered with the CT in "A". (C) A fused PET/CT image resulted from the superimposing of "A" and "B". (D) Maximal intensity projection (MIP) presentation of the fused PET/CT images. Yellow arrows: the area corresponding to interscapular brown adipose tissue.

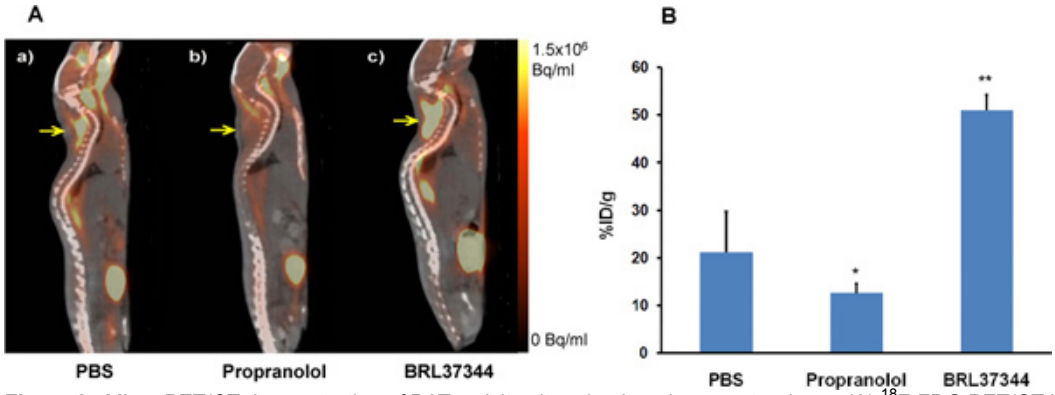

Figure 2. Micro-PET/CT demonstration of BAT activity alteration by adrenoceptor drugs. (A) ^{18}F-FDG PET/CT imaging of mice treated with different drugs. a) PBS (control). b) Propranolol (β antagonist, 5mg/kg, i.p.). Note a marked reduction of FDG uptake in BAT area. c) BRL37344 (β3 agonist, 5 mg/kg, i.p.). Note a significant increase in FDG accumulation in BAT. Yellow arrows: the area corresponding to interscapular brown adipose tissue. (B) Quantitative analysis of the FDG uptake in BAT. Values of %ID/g (the percentage injected dose per gram of tissue) are presented (mean ± SD). n=10 for the PBS group; n=5 for both Propranolol and BRL37344 groups. *, $p < 0.05$; **, $p < 0.005$, as compared with the PBS group.

Discussion

In this study a micro-PET/CT-based imaging method has been developed for detecting BAT activities in adult mice which simply requires a cold treatment and one injection of commercially available ^{18}F-FDG. The whole procedure can be done in one day following a treatment and imaging sequence which starts every 30 minutes until all animals are treated and imaged. Under the experimental conditions outlined, a total of 10 mice (or 2 groups of 5 mice) can be tested on the same day with a single imaging system. The limitation to this type of throughput can be lifted if 2 or more animals can be scanned simultaneously on a specially designed animal bed as it has been previously reported [17]. To complete the study we rely on the use of a combined micro-PET/CT imaging system which takes advantage of the detailed anatomical information provided by the CT. However, a standalone micro-PET is also able to fulfill the task when a ^{57}Co transmission scan is added to the workflow prior to the F18 emission data acquisition. The ^{57}Co transmission data can be used for attenuation correction during PET image reconstruction and can also be reconstructed for anatomical localization.

A critical step of this protocol is to optimize the duration of the cold treatment (*e.g.*, 5 hours in this study). A shorter duration time or the elimination of cold exposure may produce an activity close to the background and the method can be insensitive to any down-regulation of BAT (the floor effect). In contrast, an elongated cold exposure (such as overnight, or 24 hours) may maximize the induction and the method can become saturated masking any differences in the up-regulation of BAT (the ceiling effect). The optimized conditions described in this protocol have been validated through the propranolol suppression and β3 agonist BRL37344 stimulation tests (**Figure 2**), suggesting that this method is considerably sensitive and consistent in detecting alterations of BAT activities in mice. Applications of this method will include various basic studies using mouse models towards a better understanding of BAT differentiation and regulation, as well as preclinical research aiming to the discovery of safe BAT-stimulating drugs that may benefit the treatment of obesity and diabetes.

Disclosures

No conflicts of interest declared.

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