Supporting Information

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SI Materials and Methods

Xenon Enhancement Calibration Studies. Determination of the concentration of xenon in tissues by CT requires measurements of the density enhancement as a function of xenon concentration. These measurements (Fig. S1) were obtained by scanning a glass vessel containing different concentrations of xenon gas at different peak kilovoltages (kVp). For these measurements, the glass vessel was connected to a gas-charging system connected to a xenon gas cylinder equipped with a pressure regulator, a rotary vacuum pump, and a precision pressure gauge (precision up to 0.01 psi). The rotary pump was used to remove air from the system to achieve a baseline pressure of 0.0007 atm, before stable xenon gas was introduced. Gas pressures were then changed between 0.1 and 3 atm. To calculate the gas molarity from pressures and volume readings, the following van der Waals equation was used:

$$\left(P + \frac{an^2}{V^2}\right)\left(V - \frac{n}{b}\right) = nRT,$$

with coefficient $a = 4.1333$ atm·L^2/mol^2, $b = 0.0516$ L/mol, and a room temperature value of $T = 294.65$ K.

Modeling of Xenon Uptake in BAT. The time-dependent xenon concentration in a given tissue can be described by the Kety–Smith equation (1):

$$C_i(t) = \lambda_i C_a \left(1 - e^{-\frac{F_i}{\lambda_i}t}\right),$$

where $C_i(t)$ is the xenon concentration in the analyzed tissue, λ_i is the tissue/blood partition coefficient of xenon, C_a is the concentration of xenon in arterial blood (which is assumed to be constant in our case), and F_i is the tissue's blood flow per unit volume. According to this equation, xenon tissue absorption depends not only on the degree of tissue perfusion, but also on the solubility of xenon in the given tissue (partition coefficient). Upon xenon inhalation, in any given tissue, xenon concentration rises toward its equilibrium value of $\lambda_i C_a$, with a time constant equal to xenon solubility in the tissue divided by tissue blood flow $\frac{\lambda_i}{F_i}$. This means that an increase in blood flow rate is expected to decrease the time at which xenon steady state concentration is reached (the "wash-in rate"), but not its absolute value, which is determined solely by the arterial blood concentration and by the blood tissue partition coefficient $\lambda_i C_a$. According to this model, a radiodensity enhancement during NST$^+$ can only be caused by an increase in tissue blood flow if steady state is not reached during NST$^-$.

For modeling xenon uptake in BAT, we used the following parameters. For xenon concentration in arterial blood, we used a xenon concentration of 6 mM, as calculated from the measured average enhancement of 42 HU found in the heart of these animals. For the xenon brain/blood partition coefficient, we used the previously measured value of 1 (2). For BAT, we assumed a tissue/blood partition coefficient of 3.5 for BAT in lean animals and 7 for BAT in obese animals. These values are consistent with the lower fat fraction value of BAT in lean mice (fat fraction typically ranges from 20 to 40%) vs. obese animals (fat fraction typically ranges from 40 to 90%). These values are also similar to those reported for xenon in perirenal adipose tissue [which we know to contain BAT (3, 4)] with a fat fraction ranging from 29 to 90% (2). The blood flow values used here were those

measured in these phenotypes by using the radioactive microsphere technique (5). Specifically, at baseline, blood flow to BAT was set to 0.18 and 0.13 mL/g·min^{-1} in lean and obese mice, respectively. During stimulation of NST, these values were increased to 6.00 and 1.35 mL/g·min^{-1}, respectively, as reported (5). In the brain, blood flow was set to 0.50 mL/g·min^{-1} at baseline, and to 1.00 mL/g/min during stimulation of thermogenic activity (5).

Mouse Studies. All animal experiments were performed under an approved protocol and according to the guidelines from the Institutional Animal Care and Use Committee (IACUC) at the University of North Carolina at Chapel Hill, and conducted in compliance with the NIH's *Guide for the Care and Use of Laboratory Animals* (6). For these studies, 12 C57BL/6J and 12 B6.V-Lep^{ob} (ob/ob), between 10 and 12 wk of age, were acquired from Jackson Laboratories. The leptin-deficient ob/ob mouse model is the most commonly used animal model of obesity, characterized by BAT with a lower UCP1 (the mitochondrial protein responsible for BAT thermogenesis) content but greater lipid accumulation compared with the wild-type (WT) lean mouse model (7, 8). The extensive lipid accumulation in BAT of obese mice leads to BAT having a morphology similar to that of WAT, ultimately making this tissue undetectable by conventional tomographic imaging techniques.

For all imaging studies, fasted (6 h of fasting) animals were first anesthetized with pentobarbital at a dose of 70 mg/kg and kept under anesthesia for the entire duration of the experiment (40 min for the XECT and ~2 h for the combined XECT and ^{18}F-FDG-PET/CT imaging protocol) by continuous injection of pentobarbital at a 1/4 of the initial dose, every 40–50 min as needed. Anesthetized animals were mechanically ventilated with 30 vol % O$_2$ and 70 vol % N$_2$ at a rate of 60 breaths per minute with a tidal volume of ~0.20 mL. At 20 s before XECT imaging, to ensure that xenon concentration in arterial blood reached equilibrium before image acquisition, breathing gas was switched to a mixture of 70 vol % xenon and 30 vol % O$_2$ to achieve a tidal volume of 0.20 mL. Right after each image acquisition, the breathing gas was switched back to 30 vol % O$_2$ and 70 vol % N$_2$. During the CT examination, BAT thermogenesis was stimulated by a s.c. injection of norepinephrine bitartrate (Levophed; Hospira, Inc.) at a dose of 1 mg/kg. XECT images were then re-acquired 15 min after norepinephrine injection.

During the same imaging session, half of the mice (six lean and six ob/ob) underwent also an ^{18}F-FDG-PET study. For these studies, 10 min after the i.p. injection of norepinephrine (1 mg/kg) and before the last XECT study, animals received an i.v. injection of the ^{18}F-FDG radiotracer (at a dose of 7.4 MBq in 0.2 mL). At the end of the XECT study, the animal was transported to the GE eXplore Vista system, where PET images were acquired 1 h after radiotracer injection.

Histological and Histochemical Analyses of Enhanced Fat Tissue. Right after euthanasia, all tissue that displayed an enhancement of >30 HU between images acquired prestimulation and poststimulation of NST was carefully excised under a surgical microscope, fixed overnight in 4% paraformaldehyde at 4 °C, dehydrated, cleared, paraffin-embedded, and sectioned in 4-μm slices. After the sections were dewaxed and rehydrated in a series of graded alcohols, heat-induced epitope retrieval was performed in citrate buffer (pH 6.0) for 20 min. The blocking procedure was carried out by using Cyto-Q Background Buster (NB306; In-novex) for 30 min, followed by UCP1 primary antibody incubation for 1 h at room temperature (1:250; catalog no. ab10983; Abcam). Secondary antibody incubation

and detection was performed for 30 min with biotinylated goat anti-rabbit (1;200; catalog no. BA100; Vector) and Vectastain Elite ABC complex, respectively (Vector). The Research Immunohistology Lab at Duke University performed all tissue processing and procedures.

Microangiography of BAT. For arterial casting of BAT, we used a total of 11 C57BL/6J mice, between 8 and 12 wk of age. For these experiments, a silicone rubber injection compound (MICROFIL; Flow Tech, Inc.) mixture was prepared by vortexing 0.6 mL of MICROFIL MV-122 in 0.2 mL of diluent and curing agent (provided as a kit). Mice were divided into two groups: a control group (five mice) and an experimental group (six mice). Control mice were first euthanized with 250 mg/kg pentobarbital after receiving an i.p. injection of heparinized saline, 10 min before euthanasia. The experimental group received a s.c. injection of 1 mg/kg norepinephrine, in addition to an i.p. injection of heparinized saline, 10 min before euthanasia. Thoracotomy was then performed immediately after euthanasia, followed by cannulation of the aorta. Blood was flushed with a solution of sodium nitroprusside in PBS, to maximally dilate the vasculature. After perfusion, 2% paraformaldehyde in PBS was applied on exposed interscapular BAT and surrounding tissue. Animals in the experimental group were perfused with an additional 30 mL of norepinephrine in saline at a concentration of 2 μg/mL. Microfil was perfused by hand injection retrograde through the aorta until the capillaries of the superficial muscle tissue were filled on visualization under a surgical microscope.

After allowing 30 min at room temperature for Microfil to cure, the interscapular BAT and surrounding WAT depots were then dissected and placed in 2% paraformaldehyde overnight at 4 °C. Tissues were dehydrated with an increasing gradient of ethanol and water up to 100% ethanol, followed by optical clearing of the tissue in 100% methyl salicylate for 2 d at room temperature. The 3D imaging of the vasculature was obtained by using a Leica stereomicroscope.

NHP Studies. Two 10-y-old female rhesus macaques, weighing 7.5 kg (NHP-1) and 7.95 kg (NHP-2), were used for these studies. For imaging, the animals were first sedated with an injection of 80 mg/kg ketamine. Anesthesia was induced with propofol (2 mg/kg i.v. bolus), before the animal was orotracheally intubated. Anesthesia was maintained during the entire imaging session with propofol at a dose of 0.15 mg/kg·min^{-1} via calibrated syringe pump and titrated to maintain immobilization and spontaneous ventilation. XECT scans were acquired before and after the beginning of norepinephrine infusion, which was administered at a dose of 0.4 mg/kg·min^{-1} and which lasted until the end of the acquisition of PET images. Approximately 15 min after the beginning of norepinephrine infusion, a marked increase in mean blood pressure of 50%, and an increase in heart rate of ~20–30% was observed. Approximately 20 min from the beginning of norepinephrine infusion, ^{18}F-FDG was injected i.v. at a dose of 185 MBq, and XECT scans were performed. For the enhanced CT scans, the intratracheal tube was connected to closed-circuit xenon rebreathing system (Biodex Medical Systems, Inc.), containing 40 vol % xenon in oxygen. The xenon rebreathing system was connected, during inhalation, to an oxygen source to offset oxygen consumption. The XECT images were acquired toward the end of an 8-min xenon inhalation period.

CT and PET/CT Imaging Parameters in Mice. All mouse XECT imaging scans were performed by using a GE eXplore CT120 system. For the acquisition of XECT images, the tube peak voltage was set at 80 kVp and the current was set constant at 32 mA. Animals were scanned in a 75-mm (obese phenotype) or 30-mm (lean phenotype) wide specimen holder with a resolution of 100 μm per pixel. The number of views, which represent the number of projection data collected during a single 360° rotation around the object, was kept at 220. For each 3D image dataset, mean acquisition time was 4 min, and, according to the manufacturer, the estimated radiation exposure remained <40 mSv.

PET images were acquired on a GE eXplore Vista small animal PET/CT system, with a center spatial resolution of 1.2 mm and reconstructed by using a 2D ordered subset expectation maximization reconstruction algorithm.

CT and PET/CT Imaging Parameters in NHP. XECT and 18F-FDG-PET studies in NHP were performed on a hybrid human PET/CT system (Biograph mCT; Siemens Healthcare). XECT and nonenhanced CT images in NHP were acquired in spiral acquisition mode and reconstructed with a 0.977- × 0.977-mm in-plane resolution and 1-mm through-plane resolution. For the acquisition of these images, the following parameters were used: 100 kV; 96 mAs; single collimation width 0.6 mm; slice width 1 mm; feed 15.3 mm per rotation; pitch 0.8; scan rotation time 1 s; data collection diameter 50 cm; reconstruction diameter 50 cm; number of slices 85; exposure time 1 s; convolution kernel I26s; reconstruction resolution 512 × 512. CT images for attenuation correction of glucose uptake maps were acquired in spiral acquisition mode; 120 kV; 10 mAs; single collimation width 0.6 mm; slice width 5 mm; feed 17.2 mm per rotation; pitch 0.9; scan rotation time 0.5 s; data collection diameter 50 cm; reconstruction diameter 78 cm; number of slices 234; exposure time 0.5 s; and convolution kernel B19f; reconstruction resolution 512 × 512. The PET/CT images were acquired with a field-of-view covering from the crown of the head to the upper thighs by using four bed positions (12 min per bed position). Each PET scan was corrected for attenuation with the associated fused CT scan. Reconstructed PET and CT voxel sizes were as follows: PET, 4.07 mm × 4.07 mm in-plane and 3 mm through-plane; CT, 1.52 mm × 1.52 mm in-plane and 5 mm through-plane.

Image Analysis. In mice, quantification of radiodensity enhancement in different tissues was performed by using OsiriX. For these measurements, 20 regions of interest (ROIs) were carefully drawn on transaxial CT slices to encompass the central portion of each tissue analyzed, while carefully excluding visible blood vessels. The ROI analysis was conducted for the trachea, brain, liver, muscle, WAT, and BAT. Mean tissue enhancement across all animals was reported as mean ± SD. Xenon concentration ([Xe]) in millimolar in each tissue was calculated by using the following equation: $[Xe] = (HU_E - HU_i)/7.1$, where HU_E represents the change in tissue radiodensity during xenon inhalation and HU_i represents baseline tissue radiodensity.

In NHP, for quantification of BAT by XECT, enhanced and nonenhanced xenon CT images were first thresholded by using a −150- to 50-HU range, to identify fat depots within the supraclavicular region, while excluding muscle and blood vessels. From the two datasets, after excluding all visible blood vessels, a difference map was created, from which BAT was identified as fat tissue that underwent an enhancement >20 HU. Average tissue enhancement was then quantified in BAT, muscle, WAT, and in the central portion of the trachea by drawing, in all tissues, 20 ROIs large enough to minimize noise, while avoiding partial volume effects and excluding blood vessels. For BAT, ROIs were drawn within the segmented BAT, whereas for WAT, ROIs were drawn within the s.c. fat depot. Mean enhancement and SD of the mean enhancement were then calculated for the trachea, muscle, WAT, and BAT. Mean BAT enhancement was also computed over the entire BAT depot, segmented as described above.

In ^{18}F-FDG-PET/CT, BAT was identified as a tissue with an HU between −100 and −50 HU and with an SUV > 0.75, since SUVs in NHP-1 were well below 1.5 SUV.

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Fig. S1. Enhancement calibration. Mean HU values from the glass phantom study, plotted as a function of xenon concentration, are shown. The HU enhancement per millimolar xenon is 7.8 at 70 kVp, 7.1 at 80 kVp, and 6.7 at 100 kVp.