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Quantification of Human and Rodent Brown Adipose Tissue Function Using ^{99m}Tc-methoxyisobutylisonitrile SPECT/CT and ^{18}F-FDG PET/CT

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Abstract

For brown adipose tissue (BAT) to be effective at consuming calories, its blood flow must increase enough to provide sufficient fuel to sustain energy expenditure and also transfer the heat created to avoid thermal injury. Here we used a combination of human and rodent models to assess changes in BAT blood flow and glucose utilization.

Methods—^{99m}Tc-methoxyisobutylisonitrile (MIBI) SPECT (n=7) and SPECT/CT (n=74) scans done in adult humans for parathyroid imaging were reviewed for uptake in regions consistent with human BAT. Site-directed biopsies of subcutaneous and deep neck fat were obtained for electron microscopy and gene expression profiling. In mice, tissue perfusion was measured with ^{99m}Tc-MIBI (n=16) and glucose uptake with ^{18}F-FDG (n=16). Animals were fasted overnight, anesthetized with pentobarbital, and given intraperitoneally either the β3-adrenergic receptor agonist CL-316,243 (CL) 1mg/kg (n=8) or saline (n=8) followed by radiotracer injection 5 minutes later. After 120 minutes, mice were imaged using SPECT/CT or PET/CT. Vital signs were recorded over 30 minutes during the imaging. BAT, white adipose tissue (WAT), muscle, liver, and heart were resected, and tissue uptake of both ^{99m}Tc-MIBI and ^{18}F-FDG were

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quantified by percent injected dose (%ID) per gram of tissue and normalized to per kilogram of total body weight (%ID/g*kg).

Results—In 5.4% (4/74) patients, ^{99m}Tc-MIBI SPECT/CT showed increased retention in cervical and supraclavicular fat that displayed multilocular lipid droplets, dense capillary investment, and a high concentration of ovoid mitochondria. Expression levels of the tissue-specific uncoupling protein-1 (UCP1) were 180x higher in the BAT compared to the subcutaneous WAT ($P < 0.001$). In mice, BAT tissue perfusion increased by 61% ($P < 0.01$), with no significant changes in blood flow to WAT, muscle, heart, or liver. CL increased glucose uptake in BAT even more, by 440% ($P < 0.01$).

Conclusion—Pharmacologic activation of BAT requires increased blood flow to deliver glucose and oxygen for thermogenesis. However, the glucose consumption far exceeds the vascular response. These findings demonstrate that activated BAT increases glucose uptake beyond what might occur by increased blood flow alone and suggest that activated BAT likely uses glucose for non-thermogenic purposes.

Keywords

brown fat; blood flow; glucose uptake

INTRODUCTION

The contemporary pandemics of obesity and diabetes are devastating in size, breadth, and rate of growth (1). Central to these pathological processes is the adipose tissue depot. White adipose tissue (WAT) stores energy and in excess results in obesity and insulin resistance, while brown adipose tissue (BAT) consumes calories for the purpose of thermogenesis via tissue-specific UCP1 (2). BAT was initially known for its role in non-shivering and diet-induced thermogenesis in infants and small mammals (3), but it has been recently shown that adult humans have detectable BAT (4–10). When activated by mild cold exposure, human BAT consumes more glucose per gram than any other tissue (11), suggesting that BAT activity may be utilized to treat metabolic dysfunction.

Although there is great therapeutic potential in the use of human BAT energy expenditure, very little is known about the details underlying its physiological response to stimulation. Currently, BAT is routinely imaged via ^{18}F-FDG PET/CT, which has been useful in epidemiological (12) and functional (13–16) studies. This approach has its limitations since glucose retention may not reflect the actual energy expenditure of the tissue (17). PET studies using ^{15}O and ^{11}C have been helpful in demonstrating BAT perfusion (11) and aerobic respiration (18), respectively, but their short half-lives substantially reduce their utility. An attractive alternative is ^{99m}Tc-methoxyisobutylisonitrile (MIBI), a perfusion-mediated radiotracer that is readily accessible and can provide essential information about BAT blood flow in adult humans (19), (20).

BAT activity in both humans and rodent models is routinely achieved through cold exposure (6), (8),(21), which acts centrally to stimulate the sympathetic nervous system to release norepinephrine from postganglionic neurons and activate UCP1 uncoupling and

thermogenesis in BAT (22). Observational studies support the possibility that ^{99m}Tc-MIBI could localize to human BAT (23–26). However, it is not yet known how BAT tissue perfusion responds to pharmacological stimulation. In the current study, we provide the first direct physical evidence that ^{99m}Tc-MIBI localizes to adult human BAT *in vivo*. We then use a rodent model to evaluate uptake following stimulation with CL-316,243 (CL) (27), an agonist for the β3-adrnergic receptor, which is highly expressed in adipose tissue. Using ^{99m}Tc-MIBI and ^{18}F-FDG, we establish the relationship between the increase in tissue perfusion and glucose uptake after pharmacological stimulation of mouse BAT.

MATERIALS AND METHODS

Patients

This study followed institutional guidelines and was approved by the ethics committees of Beth Israel Deaconess Medical Center, Partners HealthCare, and Joslin Diabetes Center, all in Boston, MA. For the medical records review and discarded material analyses, the consent of patients was not required. The Harvard Medical School Shared Pathology Informatics Network Patients was used to identify patients at Massachusetts General Hospital who had undergone both ^{99m}Tc-MIBI SPECT to locate parathyroid adenomas and also had tissue processed for clinical indications during subsequent parathyroidectomies that were officially documented by staff pathologists as having brown adipocytes. For the ^{99m}Tc-MIBI SPECT/CT dataset at Beth Israel Deaconess Medical Center, consecutive studies over a 22-month period were assessed for the presence of activity in regions associated with brown fat. Informed consent was obtained from the patient from whom the prospective biopsy was obtained.

Human Parathyroid Imaging with ^{99m}Tc-MIBI

For planar imaging, patients were injected intravenously with 20 mCi (740 MBq) of ^{99m}Tc-MIBI. Immediately and then 1.5 hours after injection, 10-min anterior, 35° right anterior oblique, and 35 left anterior oblique planar images were acquired in a 128 × 128 matrix, with a 20% window centered around the 140-keV photopeak, using a low-energy, high-resolution parallel collimator in a Siemens e.cam nuclear gamma camera (Siemens Medical Systems).

For SPECT/CT, imaging was carried out as described (28)(Supplementary Methods). All patients were injected intravenously with 20 mCi (740 MBq) of ^{99m}Tc-MIBI, and planar dual-phase imaging was performed with standard parameters after 20 minutes and 2 hours using a Philips Medical Systems (Andover, MA) Precedence SPECT/CT. CT Images were used for attenuation correction and anatomic localization. Static views were done 20 minutes after tracer injection, then SPECT/CT imaging after the 20-minute static views, and finally 2-hour planar views.

Processing of Human Tissue

^{99m}Tc-MIBI SPECT/CT scans were done for the clinical purpose of identifying parathyroid adenomas. Patients whose scans indicated the presence of BAT were selected. The precise anatomical location of the adipose tissue with increased uptake of ^{99m}Tc-MIBI was

reviewed with the surgeon who then resected the fat tissue providing site-directed biopsies of human neck subcutaneous white fat and deeper brown fat. The fat was prepared for light and electron microscopy as described (29).

Human Gene Expression

These experiments were carried out as described (30). BAT and WAT tissue was collected and stored in liquid nitrogen at −80 °C. RNA was extracted from tissue using Qiazol and RNEasy kit (QIAGEN). cDNA was made using Applied Biosystems High Capacity cDNA kit, and quantitative real-time PCR was performed using cDNA with SybrGreen Master Mix (Fisher)(Supplementary Table 1). PCR was run using ABI Prism 7900 sequence detection system (Applied Biosystems). Expression levels of genes associated with BAT (*UCP1*, *DIO2*, *CideA*, *PGC1α*) and WAT (*leptin*) were normalized to 36B4.

Rodent Imaging Protocol

All animal studies were approved by the Institutional Animal Care and Usage Committees at Joslin Diabetes Center and Beth Israel Deaconess Medical Center. For the full details of the imaging protocols, please see the Supplementary Methods. In brief, a total of 32 129SVE mice (Taconic), 8–12 weeks old, 20–30g, was fasted overnight, then anesthetized with sodium pentobarbital (50–75mg/kg intraperitoneally, followed by 20mg/kg intraperitoneally every 30 minutes starting 1 hr after the initial dose), then given either the β3-adrenergic receptor agonist CL-316,243 (CL) 1mg/kg (n=16) or saline (n=16) followed 5–10 minutes later by either ^{18}F-FDG, 275 μCi (n=8 for each of the two treatments) or ^{99m}Tc-MIBI, 225 μCi (n=8 for each of the two treatments). Two hours later, mice were imaged using PET/CT or SPECT/CT, respectively. Imaging was performed on a NanoSPECT/CT (Bioscan, Washington, DC) and a NanoPET/CT (Mediso Medical Imaging Systems, Budapest, Hungary). Helical micro-SPECT was performed using a four-headed gamma camera outfitted with multi-pinhole collimators 1.0mm diameter pinholes for a single mouse scan. Reconstruction was performed using Nucline software (Mediso Medical Imaging Systems, Budapest, Hungary) in 2D mode. OSEM reconstruction algorithm was used with a SSRB 2DLOR rebinning method. Respiratory rate was measured using the Small Animal Monitoring and Gating System; Model 1025T (SA Instruments, Stonybrook, NY), and heart rate using 3M™ Red Dot™ Neonatal, Pre-Wired, Radiolucent Monitoring Electrode with clear tape (3M, St. Paul MN). Vital signs were recorded over 30 minutes during the imaging. At the end of the imaging session, the mice were sacrificed and interscapular BAT, posterior subcutaneous WAT, muscle, liver, and heart tissue were dissected and weighed. Tissue radioactivity for both ^{99m}Tc-MIBI and ^{18}F-FDG was measured using percent injected dose (%ID) per gram of tissue and normalized to per kilogram of total body weight (%ID/g × kg).

Statistical Analyses

Data were analyzed using JMP Pro 9.0.0 software (SAS Institute, Inc., Cary, NC). All *P* values presented are two tailed, and $P < 0.05$ was considered to indicate statistical significance. Comparison of the gene expression in different anatomical depots was done using the Student's *t*-test.

RESULTS

^{99m}Tc-MIBI Localizes to BAT in Adult Humans

To prove that ^{99m}Tc-MIBI was localizing to BAT, we first reviewed all SPECT scans done over a three year period in which biopsy tissue was available and showed the presence of brown adipocytes. Nine patients met these criteria. The delayed images showed increased tracer retention in a mantle-like appearance in the regions of the neck and upper thorax, areas known to be enriched with human BAT typically (Fig. 1A–B). Biopsy of the tissue revealed brown adipocytes with typical multilocular lipid droplets and polygonal cells (8), (31)(Fig. 1C). We next turned to SPECT/CT to provide a definitive correlation between adipose tissue identified as MIBI-avid and histological proof of brown adipocytes. Every ^{99m}Tc-MIBI SPECT/CT scan done for the purpose of localizing parathyroid adenomas over a 22-month interval was reviewed. Of the 74 patients (19M/49F, age 58 ±15y), four (5.4%) demonstrated substantially increased uptake in the distinct fascial plane within the cervical and supraclavicular regions where BAT is located (7) (Fig. 2A–B). From a representative patient, biopsies from the subcutaneous WAT and MIBI-avid deeper neck adipose tissue were obtained. The microscopic and ultrastructural appearances were consistent with WAT and BAT, respectively (Fig. 2C–D). WAT from the subcutaneous depots was yellow, with large unilocular adipocytes and little cytoplasm. In contrast, the MIBI-localized fat was brown and multilocular with a very high density of ovoid mitochondria.

Gene Expression Profile of ^{99m}Tc-MIBI-avid Adipose Tissue

Quantitative reverse transcriptase PCR (qRT-PCR) was used to examine the gene expression profile of the two different adipose tissue depots. Compared with subcutaneous WAT, MIBI-avid fat had 180x greater tissue-specific *UCP1* expression levels ($P < 0.001$) (Fig. 3). Additionally, genes found enriched in human BAT such as *DIO2*, *PGC1α*, *CideA* (8) were increased, while WAT-associated *leptin* (32), (33) had lower expression levels in the deeper fat.

Comparing Blood Flow and Glucose Uptake in Activated Rodent BAT

After locating -MIBI-avid adipose tissue is BAT in human tissue, we turned to the mouse model to define the physiological changes resulting from pharmacological stimulation of BAT. For each tracer, male 129SVE mice were administered either the β3-adrenergic receptor agonist CL (1mg/kg) (n=16) or saline control (n=16). ^{18}F-FDG PET/CT (n=8 for each of the two treatments) was used to reflect glucose uptake, and ^{99m}Tc-MIBI SPECT/CT (n=8 for each of the two treatments) was given to measure tissue perfusion. CL increased heart rate by 23% and respiratory rate by 85% ($P < 0.001$) (Supplementary Figure 2). ^{18}F-FDG PET/CT imaging done 2 hours post-stimulation revealed significantly increased uptake in the interscapular BAT compared to saline control (Fig. 5A–B). Uptake in the myocardium was also significantly elevated. To confirm the observed changes, we resected BAT, WAT, skeletal muscle, heart, and liver and quantified the percentage injected dose (%ID) per gram of tissue normalized by kilogram of body weight (%ID/g × kg [%ID/body weight]) (21). Even prior to stimulation, BAT retained more FDG than the other tissues and rose by 440% when stimulated by CL ($P < 0.01$) (Fig. 4C). Other tissues showing CL-induced increases in

FDG were WAT, skeletal muscle, and heart. When the analogous study was done with ^{99m}Tc-MIBI, uptake in BAT was comparatively less than in liver and heart (Fig. 5A–B). The retained tracer in the resected tissues confirmed these findings, as %ID/body weight in BAT increased by only 61% ($P < 0.01$), and there were no significant changes in WAT, skeletal muscle, liver, or heart (all $P > 0.05$) (Fig. 5C). Thus, specific pharmacological stimulation of BAT led to a much greater increase in glucose uptake than tissue perfusion.

DISCUSSION

From the time that it was identified as a functional tissue in adult humans (6–10), there has been great interest in utilizing BAT energy expenditure to treat obesity and metabolic dysregulation. When activated by mild cold exposure, adult human BAT has an energy expenditure of up to several hundred kcal/day (18). The broad reliance on ^{18}F-FDG PET/CT imaging has allowed the comparison of results among investigators, but the ability to describe other features of BAT physiology becomes more limited. Progress is being made in this area, with the introduction of new tracers such as ^{18}F-fluorobenzyl triphenyl phosphonium to measure thermogenesis (34) and the simultaneous use of combinations of ^{15}O-H_2O, ^{15}O-O_2, and ^{11}C-acetate along with ^{18}F-FDG to quantify blood flow, aerobic respiration, and glucose uptake (11), (17),(18). However, these and other radiotracers are either not yet approved for use in humans or are not readily available. ^{99m}Tc-MIBI meets these criteria and provides the opportunity to measure BAT mass and activity using SPECT/CT imaging. Prior studies have already indicated the possibility that ^{99m}Tc-MIBI could be used to detect BAT in adult humans (23–26). Here we have presented evidence strongly suggestive that ^{99m}Tc-MIBI localizes to human BAT and have demonstrated its utility in measuring BAT activity in response to pharmacological activation.

Both the rate of detection of unstimulated BAT (5.4%) and the anatomical location seen using ^{99m}Tc-MIBI SPECT/CT imaging match the findings reported with ^{18}F-FDG PET/CT (7),(35), indicating that both tracers are localizing to the same tissue. This pattern of tracer avidity is consistent with their molecular structures. The glucose analog FDG localizes to tissues with high metabolic activity. MIBI is a lipophilic cation that is retained in tissues with high perfusion rates, particularly those enriched with mitochondria, which have a large negative transmembrane potential (36). The ultrastructural and gene expression findings presented here demonstrate how human brown adipocytes can be particularly avid for both FDG and MIBI. These features also have direct clinical relevance since human neck BAT lies close to the parathyroid glands. Clinicians should be careful to ensure that increased ^{99m}Tc-MIBI uptake is from parathyroid tissue and not a false-positive signal from BAT.

As an organ designed to generate heat at a rate of up to 300W/kg (32), a high rate of BAT perfusion is necessary to provide oxygen and fuel and transfer away the heat before suffering thermal injury. When studied in response to cold exposure, BAT tissue perfusion increases, but less than glucose uptake (11). These two independent measures of metabolism correlate significantly, which justifies the wide use of FDG to measure relative changes in BAT activity. However, it remains unclear what the fate of the glucose is. The fact that glucose utilization exceeds that of blood flow indicates that activated BAT likely uses

glucose for several metabolic purposes. Internal lipid stores are the primary fuel for BAT and are used rapidly in response to cold exposure (32), (37). The glucose may be used for lipogenesis, glycolysis, anaplerosis, and uncoupled aerobic respiration (38). The simultaneous activity of these multiple pathways could explain the relatively higher rate of glucose utilization seen during BAT activation. Additional studies are required to determine how glucose is utilized in quiescent and activated BAT and how the measured uptake with FDG correlates with these different processes.

A limitation of this study is that we did not prospectively administer ^{99m}Tc-MIBI to humans to show changes in tracer uptake in response BAT stimulation. Rather, we relied on the site-directed biopsies to support the presence of human BAT and used the rodent studies to show the dynamic changes in BAT function. In addition, we did not simultaneously evaluate the changes in uptake of ^{99m}Tc-MIBI and ^{18}F-FDG. In the mice, to permit detection of both tracers, this approach would have required doing the studies sequentially and not being able to accurately measure each tracer's uptake on biopsy. However, the degree of BAT stimulation was likely to be similar with both tracers as evidenced by the non-significant difference between the heart and respiratory rates seen after treatment with CL and vehicle. Another issue is that uptake of ^{99m}Tc-MIBI is driven not only by perfusion but also the expression of p-glycoprotein (39), levels of which are unknown in BAT. Future studies should examine the expression of p-glycoprotein in rodent and human BAT. Furthermore, stimulation of BAT leads to mitochondrial uncoupling and a lowering of the transmembrane potential, which could reduce MIBI uptake. An alternative measure to quantify blood flow is contrast ultrasound, which has been used in rodent BAT (40) and could be correlated with MIBI uptake. Nevertheless, our findings are qualitatively similar to what has been reported with other markers of blood flow after cold-mediated activation of BAT in humans (11) and rodents (21).

CONCLUSION

We demonstrate that the accessible tracer ^{99m}Tc-MIBI may be used to detect BAT via SPECT/CT in humans and can provide information about changes in its blood flow. Understanding the physiological function and clinical utility of BAT energy expenditure will likely require multiple complementary tracers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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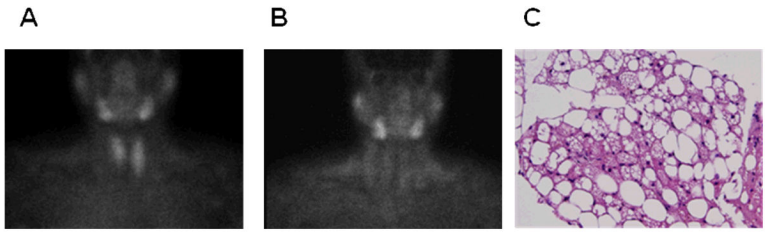

Figure 1.

A 54 year-old woman with clinical hyperparathyroidism. Coronal images of parathyroid adenomas using ^{99m}Tc-MIBI SPECT at (A) 20 minutes and (B) 2 hours showed increased uptake in neck and upper thorax, areas associated with human BAT. (C) The tissue was resected and studied using hematoxylin and eosin staining.

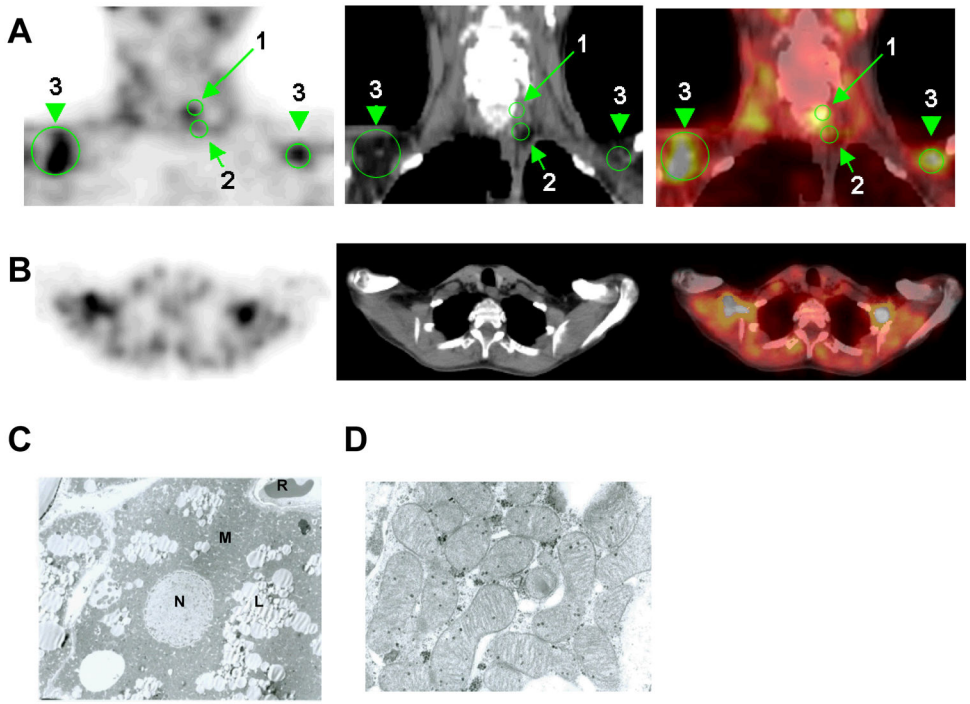

Figure 2.

57 year-old woman with clinical hyperparathyroidism. (A) Coronal and (B) transaxial slices of SPECT, CT, and fused SPECT/CT imaging of ^{99m}Tc-MIBI uptake. Scans performed showed focal abnormal uptake posterior to mid pole of the left thyroid lobe, compatible with the reported parathyroid adenoma. The green arrows indicate (1) the parathyroid adenoma; (2) site of deep tissue biopsy; and (3) the main depot of BAT in the supraclavicular space. Osmium tetroxide staining of the biopsied tissue shows individual (C) individual brown adipocyte; (D) focus on ovoid mitochondria with dense cristae. Labels are the following: lipid droplets (L), mitochondria (M), nucleus (N), and red blood corpuscle (R).

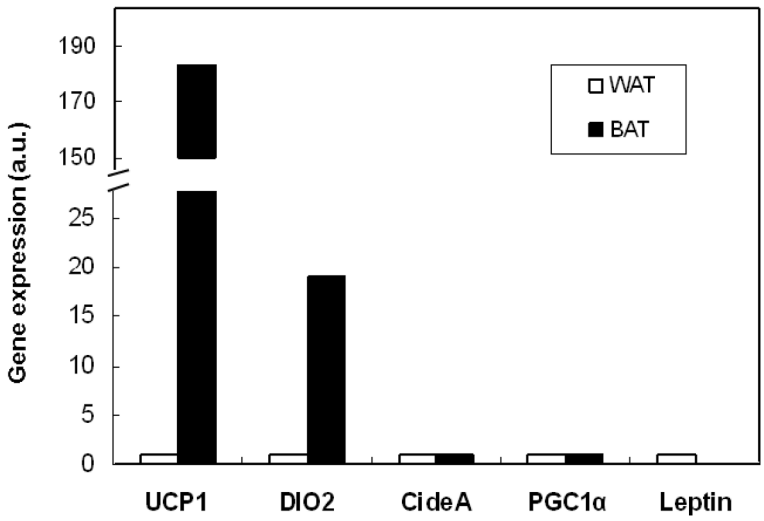

Figure 3.

Quantitative reverse transcriptase PCR (qRT-PCR) of subcutaneous WAT and site-directed biopsy of MIBI-avid tissue from a single volunteer. Letpin is a marker of WAT and UCP1 is a marker of BAT. Expression levels are in arbitrary units normalized to TBP.

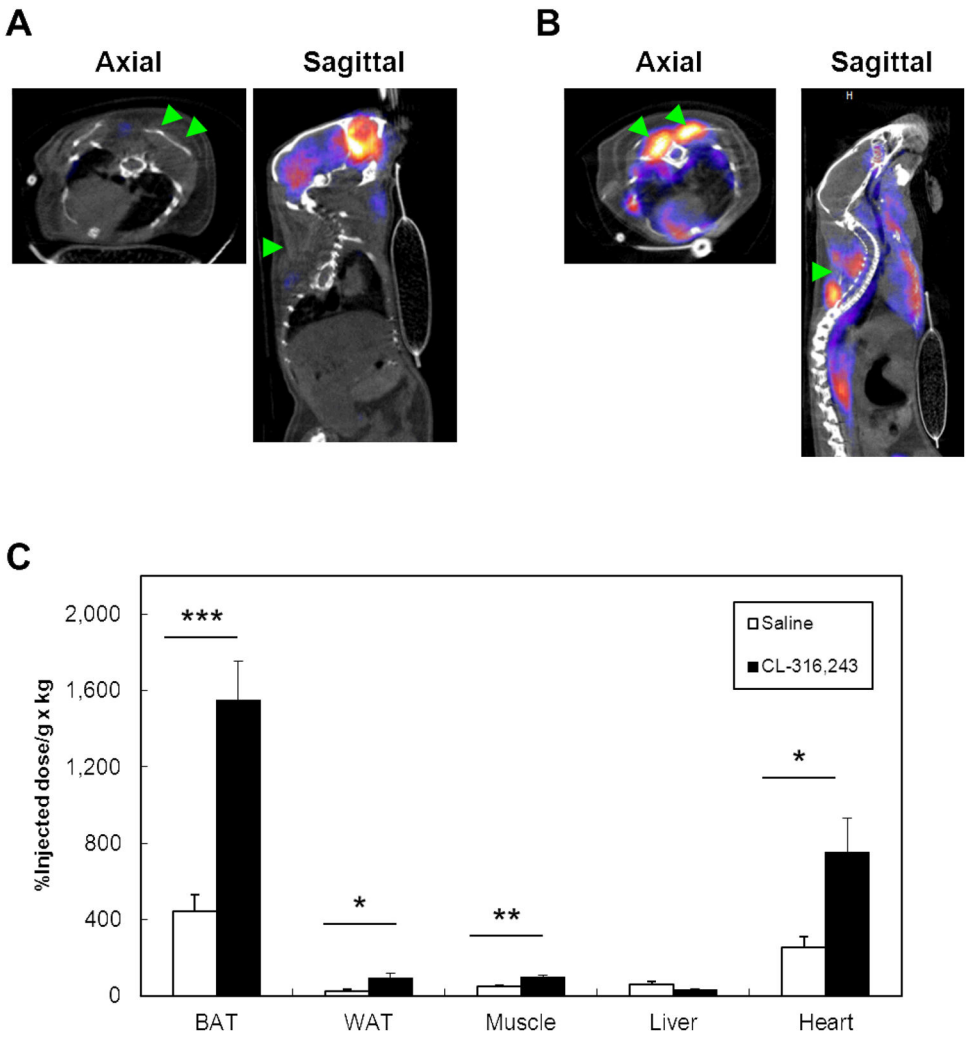

Figure 4.

PET-CT axial and sagittal images of 129SVE mice demonstrating uptake in interscapular BAT (*green arrows*) 120 minutes after injection of ^{18}F-FDG. Mice were given either (A) saline control or (B) CL-316,243 (1 mg/kg). After imaging, the tissues were resected, and activity was quantified (C) as the percent injected dose (%ID) per gram of tissue and normalized to per kilogram of total body weight (%ID/g × kg). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

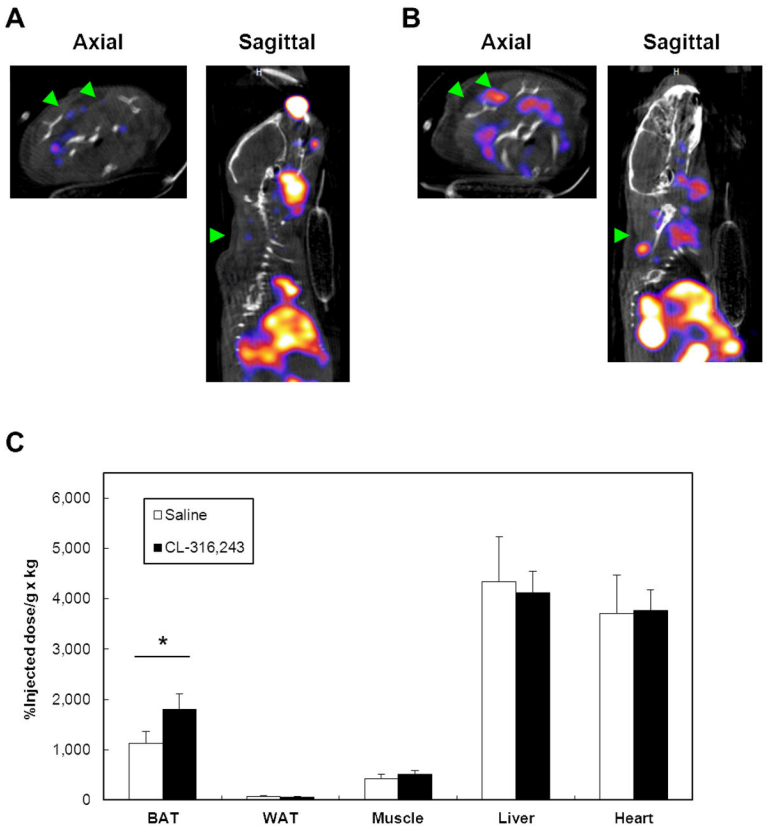

Figure 5.

SPECT-CT axial and sagittal images of 129SVE mice demonstrating uptake in interscapular BAT (*green arrows*) 120 minutes after injection of ^{99m}Tc-MIBI. Mice were given either (A) saline control or (B) CL-316,243 (1 mg/kg). After imaging, the tissues were resected, and activity was quantified (C) as the percent injected dose (%ID) per gram of tissue and normalized to per kilogram of total body weight (%ID/g × kg). *, $P < 0.05$.