Published in final edited form as:

Int J Obes (Lond). 2020 April ; 44(4): 964–967. doi:10.1038/s41366-019-0471-4.

Norepinephrine transporter availability in brown fat is reduced in obesity: a human PET study with [^{11}C] MRB

Elizabeth Sanchez-Rangel¹, Jean-Dominique Gallezot², Catherine W. Yeckel³, Wai Lam¹, Renata Belfort-DeAguiar¹, Ming-Kai Chen², Richard E. Carson², Robert Sherwin¹, Janice J. Hwang¹

¹Department of Internal Medicine/Section of Endocrinology, Yale University School of Medicine, New Haven, CT 06519, USA

²Department of Radiology and Biomedical Imaging, Yale PET Center, Yale University School of Medicine, New Haven, CT 06519, USA

³Department of Public Health, Yale University, New Haven, CT 06519, USA

Abstract

The energy-dissipating properties of brown adipose tissue (BAT) have been proposed as therapeutic targets for obesity and diabetes. Little is known about basal BAT activity. Capitalizing on the dense sympathetic innervation of BAT, we have previously shown that BAT can be detected in humans under resting room temperature (RT) conditions by using (S,S)- ^{11}C -O-methylreboxetine (MRB), a selective ligand for the norepinephrine transporter (NET). In this study, we determine whether MRB labeling of human BAT is altered by obesity. Fifteen healthy, nondiabetic Caucasian women (nine lean, age 25.6 ± 1.7 , BMI $21.8 \pm 1.3 \text{ kg/m}^2$; six obese age 30.8 ± 8.8 BMI $37.9 \pm 6.6 \text{ kg/m}^2$) underwent PET-CT imaging of the neck/supraclavicular region using ^{11}C -MRB under RT conditions. The distribution volume ratio (DVR) for ^{11}C -MRB was estimated via multilinear reference tissue model 2 (MRTM2) referenced to the occipital cortex. Two women (one lean and one with obesity) had no detectable BAT. Of the women with detectable BAT, women with obesity had lower ^{11}C -MRB DVR (0.80 ± 0.12 BAT DVR) compared to lean (1.15 ± 0.19 BAT DVR) ($p = 0.004$). Our findings are consistent with reports that NET is decreased in obesity and suggest that the sympathetic innervation of BAT is altered in obesity.

Introduction

Brown adipose tissue (BAT) has emerged as a potential target for modulating human energy homeostasis [1]. In addition to the thermogenic characteristics of BAT, activation of BAT has also been proposed to play a role in glucose control [2] making it a potential target for the treatment of metabolic diseases. Most studies have focused on activating BAT either with cold [3] or food [4]. Less attention has been paid to the metabolic activity of BAT in its basal

Janice J. Hwang, Janice.hwang@yale.edu.

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

state despite reports that BAT is tonically active and consumes oxygen on average 300% higher than subcutaneous white adipose tissue (WAT) under basal conditions [5].

We have previously shown that BAT can be reliably detected in humans under both room temperature (RT) and cold conditions with ^{11}C -MRB ((S,S)- ^{11}C -O-methylreboxetine) [6], a selective positron emission tomography (PET) ligand for the norepinephrine transporter (NET). BAT is largely regulated by the sympathetic nervous system (SNS), which is mediated principally by norepinephrine and relies on the recycling of norepinephrine by NET [7]. Sympathetic tone modulates BAT physiology, including acute activation, brown adipocyte differentiation, recruitment, apoptosis and efficacy of BAT for thermogenesis [8]. Basal SNS tone has been shown to be necessary to achieve maximal stimulation of BAT in rats [9]. It has also been observed that in individuals with obesity, there is a decreased NET availability in the thalamus and peripheral tissues [10]. As animal models and human data indicate the potential for impaired sympathetic system activity in the setting of obesity, in this study we sought to determine whether obesity alters basal, human BAT with use of ^{11}C -MRB.

Methods

Participants

Fifteen healthy, nondiabetic Caucasian women, nine lean, and six obese were recruited through public advertisements in the greater New Haven area (Table 1). Exclusion criteria included any medical problems including type 1 and type 2 diabetes mellitus; thyroid, cardiovascular, pulmonary disease; smoking and illicit drug use; use of any medications with the exception of hormonal contraception for women. Subjects refrained from any grapefruit products, excessive physical activity beyond their normal routine or alcohol use for 3 days prior to and after each scan. Five of the lean women were previously included as part of another study [6].

The protocol was approved by the Yale University Human Investigation and Radiation Safety Committees. All subjects provided informed, written consent before participation in the study. Each subject underwent a physical exam, electrocardiogram, blood testing, and a urine toxicology screen. Baseline anthropometric measurements were obtained including height, weight, and total body fat measured by bioelectrical impedance analysis (Tanita body composition analyzer, model TBF-300).

Study design

All subjects with obesity ($n = 6$) and a subset of lean subjects ($n = 4$ out of 9 subjects studied) underwent an oral glucose tolerance (OGTT) with 75 g of glucose following a 10-h fast. Insulin resistance was estimated using homeo-static model assessment of insulin resistance (HOMA-IR). Plasma glucose concentrations were determined by the glucose oxidase method (YSI Inc., Yellow Springs, OH). Insulin was measured by the radioimmunoassay (Millipore, St. Charles, MO). On a separate day, subjects arrived following a 10-h fast for PET/CT scanning using ^{11}C -MRB under RT conditions. Vitals

signs including tympanic body temperature (Braun PRO 4000, USA), blood pressure, and heart rate were obtained during scans.

PET-CT protocols

PET imaging was performed using a mCT PET/CT scanner (Siemens). ^{11}C -MRB was synthesized at the Yale PET center based on procedures described previously [7]. The specific activity at the end of synthesis was 668 ± 370 MBq/nmol and the radiochemical purity was 99.5%. A CT scan was performed for attenuation correction and to help delineate the BAT region of interest. Images were reconstructed with an ordered subset expectation maximization algorithm using point spread function correction and time-of-flight information. To delineate fat, WAT and BAT regions of interests (ROIs), CT images were first resliced to match the resolution and location of PET images, then a supraclavicular area of interest was first manually drawn on the CT images. Within that area, a fat ROI was segmented using the CT images and an intensity window from -200 to -50 HU. Within that fat ROI, WAT, and BAT ROIs were segmented using the ^{11}C -MRB PET images: standardized uptake value (SUV) >1.25 for BAT, SUV <1.25 for WAT. The brain ROI for the occipital cortex was taken from an MR template, which was co-registered with the ^{11}C -MRB scans using an early sum image (0–10 min post injection) and an affine (12 parameters) transform. For analysis of tracer uptake, mean SUV was reported given the relative homogeneity of the adipose tissue. Because the distribution volume ratio (DVR) is a more precise measurement of uptake for reversible tracers [11] such as ^{11}C -MRB, we quantified regional ^{11}C -MRB uptake as DVR, estimated via multilinear reference tissue model 2 (MRTM2) [12, 13] ($t^* = 30$ min) using the occipital cortex, a region with low NET density [12, 14], as the reference region. MRTM2 reduces the variability of MRTM [13] parameter estimates by fixing one of the three parameters, the parameter k_2' , which is only related to the reference region time-activity curve, to a common value for all target regions. In this study, the parameter k_2' , was fixed to 0.021 min^{-1} , based on previous brain studies.

Statistical analysis

Two-sided independent sample t -tests were used to assess differences between lean and obese participants for normally distributed variables. Data analysis was performed using SPSS software (version 24.0, IBM). P values of less than 0.05 were considered statistically significant.

Results

As shown in Table 1, compared to the lean individuals, individuals with obesity had significantly higher fat mass ($p = 0.005$) and lean mass ($p = 0.026$). There were no differences in fasting glucose (lean 81.2 ± 5.0 , obese 88.3 ± 5.9 , $p = 0.087$), response to OGTT between groups (glucose at time 120 min, lean 100.5 ± 22.7 , obese 114 ± 28.3 , $p = 0.425$). Plasma catecholamines were measured in six obese subjects and four of lean subjects. We found no difference between groups in serum epinephrine levels (lean 0.19 ± 0.08 , obese 0.15 ± 0.06 , $p = 0.970$) or norepinephrine levels (lean 0.23 ± 0.00 , obese 0.27 ± 0.02 , $p = 0.436$). Furthermore, there were no differences in baseline body temperature ($p =$

0.485), resting heart rate ($p = 0.162$), but individuals with obesity had higher measures of blood pressure (Table 1).

Lean individuals had significantly higher ^{11}C -MRB uptake (DVR) compared to obese individuals (lean 1.15 ± 0.19 vs obese 0.80 ± 0.12 BAT DVR, $p = 0.004$) (Fig. 1a, b). Of note, two participants (age 40 years, BMI 42.8 kg/m^2 and age 25 years, BMI 23 kg/m^2) had no BAT detected. There were no differences in physical exam or history between these two subjects and other subjects. In an exploratory analysis to see what metabolic variables may be associated with changes in BAT, we noted a negative correlation between ^{11}C -MRB DVR in BAT and HOMA-IR ($\rho = -0.881$, $P = 0.004$). There were no relationships between ^{11}C -MRB uptake and systolic blood pressure, diastolic blood pressure, body temperature or heart rate.

Discussion

In this study, our data demonstrate that ^{11}C -MRB labeling of BAT is diminished amongst obese women compared to lean women under basal, RT conditions. Our findings could be interpreted as either diminished NET density or decreased BAT quantity in obesity and further studies will be needed to distinguish between these interpretations. Our findings using ^{11}C -MRB, which labels the degree of sympathetic innervation of BAT, are consistent and complementary to prior studies using ^{18}F -FDG (fluorodeoxyglucose) PET/CT, which have shown a negative correlation between ^{18}F -FDG PET/CT BAT uptake and BMI [15]. Because most of these FDG studies were done retrospectively and not under cold exposure [16] or lacked a standardized protocol for human BAT activation [3], it has remained unclear whether obesity is associated with decreased activity of BAT or due to differences in cold sensing and/or activation threshold of BAT. Taken together with our findings, the data suggest that obesity is associated with diminished BAT even in the absence of cold-activation. Of note, two subjects did not have any BAT detected, which may reflect low NET availability or lack of BAT as shown in others reports in which between 4 and 6% of individuals do not have detectable BAT [3, 17]. Our findings are also consistent with a recent report amongst lean, young adults using a different tracer for NET, which found that the degree of sympathetic innervation of BAT predicts the degree of BAT activation [18]. Our proof of concept study does have some limitations including a small sample size as well as the fact that we only studied women. Additional studies will be needed to determine whether individuals of varying ethnicity, sex, or age will have different patterns of SNS innervation of BAT as assessed by ^{11}C -MRB imaging.

Acknowledgements

The authors gratefully acknowledge the participants of the study as well as the nurses and staff at the Yale University PET Center. Funding for this study was supported by Regeneron, Pharmaceuticals, Inc. The funding agency had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the paper. This work was supported by NIH grant 1S10RR029245. This publication was made possible by CTSA Grant Number UL1 TR000142 from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIH.

References

1. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. 2009;360:1509–17. [PubMed: 19357406]
2. Chondronikola M, Volpi E, Borsheim E, Porter C, Annamalai P, Enerback S, et al. Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes*. 2014;63:4089–99. [PubMed: 25056438]
3. van Marken Lichtenbelt WD, Vanhomerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med*. 2009;360:1500–8. [PubMed: 19357405]
4. Saito M, Yoneshiro T, Matsushita M. Activation and recruitment of brown adipose tissue by cold exposure and food ingredients in humans. *Best Pract Res Clin Endocrinol Metab*. 2016;30:537–47. [PubMed: 27697214]
5. Vijgen GH, Sparks LM, Bouvy ND, Schaart G, Hoeks J, van Marken Lichtenbelt WD, et al. Increased oxygen consumption in human adipose tissue from the “brown adipose tissue” region. *J Clin Endocrinol Metab*. 2013;98:E1230–4. [PubMed: 23783102]
6. Hwang JJ, Yeckel CW, Gallezot JD, Aguiar RB, Ersahin D, Gao H, et al. Imaging human brown adipose tissue under room temperature conditions with (11)C-MRB, a selective norepinephrine transporter PET ligand. *Metabolism*. 2015;64:747–55. [PubMed: 25798999]
7. Lin KS, Ding YS. Synthesis, enantiomeric resolution, and selective C-11 methylation of a highly selective radioligand for imaging the norepinephrine transporter with positron emission tomography. *Chirality*. 2004;16:475–81. [PubMed: 15236345]
8. Nechad M, Nedergaard J, Cannon B. Noradrenergic stimulation of mitochondriogenesis in brown adipocytes differentiating in culture. *Am J Physiol*. 1987;253:C889–94. [PubMed: 2827500]
9. Festuccia WT, Blanchard PG, Richard D, Deshaies Y. Basal adrenergic tone is required for maximal stimulation of rat brown adipose tissue UCP1 expression by chronic PPAR-gamma activation. *Am J Physiol Regul Integr Comp Physiol*. 2010;299: R159–67. [PubMed: 20393157]
10. Straznicki NE, Guo L, Corcoran SJ, Esler MD, Phillips SE, Sari CI, et al. Norepinephrine transporter expression is inversely associated with glycaemic indices: a pilot study in metabolically diverse persons with overweight and obesity. *Obes Sci Pract*. 2016;2:13–23. [PubMed: 27812376]
11. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007;27:1533–9. [PubMed: 17519979]
12. Hannestad J, Gallezot JD, Planeta-Wilson B, Lin SF, Williams WA, van Dyck CH, et al. Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo. *Biol Psychiatry*. 2010;68:854–60. [PubMed: 20691429]
13. Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab*. 2003;23:1096–112. [PubMed: 12973026]
14. Schou M, Halldin C, Pike VW, Mozley PD, Dobson D, Innis RB, et al. Post-mortem human brain autoradiography of the norepinephrine transporter using (S,S)-[18F]FMeNER-D2. *Eur Neuropsychopharmacol*. 2005;15:517–20. [PubMed: 16139169]
15. Persichetti A, Sciuto R, Rea S, Basciani S, Lubrano C, Mariani S, et al. Prevalence, mass, and glucose-uptake activity of (1)(8)FFDG-detected brown adipose tissue in humans living in a temperate zone of Italy. *PLoS ONE*. 2013;8:e63391. [PubMed: 23667608]
16. Ouellet V, Routhier-Labadie A, Bellemare W, Lakhel-Chaieb L, Turcotte E, Carpentier AC, et al. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab*. 2011;96:192–9. [PubMed: 20943785]
17. Lee P, Swarbrick MM, Ho KK. Brown adipose tissue in adult humans: a metabolic renaissance. *Endocr Rev*. 2013;34:413–38. [PubMed: 23550082]

18. Muzik O, Mangner TJ, Leonard WR, Kumar A, Granneman JG. Sympathetic innervation of cold-activated brown and white fat in lean young adults. *J Nucl Med*. 2017;58:799–806. [PubMed: 27789721]

Fig. 1.

a BAT NET labeling visualized using $^{11}\text{C-MRB}$ under room temperature (RT) in one representative female lean participant and a female participant with obesity. Left panel, CT image to define regions of interest; middle panel, PET image; right panel, co-registered PET/CT image; **b** Individual values of $^{11}\text{C-MRB DVR}$ in Obese and Lean participants under RT

Table 1Participant characteristics. Data presented as mean $\pm$ SD

Characteristics of study participants			
Column 1	Obese (<i>n</i> = 6)	Lean (<i>n</i> = 9)	<i>P</i>
Age (years)	30.8 $\pm$ 8.8	25.6 $\pm$ 1.7	0.216
BMI (kg/m ²)	37.9 $\pm$ 6.6	21.8 $\pm$ 1.3	0.002
Fat mass (kg)	48.2 $\pm$ 17.1	15.3 $\pm$ 3.1	0.005
Body fat (%)	44.8 $\pm$ 4.0	25.5 $\pm$ 3.6	<0.001
Lean mass (kg)	50.9 $\pm$ 6.5	44.1 $\pm$ 3.9	0.026
Temp (°F)	98.5 $\pm$ 0.3	98.3 $\pm$ 0.6	0.485
Resting HR (bpm)	85.1 $\pm$ 10.6	75.2 $\pm$ 13.8	0.162
Systolic BP (mm Hg)	126.5 $\pm$ 12.6	101.4 $\pm$ 7.4	<0.001
Diastolic BP (mm Hg)	82.5 $\pm$ 9.9	66.0 $\pm$ 9.3	0.006
Mean arterial pressure	97.1 $\pm$ 9.3	77.6 $\pm$ 8.6	0.001