According to the statistical data from the National Health and Nutrition Examination Survey, 2009-2010,1,2 more than 2 in 3 adults are overweight or obese, and more than 1 in 3 adults are obese. As a result, the obesity-associated comorbidities, such as type 2 diabetes mellitus, cardiovascular disease (including coronary heart disease, stroke, and heart failure), Alzheimer disease, and cancer, as well as many other adverse health conditions, can be anticipated to increase dramatically. The cost of obesity to society is tremendous, due to obesity-associated illnesses and losses in productivity at work.3 It is imperative to develop strategies that will affect energy balance, particularly during periods of weight loss or weight loss maintenance. The recently confirmed existence of brown adipose tissue (BAT) in biopsy samples from adult humans4,5 has renewed interest in this tissue, now thought possible to be involved in conditions where energy balance is disrupted. For example, in human obesity and insulin resistance, adaptive thermogenesis is often impaired.6-9 BAT is one of the primary tissues responsible for adaptive nonshivering thermogenesis in mammals.10 Obese animal models demonstrate impaired sympathetic nervous system (SNS) activation and postsynaptic processing in BAT.11,12 However, the relative importance of BAT...
activity for whole-body energy expenditure in an adult human is still unknown.4,13 Before we can investigate the function/dysfunction of BAT in human energy balance, we need new methodological approaches that can target specific attributes of BAT in humans.

**FDG-PET for BAT detection**

BAT in adult humans was serendipitously revealed by fluorodeoxyglucose (FDG)-positron emission tomography (PET) with the radiolabeled tracer \[18F\]FDG.5,14-16 However, FDG uptake is not specific to BAT, and glucose is not the primary substrate used for BAT heat production. Nevertheless, to detect BAT, \[18F\]FDG has an advantage over other radiolabeled compounds in that \[18F\]FDG-labeling of BAT is markedly increased under mild cold conditions. However, good resolution is unachievable at room temperature.17 Indeed, in human studies, imaging performed at room temperature showed no appreciable detection of BAT, whereas imaging in those same individuals after mild cold exposure in a climate chamber did detect BAT.18,19 Therefore, the recent FDG-PET–based observations of BAT in humans may bias our basic understanding of BAT as a function of sex, obesity, and aging: individual differences in BAT activation due to different thermal responses probably complicate comparisons between obese subjects and those of normal weight. It is therefore important to develop new and more specific methods to address both the plasticity of BAT activation and the basal characteristics that do not require stimulation. Such methods could also be combined with those that highlight BAT-stimulated activity.

**NET-PET for BAT detection**

Lessons learned from steps taken to reduce undesirable \[18F\]FDG-labeling during clinical scans demonstrate that repeat scans after \(\beta\)-adrenergic blockade eliminate the interfering BAT signal.20 These data suggest that the SNS strongly activates BAT in humans and that the noradrenergic system may be involved in regulating BAT activity. This is not surprising; nor-epinephrine (NE) is the principle neurotransmitter in the SNS, and the interaction of NE, insulin, and BAT has been previously shown. Studies in animal models point to disruption of NE sensitivity in BAT activation related at least in part to insulin resistance.12,21 Direct effects of insulin on peripheral SNS have been examined in the mesentery and show that insulin may diminish NE overflow by increasing reuptake by the NE transporter (NET),22 thus lowering the NE concentration available for postsynaptic activation. Recently, insulin has been reported to regulate NET function both centrally and peripherally, and acute insulin treatment has been associated with a significantly decreased surface expression of NET.23

In the central nervous system, data also support noradrenergic dysfunction in obesity (eg, inhibition of either \(\alpha_1\)- or \(\beta_2\)-noradrenergic receptors in the ventromedial/paraventricular hypothalamus reduces endogenous levels of NE), which decreases food intake, whereas activation of \(\alpha_2\)-noradrenergic receptors of the lateral hypothalamus increases levels of NE and increases food intake.24-26 In humans, intake of high-caloric diets increases noradrenergic turnover in peripheral tissues, raising resting plasma NE levels, which may underlie a higher excretion of NE and higher rates of hypertension in obese people than in lean.27,28 These findings suggest an important role for the NE recycling system, the NET, in eating behavior, obesity, and obesity-related health conditions.28

Here, we discuss our investigations, both preclinical and clinical, into whether BAT can be visualized with NET-PET imaging and whether NET binding can be measured, by using the highly selective NET ligand (S,S)-[\(11C\)]O-methylreboxetine ([\(11C\)]MRB), under both basal and activated conditions in humans. For comparison, the gold standard method, \[18F\]FDG-BAT imaging, was also used. The radiotracer [\(11C\)]MRB has previously been used centrally for brain imaging studies in humans and nonhuman primates,29-32 as well as peripherally for NET imaging studies in nonhuman primates.33 The use of the [\(11C\)]MRB ligand offers specific advantages, as studies have shown that NET function is regulated both centrally and peripherally by insulin.23 Unlike other potential NET ligands, such
as \(^{18}\text{F}\)-fluorodopamine\(^{34,35}\) and metaiodobenzylguanidine (MIBG; a single-photon emission computed tomography [SPECT] ligand).\(^{15}\) \[^{11}\text{C}\]MRB can cross the blood-brain barrier, allowing for simultaneous central and peripheral imaging. Thus, \[^{11}\text{C}\]MRB may be useful to further elucidate mechanisms of BAT action and the role of NET in energy balance in obesity and insulin resistance by simultaneously correlating the functions of the central nervous system and the peripheral SNS.

**Preclinical NET-BAT imaging study in rodents**

We first conducted BAT imaging studies, both ex vivo and in vivo, in rats to determine \[^{11}\text{C}\]MRB uptake in BAT in the basal state (at room temperature); imaging of basal state BAT is unreliable with \[^{18}\text{F}\]FDG.\(^{36}\) In an ex vivo study, we administered intravenous \[^{18}\text{F}\]FDG or \[^{11}\text{C}\]MRB to awake male Sprague-Dawley rats after they were exposed to cold (4 °C for 4h, \(n=9\)) or room temperature conditions (\(n=9\)) and then sacrificed the rats at 20, 40, and 60 minutes after injection. Analysis of BAT samples showed that uptake of \[^{11}\text{C}\]MRB (% injected dose) was 3 times higher than that of \[^{18}\text{F}\]FDG at room temperature (\(P=0.0088\)). \[^{18}\text{F}\]FDG uptake after cold exposure was 10 times higher than in the room temperature control (1.6±0.3% injected dose per gram of tissue [ID/g] [cold] vs 0.2±0.05% ID/g [room temperature]; \(P=0.0009\)), whereas no significant thermal effect was observed with \[^{11}\text{C}\]MRB uptake (0.87±0.18% ID/g [cold] vs 0.63±0.09% ID/g [room temperature]; \(P=0.082\)). In addition to uptake in the brain and heart, BAT exhibited specific \[^{11}\text{C}\]MRB uptake that was significantly reduced to near baseline levels (\(P=0.0013\)) by pretreatment with unlabeled MRB or nisoxetine (a selective NET inhibitor). These ex vivo results were concordant with the in vivo PET imaging of anesthetized rats, which clearly demonstrated intense \[^{11}\text{C}\]MRB uptake in the interscapular BAT both at room temperature and after cold exposure; in contrast, \[^{18}\text{F}\]FDG uptake in BAT was detected only after cold exposure. Furthermore, \[^{11}\text{C}\]MRB uptake in BAT was completely abolished by pretreatment with unlabeled MRB, demonstrating the specific and saturable binding of the ligand to BAT (Figure 1).\(^{36}\) High-performance liquid chromatography analysis revealed that 94%-99% of total radioactivity in BAT represented unchanged \[^{11}\text{C}\]MRB (ie, the parent tracer and not a metabolite), further support-
(SUV) greater than 1.5 and Hounsfield units ranging from -200 to -50, as used by others. We measured uptake of $[^{11}C]$MRB as the distribution volume ratio (DVR), using the occipital cortex as the reference region; the tracer binding was well-characterized in our previous brain studies in humans and uptake of $[^{11}C]$MRB metabolites is minimal in rodent BAT. We used muscle as a peripheral reference and calculated the ratio of BAT-DVR to muscle-DVR (BAT/muscle). Total body fat and lean body mass were assessed via bioelectrical impedance analysis.

Consistent with previous studies, we found that $[^{18}F]$FDG uptake in BAT was difficult to detect at room temperature, but easy to detect after cold stimulation ($P=0.01$). In contrast, BAT $[^{11}C]$MRB uptake (also normalized for muscle) was equally evident both under room temperature and cold conditions (BAT-DVR: $1.0\pm0.3$ [room temperature] vs $1.1\pm0.3$ [cold], $P=0.31$; BAT/muscle-DVR: $2.3\pm0.7$ [room temperature] vs $2.5\pm0.5$ [cold], $P=0.61$) (Figure 2). Importantly, core body temperature correlated positively with percent body fat ($r=-0.74$, $P=0.04$) and positively with lean mass ($r=0.71$, $P=0.05$), which showed a gender difference, most likely reflecting the difference in body composition and body temperature between men and women. There were no relationships between change in $[^{18}F]$FDG uptake in response to cold and body composition, BMI, body temperature, heart rate, or blood pressure.

**Conclusion**

Our studies, preclinical in rodents and clinical in humans, provide the first evidence that $[^{11}C]$MRB PET imaging can be used to visualize BAT by targeting the NET element of the SNS innervation of such tissue. Furthermore, this imaging modality can be used under both cold-stimulated and basal conditions. Thus, $[^{11}C]$MRB PET imaging offers an alternative and complimentary approach to standard $[^{18}F]$FDG imaging, which—although well validated for assessing BAT after cold exposure—is unreliable when assessing basal BAT. Our proof-of-concept study does have some limitations, including a small sample size and a relatively homogeneous subject population, which was young, relatively lean, and white. Additional studies will be needed to determine whether individuals with differences in body mass, ethnicity, gender, or age will have different patterns of SNS innervation of BAT as assessed by $[^{11}C]$MRB imaging.

BAT is the principal thermogenic tissue responsible for adaptive thermogenesis, including cold-induced (CIT) and diet-induced thermogenesis (DIT). By far, most of the imaging studies have focused on CIT. The results from the limited number of DIT studies using $[^{18}F]$FDG have shown the difficulties in determining the contribution of BAT to DIT, relative to other tissues, such as skeletal muscle. Whether $[^{11}C]$MRB imaging would provide a better outcome in determining the contribution of BAT to DIT remains to be seen.
The idea of using the thermogenic properties of BAT to combat diabetes and obesity is drawing increasing amounts of interest in clinical circles. However, understanding the mechanisms behind BAT’s role in these metabolic diseases is more complex than simply determining what factors activate existing BAT. We must also discover the factors that regulate not only the amount of BAT in the body, but also the effectiveness of BAT with regard to thermogenesis. Understanding the thermogenic potential of human BAT requires that we assess BAT both after acute stimulation and in its basal state (without cold stimulation). Here, we show that [11C]MRB can be used for this purpose, as its uptake can be visualized under both these conditions. Furthermore, because it directly assesses the degree of tonic sympathetic activity in BAT, imaging with [11C]MRB offers unique advantages for further investigation into other factors, besides cold stimulation, that may be involved in regulating human BAT.

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