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Review

A synopsis of brown adipose tissue imaging modalities for clinical research

L. Sun [a,1], J. Yan [b,c,1], L. Sun [d], S.S. Velan [e,f], M.K.S. Leow [a,f,g,h,i,j,k,*]

[a] *Clinical Nutrition Research Centre (CNRC), Singapore Institute for Clinical Sciences (SICS), Agency for Science, Technology and Research (A*STAR) and National University Health System, Singapore*
[b] *Department of Nuclear Medicine, First Hospital of Shanxi Medical University, China*
[c] *Molecular Imaging Precision Medicine Collaborative Innovation Center, Shanxi Medical University, China*
[d] *Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, Singapore*
[e] *Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, A*STAR, Singapore*
[f] *Department of Medicine and Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[g] *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*
[h] *Office of Clinical Sciences, Duke-NUS Medical School, Singapore*
[i] *Clinical Trials and Research Unit, Changi General Hospital, Singapore*
[j] *Department of Medicine, National University Hospital, Singapore*
[k] *Department of Endocrinology, Tan Tock Seng Hospital, Singapore, Singapore*

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ABSTRACT

Body weight gain results from a chronic excess of energy intake over energy expenditure. Accentuating endogenous energy expenditure has been accorded considerable attention ever since the presence of brown adipose tissue (BAT) in adult humans was recognized, given that BAT is known to increase energy expenditure *via* thermogenesis. Besides classic BAT, significant strides in our understanding of inducible brown adipocytes have been made regarding its development and function. While it is ideal to study BAT histologically, its relatively inaccessible anatomical locations and the inherent risks associated with biopsy preclude invasive techniques to evaluate BAT on a routine basis. Thus, there has been a surge in interest to employ non-invasive methods to examine BAT. The gold standard of non-invasive detection of BAT activation is ^{18}F-fluorodeoxyglucose positron emission tomography (PET) with computed tomography (CT). However, a major limitation of PET/CT as a tool for human BAT studies is the clinically significant doses of ionizing radiation. More recently, several other imaging methods, including single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), infrared thermography (IRT)/thermal imaging and contrast ultrasonography (US) have been developed in hopes that they would allow non-invasive, quantitative measures of BAT mass and activity with lower costs. This review focuses on such methods to detect human BAT activation and white adipose tissue (WAT) browning to prompt the establishment of BAT-centric strategies for augmenting energy expenditure and combatting obesity. Clinical validation of these methods will most likely expand the scope and flexibility of future BAT studies.

Introduction

Obesity is a worldwide epidemic associated with debilitating metabolic and cardiovascular sequelae, including diabetes, hypertension and dyslipidemia. The fundamental basis of the obesity crisis is a surplus of energy intake over energy expenditure. Excess calories are stored preferentially as triglycerides in white adipose tissue (WAT), a conserved evolutionary adaptation for maximum efficient energy reserves stored in fat tissue. Thus, WAT is an energy-storing tissue, whereas brown adipose tissue (BAT) dissipates energy in the form of heat. Indeed, the thermoregulatory function of BAT in small and hibernating mammals, including human neonates and infants, has been known for decades [1,2]. Beyond the survival advantage conferred by this adaptation, overwhelming evidence that BAT activation can improve whole-body metabolism [3–5] has brought about a resurgence of research

* Corresponding author at: Centre for Translational Medicine, 14 Medical Drive #07-02, MD 6 Building, Yong Loo Lin School of Medicine, Singapore 117599, Singapore.
E-mail address: melvin_leow@sics.a-star.edu.sg (M.K.S. Leow).
[1] These authors contributed equally to this article as co-first authors.

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interest in BAT, especially since its presence was demonstrated in human adults by fluorine-18 fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) with computed tomography (CT) in 2002 [6]. Recent evidence has also described the presence of brown adipocyte-like cells within WAT harbouring a similar phenotype to BAT called 'beige' or 'brite' (brown in white) adipocytes as distinct from 'classic constitutive BAT' [7]. As both classic brown and beige/brite adipocytes are thermogenic and expend stored energy, they add to the growing arsenal against obesity and diabetes [8]. There are many excellent reviews of BAT biology and its catabolic processes [9–11], whereas the present review focuses on the methodology for imaging BAT activation and WAT browning, with an emphasis on human applications, and highlights the advantages and drawbacks of each method.

BAT characteristics and WAT browning

In infants, classic brown adipocytes reside in depots anatomically localized to the interscapular, supraclavicular, pericardial, suprarenal and para-aortic regions. Beige/brite adipocytes in human adults can be found in fat tissue in the neck, supraclavicular areas, mediastinum (para-aortic), paravertebral and suprarenal regions. Supraclavicular and cervical BAT constitutes the two most abundant and readily inducible depots in most people [12]. At those sites, BAT is predominantly composed of inducible beige/brite adipocytes [9]. BAT prevalence and activity have been demonstrated to correlate negatively with age, body mass index (BMI), diabetes status and external temperatures [13,14]. While BAT is thought to be present in most, if not all, adults, its activation capacity in any given subject remains questionable [15]. Cold- and diet-induced thermogenesis are both mediated by BAT. Activated BAT preferentially oxidizes lipids for fuel, although it also uses glucose as a metabolic substrate. BAT might therefore be exploited therapeutically for its anti-obesity lipid- and glucose-lowering effects [3,16].

BAT biopsy is the definitive method for identifying its histological features and distinguishing it from WAT. Morphologically, brown adipocytes are characterized by their numerous small lipid droplets, polygonal shape, smaller size and iron-containing mitochondria in significantly higher numbers, which are responsible for their brownish, rather than white, appearance [17]. BAT also has a remarkably greater density of sympathetic innervation compared with WAT [14].

The 32-kDa uncoupling protein 1 (UCP1)—a long-chain fatty-acid-activated protein known as 'thermogenin'—is the attribute *sine qua non* that characterizes the heat-dissipating mitochondria of BAT. UCP1 sits in the inner mitochondrial membrane and behaves like a protonophore by facilitating proton leaks from the intermembrane space back into the mitochondrial matrix, which physiologically uncouples the respiratory chain and, instead of ATP biosynthesis, releases energy as heat in a process known as 'adaptive thermogenesis' [18]. UCP1 thus increases fuel oxidation independent of intracellular levels of ATP and generates remarkable thermal power—in the order of 300 W/kg in rodent experiments [19]. If extrapolated to humans, approximately 50 g of BAT can account for up to 20% of total energy expenditure [20], while nearly 4 kg of weight loss over the course of a year has been estimated to be due to roughly 60 g of BAT in human PET studies [21].

Under the appropriate stimuli, WAT can transform into inducible BAT (beige/brite fat). Notably, this WAT 'browning' may arise through either differentiation of adipocyte stem cells within WAT towards a beige phenotype [22] or the more controversial process of transdifferentiation of white into brown adipocytes [23]. WAT browning is often observed in mouse models, but not in humans. Recently, however, Sidossis et al. [24] reported that human subcutaneous white adipose tissue (sWAT) can transform from energy-storing to energy-dissipating tissue after severe adrenergic stress, which demonstrates that human sWAT exhibits the plasticity to undergo browning. While the molecular pathways governing this browning process are still being unravelled, intracellular signals have been established: peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha (PGC-1α) is the key regulator of mitochondrial biogenesis and oxidative metabolism [25]. In humans, PGC-1α mRNA expression is highly expressed in BAT compared with WAT [26], which correlates with BAT higher energy production rates [27]. PGC-1α is not only enriched in classic BAT depots, but its expression can also be induced in beige/brite adipocytes by exposure to cold [28]. By inducing the expression of UCP1, PGC-1α is the master regulator of brown adipogenesis.

The PR domain-containing protein 16 (PRDM16) has been found to specifically promote classic brown fat rather than WAT, and plays an important role in promoting browning of visceral fat under β-adrenergic stimulation [29]. Ectopic expression of PRDM16 in WAT induced specific BAT gene expression, including UCP1 and PGC-1α, complemented by an increase in mitochondrial volume and oxygen consumption [29]. PRDM16 abundance is significantly higher in supraclavicular BAT than in WAT in humans [26].

Theoretically, WAT browning could be harnessed as a strategy for obesity treatment if the stimulus to recruit and activate beige adipocytes from white adipocytes is safe and efficacious. There are several such stimuli to increase BAT activity, including exposure to cold, and β-adrenergic receptor agonists and other pharmacological agents, which may be used in conjunction with PET imaging studies. The PPAR-γ activator thiazolidinedione can recruit BAT depots and facilitate WAT browning [30,31]. In recent years, many novel non-adrenergic soluble molecules have been identified as capable of inducing WAT browning [32]. These browning-inducing molecules are summarized in Fig. 1. Cold exposure is the best and most widely used stimulus of BAT activity. Subjects exposed to temperatures of 16–19 °C for 1–2 h have higher positive BAT detection rates [33,34]. Indeed, a given individual can switch from BAT-negative to -positive and *vice versa*, depending on the conditions. Numerous studies have also confirmed the positive association between cold-activated BAT detected by PET/CT and energy expenditure [35–40]. Women exposed to ambient temperatures of 19 °C for 12 h showed a 5% increase in energy expenditure compared with 24 °C exposures [41]. An estimated 250 kcal/day increase in energy expenditure was also observed in healthy young men exposed to 19 °C for 2 h [42], while 6 weeks (17 °C for 2 h/day) of chronic cold exposure increased ^{18}F-FDG uptake and energy expenditure in subjects with absent or low ^{18}F-FDG on baseline PET/CT scans [42]. This shows that BAT in adults can be either activated or recruited to tackle obesity.

PET/CT imaging

Nowadays, PET/CT is an important cancer imaging tool for staging, restaging, treatment-monitoring and prognostication [43], surpassing either PET or CT alone and minimizing their individual limitations. Fluorodeoxyglucose (FDG) is a glucose analogue labelled with radioisotope fluorine-18 (^{18}F). Like glucose, ^{18}F-FDG can enter cells, mediated by structurally related glucose transport proteins (GLUTs), and then be phosphorylated by hexokinase as the first step towards glycolysis. Unlike glucose, the 6-phosphate derivative of FDG cannot be further metabolized downstream for energy production and, thus, remains trapped within metabolically active cells [44]. Classic BAT expresses high GLUT 1 and 4 and are therefore FDG-positive [45]. Many studies have suggested the use of ^{18}F-FDG PET imaging as a reference for

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