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Brown Fat in Humans: Consensus Points and Experimental Guidelines

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Summary

As part of a current worldwide effort to understand the physiology of human BAT (hBAT) and whether its thermogenic activity can be manipulated to treat obesity, a workshop “Exploring the Roles of Brown Fat in Humans” was convened at the National Institutes of Health on February 25–26, 2014. Presentations and discussion indicated that hBAT and its physiological roles are highly complex and research is needed to understand the health impact of hBAT beyond thermogenesis and body weight regulation, and to define its interactions with core physiological processes like glucose homeostasis, cachexia, physical activity, bone structure, sleep and circadian rhythms.

Introduction

More than sixty years of studies in rodent models have shown that the presence and activation of brown adipose tissue (BAT) via cold stimulation or β3-adrenergic receptor (AR) treatment provides significant health benefits for experimental animals (Harms and Seale, 2013). Although observed many decades ago in cadaver tissues from winter outdoor workers (Huttunen et al., 1981), it is only more recently that BAT has been consistently detected in living adult humans (Nedergaard et al., 2007). This has led to a concerted effort worldwide to understand the physiology of human BAT (hBAT) and to investigate whether its thermogenic activity can be manipulated to treat metabolic disease. As part of this effort, a workshop entitled “Exploring the Roles of Brown Fat in Humans” (http://www.niddk.nih.gov/news/events-calendar/Pages/HumanBAT-2013.aspx) was convened at the National Institutes of Health (NIH) by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) on February 25–26, 2014. The meeting brought together 180 renowned investigators from around the world to discuss state-of-the-art technology for monitoring hBAT mass and activity, to present recent discoveries in the cell biology and

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endocrine pathways associated with BAT, and to showcase clinical data of hBAT and its implications in metabolic studies.

Much of the interest in hBAT has been driven by the hope that it represents a novel, easily assessable target for the treatment of obesity (Bachman et al., 2002). There were several widely held ideas about BAT that supported this concept, that now appear simplistic and limiting given the emerging state of knowledge. First, the original evidence for functional adult hBAT came from the detection of bilaterally symmetric patches of intense radio-labeled glucose uptake in the neck and supraclavicular region in some oncology patients during diagnostic ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) scans that could be suppressed by warming the patient. After confirmation in healthy adults, a positive ^{18}F-FDG PET/CT scan quickly came to define the presence of hBAT. Second, from rodent studies it was known that BAT is a form of “good fat” in which uncoupling protein 1 (UCP1) is activated in response to cold in order to facilitate a high rate of fuel oxidation and heat production. Therefore, it seemed appropriate to posit that the primary stimulus for hBAT activation is via cold temperature sensing, and that its physiologic role is thermogenesis. Third, although abundant in newborn babies and detectable upon cold stimulation with ^{18}F-FDG PET/CT in some young lean adults, hBAT appeared to be missing in obese and elderly people using this method of detection. This lack of hBAT could perhaps underlie a state of metabolic efficiency that supports excess fat deposition, and restoration and activation of hBAT through pharmacologic or environmental means could then be a route to reduce obesity.

Presentations and discussion at the recent NIH hBAT workshop indicated that each of these concepts is too simple to explain newer observations, and that additional research is needed to elucidate the likely more complex physiological roles of hBAT. Research in humans is still limited by the paucity of available noninvasive tools that can quantify the mass, activity, and potential for activation of hBAT in all its forms, but efforts to develop such methods that synergize with emerging biological information are quickly gaining momentum.

At the conclusion of the workshop, an engaging open-floor dialogue among the participants was held. The following summary represents the principal concepts that arose from that discussion and identifies unanswered questions, unmet needs, and some critical areas of future research.

The Definition of Human BAT is Evolving

For the purposes of clarity, the term `BAT' will be used in this document to refer to any region of fat that contains UCP1 positive adipocytes. Currently in adult humans, this includes tissue in the neck, above the clavicles and along the spine that can be visualized using ^{18}F-FDG PET/CT. There are at least two known types of brown adipocytes in BAT as well as white adipocytes in varying proportions. Data from multiple laboratories indicate that in rodents, brown adipocytes derive from two distinct mesenchymal lineages. Brown adipocytes found in the rodent interscapular and perirenal regions share an origin with myocytes and are present constitutively from the embryonic period. These brown adipocytes have been called “brown,” “classical,” or “constitutive”, and in this document the term

"classical brown adipocyte" will be used. A second type of brown adipocyte arises in rodent white adipose depots in response to stimuli such as sympathetic activity during cold acclimation, chronic administration of β3-AR agonists, or exercise, and may share a developmental origin with white adipocytes. These brown adipocytes have been called "beige," "brite," (for "brown-like in white"), "inducible," or "recruitable." Presently, it is not settled what the preferred nomenclature will be for this second type of brown adipocyte, but they will be called "beige adipocytes" in this document. Both cell types are polygonal with central nuclei, multilocular lipid droplets, a high mitochondrial content, and contain UCP1, but each type has a distinct gene expression profile (Harms and Seale, 2013). Human brown adipocytes from both lineages have been documented in biopsied fat from the human neck, supraclavicular, and interscapular regions. As data continues to accrue, it seems likely that most, if not all, adult humans have some mature brown adipocytes from both lineages, and there may be several phenotypically distinct subtypes (Lee et al., 2011; Wu et al., 2012; Sharp et al., 2012; Cypess et al., 2013; Jespersen et al., 2013).

A few more definitions are needed. All brown adipocytes can be `activated', meaning that they have UCP1 that can be turned on in response to sympathetic stimuli such as cold, resulting in heat production. Activation is proportional to a thermic challenge, such that these tissues are partially activated even at ambient temperature (Cohen et al., 2014). In addition, brown and beige adipocytes can be `induced' in rodents in response to sympathetic stimuli, such that new adipocytes arise from tissue-resident stem/progenitor cells resulting in an increase in the number of brown adipocytes. In humans, there is evidence accumulating that hBAT tissues in the neck are plastic and can therefore be induced (Huttunen et al., 1981; Blondin et al., 2014; Lee, Werner et al., 2014; Lee, Linderman et al., 2014; van der Lans et al., 2013). In mice, this `browning' can also be observed in subcutaneous fat depots (Young et al., 1984; Wu et al., 2012). It remains unknown whether humans, like rodents, have the potential for appreciable induction of beige adipocyte mass in depots outside of the neck, supraclavicular and thoracic depots noted in ^{18}F-FDG PET/CT scans, although one observation has been made in a patient treated with thyroid hormone (Skarulis et al., 2010). If browned white fat is commonly found in people, it will be difficult to investigate all the biological, behavioral and environmental triggers for such `subcutaneous inducible brown adipose tissue' (siBAT) until non- or minimally-invasive means to measure its mass and activity are developed. It will be important to determine if there is a correlation between the presence of BAT and siBAT, and if their functions are distinct.

The present discussion is focused on the hBAT depots that can currently be detected, in order to explore their mass and function in normal human health and disease, and to inform efforts to target them therapeutically. Given the current early state of knowledge, it is likely not crucial to develop technologies that can distinguish between human classical brown and beige adipocytes. It is however very important to understand if hBAT, whether in the neck/subclavicular regions or in subcutaneous white depots, can be induced to dramatically increase the potential for therapeutic thermogenesis.

^{18}F-FDG-PET/CT is Informative but Not Sufficient

Central to gaining a better understanding of hBAT physiology is the proper application of noninvasive whole-body imaging. While ^{18}F-FDG-PET combined with CT has been instrumental in advancing our understanding of hBAT over the past decade, it is becoming apparent that this technique by itself provides an incomplete picture of hBAT morphology and metabolic activity, and could at times even be misleading. There are several well-recognized limitations to ^{18}F-FDG-PET/CT. First, while the Standard Uptake Value (SUV) of the radiolabeled glucose analogue accurately reflects glucose uptake into metabolically active tissues, when a tissue such as BAT is inactive or weakly activated, it remains largely invisible. The intensity of the FDG-PET signal is a function of the extent of hBAT activation, and individuals subjected to the same cold stimulus may not respond the same. Second, even when PET time and activity curves are collected and calibrated using arterial blood ^{18}F-FDG specific activity, the resultant measure of glucose uptake is not necessarily a quantitative reflection of hBAT thermogenesis. Although plasma glucose is taken up upon hBAT activation, the initial substrate is believed to be endogenously derived fatty acids, followed by a preference for circulating free fatty acids and triglycerides from chylomicrons and VLDL. Therefore, glucose uptake measured by ^{18}F-FDG-PET likely underestimates BAT activity under physiologically meaningful conditions such as mild cold.

On the other hand, glucose uptake into BAT can potentially be increased by insulin without accompanying increases in thermogenesis, local blood flow and UCP1 activation, and can therefore be a poor measure of thermogenesis, particularly in post-prandial and disease states (Orava et al., 2011). It should be emphasized that in addition to its role as a fuel for oxidation, glucose is a substrate for other processes such as anaerobic glycolysis, lipogenesis, and anaplerosis. These processes are not necessarily linked to thermogenesis, and ^{18}F-FDG-PET/CT is currently not able to distinguish among these different pathways of glucose utilization. Thus, while it was agreed that ^{18}F-FDG uptake as observed in ^{18}F-PET/CT images can indicate the presence of metabolically active hBAT (e.g. high sensitivity), it is not a definitive imaging biomarker (e.g. low specificity) for hBAT presence and thermogenesis.

Another recognized limitation of ^{18}F-FDG-PET/CT that emerged from discussion among workshop participants was the difficulty of quantifying BAT glucose uptake in the presence of many confounding factors. For example, the PET SUV endpoint is affected by the amount and rate of glucose uptake into other tissues and organs, the dosage administered, the subject's weight and fat percentage, prandial state, radiotracer specific activity, partial volume effects, and the glucose uptake kinetics in BAT. Estimates of SUV and activated tissue volume are dependent on the specific parameters chosen for the experiment and analysis, and can vary from one observer to another. One possible approach towards more meaningful measurements is to employ quantitative dynamic PET methods combined with modeling of the imaging data by graphical analysis.

Lastly, PET/CT is relatively expensive and involves significant exposure of a subject to ionizing radiation beyond minimal risk, and therefore cannot be used in large-scale serial studies, infants and children, and healthy cohorts. While ^{18}F-FDG PET/CT has arguably

advanced our understanding of hBAT physiology over the past decade, the modality alone should not be considered a gold standard for characterizing hBAT properties, including mass, thermogenesis, and the potential for activation.

Novel hBAT Detection Methods are Under Development

A variety of physiological, biochemical, and metabolic features of hBAT can be exploited to provide signal contrast, and monitored with available imaging and biosensor methods (for reviews, see Bauwens et al., 2014 and Hu and Kan, 2013). For example, magnetic resonance imaging (MRI) and CT can measure fat fraction and thereby identify BAT, since the fat content of brown adipocytes is different than that of lean muscle and lipid-rich white adipocytes (Hamilton et al., 2011; Chen et al., 2013; Baba et al., 2010). hBAT is densely permeated by capillaries and sympathetic neurons that participate in its unique function. The change in blood flow that occurs upon cold-stimulated sympathetic activation can be imaged using PET, MRI or ultrasound and may serve as a surrogate for tissue thermogenesis (Orava et al., 2011; Khanna and Branca, 2012; Clerte et al., 2013). PET tracers have been produced that can bind to the neurotransmitter norepinephrine transporter (Lin et al., 2012) or read the changes in mitochondrial membrane potential that occur upon tissue activation (Madar et al., 2011). A sampling of BAT detection methods was presented at the workshop, and Table 1 lists detection methods under development along with the biological feature of BAT that each measures. Many were developed with support from RFA-DK10-002 in 2010, “Human Brown Adipose Tissue: Methods for Measurement of Mass and Activity” (http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-10-002.html). A majority of these methods are still in their infancy. Most of them have yet to be validated in people, remain cost prohibitive, and have yet to reach widespread availability among the research community.

It will be highly advantageous to have a substantial menu of validated tools for the study of hBAT. Some of these tools will detect properties that correlate with tissue mass but not its activation state. Other methods will detect only activated tissue, while others will yield information about both the mass and thermogenic activity of BAT. Some are quantitative while others are extremely sensitive but cannot yield absolute values for mass or heat production. It became apparent during workshop discussion that no single modality or technique currently exists to comprehensively characterize BAT morphology and measure its mass and activity, and a combination of techniques is likely to be more informative than any single entity alone. Even within a single modality such as magnetic resonance, a suite of complementary spectroscopy and imaging experiments sensitive to either tissue mass and morphology (fat fraction) or function (blood flow) should be compiled to fully characterize hBAT mass and metabolic activity. The emerging fusion modality of PET/MR is progressively becoming available at research institutions worldwide and was acknowledged at the workshop as a very promising multi-tool platform that can potentially provide a “one-stop shop” for elucidating hBAT properties within a single examination.

It is hoped that tests intended for widespread use in the clinic, for large epidemiological studies or for drug development will be inexpensive, non-invasive, and take advantage of technology that is widely available. Measurements designed for use in free-living individuals could yield important insight into tissue function. For example, although it is

difficult to translate temperature into quantitative measures of thermogenesis, radiometers that are sensitive to tissue temperature may offer the advantage of portability and home-based usage. When coupled with telemetry, radiometers could be used to estimate hBAT thermogenesis in large populations, to monitor the effects of climate and other environmental stimuli, and to investigate circadian responses (Arunachalam et al., 2008; Rodrigues et al., 2013). Ultimately, comparative studies will be needed to identify the strengths, limitations, and cost-effectiveness of the most promising technologies. It is hoped that researchers will soon be able to assemble suites of complimentary techniques that at a minimum provide independent measures reflective of hBAT mass and activity, and that are tailored for use in different experimental and clinical environments.

Standardized Protocols are Needed for hBAT Activation, Detection and Reporting of Results

Workshop participants felt that additional concrete steps should be taken by the research community to enhance the reproducibility, repeatability, accuracy, and precision of hBAT measurements. In particular, there is an immediate need to develop minimum standards for ^{18}F-FDG-PET/CT data acquisition and operator-independent image analysis (for instance, see Chen et al., 2013) so that the tissue volume and intensity of tracer uptake from different laboratories can be compared (also suggested by Bauwens et al., 2014 and van der Lans et al., 2014). Likewise, as each emerging method is developed and validated, guidelines for parameters used, and minimum standards for data acquisition, analysis, and literature reporting should be established. It was also noted that sharing of validated experimental protocols in a common repository is one way that multi-institutional collaboration and consensus can be stimulated. Since exposure of humans to cold temperatures is likely to continue to be one way researchers maximally activate hBAT for measurement of its volume, numerous times during the two day meeting there was a call to standardize the cold-exposure protocols. The spectrum of existing valid approaches for cold stimulation of people presently include the use of climate-controlled rooms, water-circulating vests, and exposure of a limb to cold water. Some groups rely on exposure to a fixed temperature while others use a personalized cooling protocol where a subject is cooled to shivering, then warmed slightly (van der Lans, et al., 2014). Such differences in the cooling goals likely affect the degree of BAT activation and therefore the ability to detect it, and any quantitative measure of thermogenesis. This makes it difficult to compare measures of hBAT volume or maximal activation potential among individuals or across populations, and should be addressed. The alternative of using pharmacological agents to reliably activate hBAT was discussed (Broeders et al., 2014). Hopefully as pharmacological agents and other means that can easily, reliably and reproducibly activate hBAT become available, protocols using these agents will also be standardized. While such minimal standards are needed to compare data regarding hBAT incidence, volume and activation potential across laboratories and populations, experimental parameters must ultimately be chosen to best answer the specific research question under study (Chen et al., 2013).

The preceding discussion focused only on technologies for monitoring hBAT. There are presently no approaches that can non-invasively differentiate between classical brown and

beige adipocytes within hBAT, although molecular imaging with targeted cell-specific probes may be a promising approach (Azhdarinia et al., 2013). More importantly, there are no current means other than histology of biopsied fat tissue to unambiguously detect beige fat cells that are induced in subcutaneous white fat depots. It seems unlikely that current imaging approaches will be useful in the short term in subcutaneous fat depots, where the ratio of brown to white adipocytes is likely to be very low under most circumstances. Perhaps circulating plasma biomarkers (e.g. secreted adipokines or miRNAs) could be found that reflect siBAT or total brown adipose mass or activation. These challenges were identified as critical unmet needs.

hBAT Thermogenesis can Directly Affect Energy Expenditure, but the Potential to Impact Body Weight is Less Clear

There was considerable discussion regarding the possible health benefits of pharmacologic or environmental activation of hBAT at the workshop. However, it remains to be seen whether excess thermogenesis can be activated safely, and if the resultant increase in energy expenditure is sufficient to affect weight or metabolic health. Estimates of potential maximal thermogenesis of cold-induced hBAT lie between 25 and 400 kcal/day in lean people (Muzik et al., 2013; Yoneshiro et al., 2011), but it has proven difficult to date to achieve high levels of activation with cold in obese people using a number of different time courses and cooling protocols (Orava et al., 2013). However, if brown fat cell mass in classical BAT or subcutaneous white fat depots can be induced in people, as has been observed in rodents (Schulz and Tseng, 2013), it may be possible to greatly increase the thermogenic potential of whole body hBAT. This could then change the emphasis of obesity treatment research from finding a safe means of activating existing hBAT, to inducing brown fat cell adipogenesis in multiple fat depots, particularly using agents other than cold exposure (Kajimura and Saito, 2014; Bonet et al., 2012). However, even if increased hBAT mass and activation state were achievable, the increase in energy expenditure is still likely to be less than that seen with even moderately intense exercise programs, which in isolation have not been effective for weight loss. Workshop participants speculated that, like exercise, sustained hBAT activation may even trigger a homeostatic response to increase calorie intake (Cannon and Nedergaard, 2009). However, either sustained or transient increases in hBAT mass and/or activity could potentially be used to protect against weight gain or help maintain weight loss, and prevent metabolic diseases such as diabetes. Whether hBAT modulation is a good target for treating obesity and preventing diabetes will require more research on large populations.

hBAT has Health Benefits Beyond its Important Role in Energy Balance and Cold Tolerance

Several talks and poster presentations at the NIH hBAT workshop touched on a number of physiological processes that appear to be regulated in some way by BAT. Beyond cold tolerance, BAT likely plays a protective role in metabolic health unrelated to weight modulation. These include potential involvement in regulating glucose homeostasis and insulin resistance (reviewed by Peirce and Vidal-Puig, 2013), liver steatosis, blood lipids, cardiovascular health and disease and immune system health. Beige fat cells appear to have

an impact on white adipose tissue health; in mice, subcutaneous white adipose depots that lack PRDM16 and therefore cannot increase brown adipocyte number contain higher levels of fat, activated macrophages and inflammatory markers (Cohen et al., 2014). hBAT mass and activity appear to be integrated with the health and function of bone in adults (Bredella et al., 2014), and with skeletal muscle mass in babies, children, and adolescents (Ponrartana et al., 2013). BAT is a primary source of heat in torpor/arousal transitions in hibernating rodents (Hindle and Martin, 2014) and in recovery from sleep deprivation in mice (Szentirmai and Kapas, 2014). Its role in circadian rhythms and sleep regulation in humans has yet to be investigated. Lastly, rodent studies have shown that multiple peptides from different organs induce the growth and activation of brown adipocytes, including FGF-21 from liver, atrial natriuretic peptide from heart, and irisin, myostatin, and TGF-β from skeletal muscle (Kajimura and Saito, 2014). In turn, BAT may secrete signaling factors that communicate back to other tissues (Villarroya et al., 2013), again, suggesting that regulated cross talk between a number of tissues and BAT is important for maintaining health.

More research is needed to understand the impact of hBAT beyond thermogenesis, and to define the interactions between hBAT and several core physiological processes: prandial states, anorexia, cachexia, physical activity, bone structure, sleep, circadian rhythms, and aging. Prospective and longitudinal studies are necessary to address these fundamental unknowns. Research on the basic biological properties of hBAT is in its infancy and many questions remain to be asked, such as whether it has an endocrine role, and how it interacts with other signaling tissues. Based on the results presented at the workshop, participants were optimistic that strategies targeting hBAT induction and activation could help to prevent weight gain, maintain weight loss, protect against the complications of obesity such as fatty liver and cardiovascular disease, improve glucose homeostasis in diabetes, and enhance overall metabolic, bone, and skeletal muscle health.

Conclusion

We are only beginning to understand the beneficial roles for adult hBAT. Our ability to conduct experiments depends on finding a combination of validated, minimally invasive technologies that can independently measure tissue mass and accurately quantitate thermogenesis capacity. An impressive array of imaging and thermometry approaches are emerging, and as they are validated these should be combined in order to provide a rich picture of hBAT mass and function. To date it is not known whether human peripheral white fat depots can be browned, and if so there will be a need for noninvasive measures that will allow this siBAT to be studied. Due to the expected low brown-to-white adipocyte ratio, it is likely that circulating molecules or other indirect biomarkers will be more useful than imaging approaches to detect siBAT. It would be advantageous if researchers could agree on a minimum set of guidelines regarding imaging data procurement, analysis and interpretation, and for the experimental protocols for cold-stimulation of hBAT so that data can be compared across laboratories and across populations. It remains to be seen whether hBAT will be an effective target for weight loss, yet emerging data indicate that this tissue plays many important roles in human health. Continued research on the cellular and molecular properties of hBAT is needed to inform on its physiological functions and provide additional strategies for manipulating its mass and activity. Finally, prospective longitudinal

studies in human populations are needed to provide a complete picture of its incidence and the environmental and physiological stimuli that regulate its activity.

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Abbreviations

BAT	brown adipose tissue
hBAT	human brown adipose tissue
AR	b3-adrenergic receptor
^{18}F-FDG PET/CT	NIH, NIDDK, ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography
UCP1	uncoupling protein 1
siBAT	subcutaneous inducible brown adipose tissue
SUV	Standard Uptake Value
MRI	magnetic resonance imaging

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Highlights

- Evidence suggests that most, if not all, adult humans have activatable hBAT in the neck region.
- It is unknown if human subcutaneous fat can be “browned” as observed in rodents.
- 18F-FDG-PET/CT is informative but not sufficient for detection of hBAT mass and activation.
- Novel methods to independently assess hBAT mass and function need to be validated.
- It is not yet known if hBAT constitutes a good target for obesity therapy.
- hBAT has health benefits beyond cold tolerance, such as in bone and metabolic health.

Table 1

Summary of Methods to Detect Human Brown Adipose Tissue (BAT)[a]

IMAGING TARGET and MEASURED PARAMETER	MODALITY	BASIS of SIGNAL	PRIMARY ENDPOINT: BAT MASS or FUNCTION	UNIQUE FEATURES	CHALLENGES	REFERENCES
Glucose uptake	PET[b]	^{18}F-Fluorodeoxyglucose	Function	Widely available and employed Large signal increase upon BAT activation	Ionizing radiation Negligible uptake in inactive BAT Unknown correlation with thermogenesis Lack of standardization	Nedergaard et al., 2007; Virtanen et al., 2009; [g]Mirbolooki et al., 2014
Fatty acid or Acetate uptake	PET	14(R,S)-^{18}F-Fluoro-6-thiaheptadocanoic acid ^{11}C-acetate	Function	Nutrients avidly take up by activated BAT Large signal increase upon BAT activation	Ionizing radiation Negligible uptake in inactive BAT Limited validation	Ouellet et al., 2012
Mitochondrial respiration	PET	Locally produced H_2 ^{15}O from inspired $^{15}O_2$	Function	Visible only in metabolically active tissues Large signal increase upon BAT activation Quantitative measure of thermogenesis	Ionizing radiation Negligible uptake in inactive BAT Limited validation	[g] Muzik et al., 2013
Mitochondrial membrane potential	PET	^{18}F-Flurobenzyl triphenyl phosphonium (FBnTP)	Mass and Function	Tracer mitochondria accumulation: inactive BAT>> muscle or WAT Signal reduced in active BAT Quantitative	Ionizing radiation Limited validation	[g] Madar et al., 2011
Norepinephrine transporter protein (NET)	PET	(S, S)-[^{11}C] O-methylreboxetine ([^{11}C] MRB)	Mass	High baseline signal in inactive BAT Signal only mildly affected by BAT activation	Ionizing radiation Limited validation	[g] Lin et al., 2012
Tissue fat content	MR[c] (imaging) CT[d]	Chemical-shift ^{1}H water-fat separation Dixon method Tissue X-ray attenuation (Hounsfield Units)	Mass	No ionizing radiation (MR) Quantitative measure of BAT morphology (e.g. fat content), BAT has lower fat content than WAT	Cannot readily distinguish inactive and active BAT Fat content not a unique signal feature of BAT Ionizing radiation (CT)	[g]Hamilton et al., 2011;[g]Chen et al., 2013Baba et al., 2010;[g]Hu et al., 2011

IMAGING TARGET and MEASURED PARAMETER	MODALITY	BASIS of SIGNAL	PRIMARY ENDPOINT: BAT MASS or FUNCTION	UNIQUE FEATURES	CHALLENGES	REFERENCES
Intracellular waterfat molecular interactions	MR (spectroscopy)	Intermolecular zero quantum coherence - spatial proximity of water and fat molecules MR resonance frequency is temperature sensitive	Mass	Signal unique for BAT Sensitivity to tissue temperature (e.g. BAT activity)	Limited commercial availability Long data acquisition time Special equipment needed for hyperpolarization Not yet validated in humans	[g] Branca, Zhang et al., 2013
^{129}Xenon	MR (imaging and spectroscopy)	hyperpolarized ^{129}Xenon gas MR resonance frequency is temperature sensitive	Mass and Function	^{29}Xenon gas dissolves in highly perfused lipid-rich BAT Sensitivity to tissue temperature (e.g. BAT activity)	Limited commercial availability Special equipment needed for hyperpolarization Not yet validated in humans	[g]Branca, White and Zhang., 2013; Branca, Zhang, White and He, 2013
Pyruvate metabolism to bicarbonate and lactate	MR (imaging and spectroscopy)	Hyperpolarized [^{13}C]1-pyruvate	Function	Visible only in metabolically active tissues A direct measures of mitochondrial oxidation	Limited commercial availability Special equipment needed for hyperpolarization Not yet validated in humans	Lau et al., 2014
BAT vascularity and blood flow	US[e] PET, SPECT[f] MR (imaging)	Echogenic microbubbles H_2 ^{15}O, ^{99m}Tcmethoxyisobutylisonitrile Blood-Oxygen-Level-Dependent (BOLD) T2* and functional imaging	Function	No ionizing radiation (US, MR) A direct measure of local tissue blood flow and perfusion (all) Appreciable signal change upon BAT activation (all)	Ionizing radiation (PET, SPECT) MR methods require large signal change to differentiate from baseline measurements Limited validation	[g]Clerte et al., 2013 Orava et al., 2011; Cypess et al., 2013 [g]Khanna and Branca, 2012;[g]Chen et al., 2013;van Rooijen et al., 2013
Targeted molecular probes specific to BAT	Near-infrared fluorescence imaging	Peptide probe selective for BAT vascular endothelium	Mass and Function	Potential high specificity for inactive and active BAT	Limited availability Not yet validated in humans	[g] Azhdarinia et al., 2013
Temperature - activated BAT produces heat via thermogenesis	Microwave radiometry Infrared imaging	Surface sensors detect radiating heat emitted from activated BAT	Function	Highly portable, wearable system that can potentially be monitored by telemetry Non-invasive, low cost	Limited availability Limited validation Heat is generated by other tissues besides BAT	[g]Rodrigues et al., 2013; Arunachalam et al., 2008 Symonds et al., 2012

[a] BAT: brown adipose tissue;

[b] PET: positron emission tomography;

[c] MR: magnetic resonance;

[d] CT: computed tomography;

[e] US: ultrasound;

[f] SPECT: Single photon emission computed tomography;

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