HHS Public Access

Author manuscript

Cell Metab. Author manuscript; available in PMC 2017 August 09.

Published in final edited form as:

Cell Metab. 2016 August 9; 24(2): 210–222. doi:10.1016/j.cmet.2016.07.014.

Brown Adipose Reporting Criteria in Imaging STudies (BARCIST 1.0): Recommendations for Standardized FDG-PET/CT Experiments in Humans

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Abstract

Human brown adipose tissue (BAT) presence, metabolic activity and estimated mass are typically measured by imaging [18F]fluorodeoxyglucose (FDG) uptake in response to cold exposure in regions of the body expected to contain BAT, using positron emission tomography combined with x-ray computed tomography (FDG-PET/CT). Efforts to describe the epidemiology and biology of human BAT are hampered by diverse experimental practices, making it difficult to directly compare results among laboratories. An expert panel was assembled by the National Institute of Diabetes and Digestive and Kidney Diseases on November 4, 2014 to discuss minimal requirements for conducting FDG-PET/CT experiments of human BAT, data analysis, and publication of results. This resulted in Brown Adipose Reporting Criteria in Imaging STudies (BARCIST 1.0). Since there are no fully-validated best practices at this time, panel recommendations are meant to enhance comparability across experiments, but not to constrain experimental design or the questions that can be asked.

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AUTHOR CONTRIBUTIONS

K.Y.C., A.M.C., M.R.L., C.R.H., H.H.H., M.A.B., S.E., P.E.K., W.D.v.M.L., F.I.L., J.J.S., K.A.V., and R.L.W. attended the meeting and presented information that formed the basis of the manuscript. K.Y.C., A.M.C., M.R.L., C.R.H., H.H.H., and R.L.W. wrote the manuscript. M.A.B., S.E., P.E.K., W.D.v.M.L., F.I.L., J.J.S., and K.A.V. edited the manuscript.

1. Introduction

With the introduction of positron emission tomography (PET) combined with x-ray computed tomography (CT) scanning technology late in the last century, it was recognized by Hany et al. (2002) and Cohade et al. (2003a) that there were unexpected metabolically-active foci seen on [18F]fluorodeoxyglucose (FDG) PET scans of hundreds of cancer patients, in regions such as the supraclavicular area, that had the characteristic CT features of fat tissue. These foci were initially called “USA-fat” (Uptake in the Supraclavicular Region) and were postulated to represent metabolically active brown adipose tissue (BAT). In the diagnostic imaging community, FDG uptake in BAT was viewed, to some extent, as an impediment to diagnostic cancer imaging, as this signal could obscure tumor visualization by increasing background signal or introduce false positives. Additional retrospective human studies confirmed the presence and activity of this fat tissue and implied that these metabolically active fat foci were more active in the winter season than the summer, more common in women than in men, and in the normal weight vs. the obese (van Marken Lichtenbelt et al., 2009; Cypess et al., 2009; Virtanen et al., 2009; Saito et al., 2009; Cohade et al., 2003b). This metabolically active fatty tissue proved to be sensitive to stimulation by cold or beta-adrenergic receptor agonists, and was suppressed by beta blockers (Baba et al., 2007b). Subsequent prospective and larger retrospective studies indicate that BAT is likely to be present and activated by chronic cold exposure in most healthy men and women (van der Lans et al., 2013; Lee et al., 2010), but is reduced in underweight (Bredella et al, 2012), overweight, and individuals with metabolic disease (Hanssen et al. 2015). BAT accumulates a range of radiotracers beyond FDG, including tracers of other metabolic substrates such as [11C]acetate and [18F]THA (Ouellet et al., 2012), tracers of adrenergic innervation such as [123I]metaiodobenzylguanidine (MIBG), and agents binding to mitochondria such as [99mTc]sestamibi (MIBI) and [99mTc]tetrofosmin (Baba et al., 2007a; Fukuchi et al., 2003), and 18F-Fluorobenzyl Triphenyl Phosphonium (FBnTP)(Madar et al. 2015). Although it has several limitations, FDG-PET/CT has become the most popular platform for investigating this unique adipose tissue and its physiological relevance to human metabolism, substrate regulation, and obesity. Despite the promise of other imaging methods (Hu and Kan, 2013; Cypess et al., 2014; Borga et al., 2014), it is evident that FDG-PET/CT will continue to have a critical role.

There are currently no widely accepted standardized protocols established for FDG-PET/CT imaging of human BAT (Betz et al. 2015; Blondin et al. 2015c). Published reports also vary extensively on the paradigm used to activate BAT, the acquisition and reconstruction settings of the PET/CT scanner, the preparation of the participants prior to imaging, the anthropometric distribution of the recruited cohorts, and the approach with which subsequent data are analyzed to estimate human BAT mass and volume, metabolic activity, and prevalence (for a summary of recent studies, see Figure 1 in van der Lans et al., 2014).

In February, 2014, an international scientific workshop entitled “Exploring the Role of Brown Fat in Humans” was hosted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in Bethesda, MD (Cypess et al., 2014). The workshop panel agreed that a unified approach toward FDG-PET/CT imaging of human BAT would improve the ability to compare data among studies and guide new researchers to the field. In

response, a one day follow-up workshop was convened by the NIDDK on November 4, 2014 to discuss a set of minimal standards and experimental guidelines pertaining to static FDG-PET/CT imaging studies of cold-activated human BAT. The result is BARCIST 1.0: Brown Adipose Reporting Criteria in Imaging STudies, version 1.0. These recommendations for experimental parameters and practices are based on currently available evidence, and on the experience of panel members. Recommendations are meant to provide guidance for the minimum requirements for acquisition, analysis, and reporting of FDG-PET/CT BAT data in humans.

The paramount recommendation is that data and experimental parameters for all future studies should be recorded and reported in a standard manner, as suggested in Table 1. Any additional methods and data analysis considered to be most appropriate for the specific study should also be recorded and reported. The intent is to ensure consistency and facilitate meaningful comparisons of biological and epidemiological observations in human BAT across publications, laboratories, and clinical sites. There is no intent to confine the types of scientific questions that can be asked, dictate experimental design, or restrict development of additional experimental methodologies.

1.1 Cell Biology of BAT and PET Imaging

BAT is heterogeneous, with at least two known types of brown adipocytes that differ by lineage, termed 'classical' and 'inducible' (also known as 'beige' or 'brite'), as well as white adipocytes in varying proportions. Uncoupling protein 1 (UCP-1) is a hallmark of brown adipocytes. When activated this mitochondrial protein uncouples the electron transport chain in cells, producing heat (Cannon and Nedergaard, 2004). The term ‘BAT’ will be used in this document to refer to any region of fat that contains detectable amounts of UCP-1-positive adipocytes. Both types of brown adipocytes are roughly polygonal with central nuclei and contain multi-locular lipid droplets, a high mitochondrial content, and UCP-1. However, each type may have a distinct gene expression profile (Harms and Seale, 2013). Brown adipocytes from both lineages have been documented in biopsied fat from the human neck, supraclavicular, and inter-scapular regions (Virtanen et al., 2009; Lidell et al., 2013; Cypess et al., 2013a; Jespersen et al., 2013; van Marken Lichtenbelt et al., 2009; Broeders et al., 2015). Currently, classification of BAT types is possible only by analysis of biopsied tissue. However, it appears as though function and detectability using FDG-PET/CT is the same for all brown adipocytes regardless of lineage. In addition to being 'activated' to produce heat in response to sympathetic stimuli such as cold, it appears that BAT tissues in the neck are plastic and can therefore be 'induced' by repeated cold exposure, resulting in an increase in the number and/or size of brown adipocytes (Huttunen et al., 1981; Blondin et al., 2014; Lee et al., 2014a; Lee et al., 2014b; van der Lans et al., 2013; Yoneshiro et al., 2013; Hanssen et al., 2015). Accumulating evidence suggests that other white adipose tissue (WAT) depots in humans such as subcutaneous fat can undergo browning (Skarulis et al., 2010; Kern et al., 2014; Petruzzelli et al., 2014; Sidossis et al., 2015).

Irrespective of their classification, brown adipocytes are most easily visualized with FDG-PET/CT imaging when FDG retention is increased in the cells compared with surrounding tissue, and this is elevated when BAT UCP-1 and thermogenesis are activated. Although

FDG-PET/CT can detect BAT FDG uptake and differences in the extent of BAT activation, it is not capable of determining BAT cell volume, cell number, or number of mitochondria per cell. Changes in BAT density due to lipid consumption after activation, as measured by CT Hounsfield Units (HU) (Baba et al., 2010), and radiotracers specific for mitochondria such at [99mTc]MIBI (Cypess et al., 2013b) or [18F]fluorobenzyl triphenyl phosphonium (Madar et al., 2011) may be better able to assess cell volume and mitochondrial density or membrane potential. Because of the signal-to-noise limitations, it also remains to be seen whether FDG-PET can reliably detect BAT when it constitutes a small fraction of cells in a fat depot. It is important to note that glucose consumption, as estimated by FDG-PET, is a proxy for thermogenesis and may not be directly linked to caloric expenditure, since BAT utilizes endogenous fat as fuel and takes up fatty acids from the circulation as well as glucose. In fact, fatty acids labeled with positron-emitters are taken up by BAT and could be used to more comprehensively estimate its activation state (Ouellet et al., 2012).

1.2 Standardization – Lessons From the Nuclear Medicine Community

Glucose uptake, estimated using the glucose analog FDG in a typical FDG-PET/CT image acquisition, is expressed as a standardized uptake value (SUV). SUV is a normalized measure of signal intensity, defined as the ratio of the radioactivity concentration in a defined region (MBq/mL) to the injected radiotracer dose that has been normalized to some index of body mass (MBq/g). If the tracer were homogeneously distributed everywhere in the body, all tissues would have SUV=1.0. SUV is typically normalized to total body mass (SUV_{bm}). However, because there is little accumulation of FDG in WAT in the fasting state, obese participants tend to have higher SUV_{bm} in non-WAT (lean) tissues, potentially confounding interpretation. A mean absolute cut-off value for SUV used for detection of BAT or other target tissue in lean participants can therefore be too low for heavier participants. This problem can be mitigated by normalizing SUV using the lean body mass (LBM) instead of total body mass (SUV_{lean}). While LBM should be directly measured whenever possible, modern PET software includes algorithms to make this correction using height and body weight. The Janmahasatian formula for LBM can accommodate very high body mass index (BMI) (Sugawara et al., 1999; Tahari et al., 2014; Graham et al., 2015), and will likely be introduced on clinical PET/CT scanners over the next several years. For now it must be applied manually. There are multiple other physiologic parameters and experimental practices that affect the PET/CT quantitative measurement (Table 2). While difficult to correct for all of these factors, tight control and documentation of experimental practices can ensure that many are reasonably constant, and should result in a general decrease in intra- and inter-site measurement variance.

FDG-PET/CT quantification of BAT metabolic activity is informed by extensive work in oncology where qualitative FDG-PET/CT is routinely used in over 3000 clinical sites to diagnose and stage cancers, and to monitor treatment. In cancer patients, foci of increased radiotracer uptake are typically interpreted as sites of active cancer, but the use of quantitative PET imaging has enriched the understanding of tumor biology and response to therapy. Efforts to provide standards in order to reduce variance in FDG-PET imaging for oncology include the Uniform Protocols for Imaging in Clinical Trials (UPICT) standardized FDG-PET/CT protocol (FDG-PET/CT Technical Committee, 2014; Graham et

al., 2015), the RSNA Quantitative Imaging Biomarker Alliance (QIBA) FDG profile (FDG-PET/CT Technical Committee, 2013; Raunig et al., 2015), European Association of Nuclear Medicine (EANM) procedure guidelines for tumour imaging (Boellaard et al., 2014), and the consensus recommendations for the use of FDG-PET as an indicator of therapeutic response in patients in National Cancer Institute Trials (Shankar et al., 2006). The UPICT, QIBA and EANM documents (and accompanying online materials) highlight a variety of factors affecting image quantitation and provide acceptable (minimum), target, and ideal standards for all phases of the imaging exam, from scheduling to quality control, in a systematic way, in order to both characterize and minimize intra- and inter-subject, intra- and inter-platform, inter-examination, and inter-institutional variability of primary and derived data.

A recent special issue of *Statistical Methods in Medical Research* describes efforts to understand and control the factors that contribute to bias and precision of quantitative imaging biomarkers (Sullivan et al., 2015). 'Bias' describes how accurately a quantitative metric such as SUV reflects true radiotracer uptake in vivo. 'Precision' is an index of the likelihood that a repeat measurement will give the same value as the first. For FDG-PET/CT imaging, factors that contribute to bias and precision can originate from the PET/CT and display hardware, quantitative corrections (scatter, attenuation, etc.), the acquisition protocol, and the downstream analysis (Kinahan and Fletcher, 2010). The bias and precision for absolute measurement of PET tracer uptake associated with the machine and software is currently quite good (approaching 1% under controlled conditions, Doot et al., 2010). The most significant origins of bias and variance are therefore likely biological and operational (i.e. operator errors including in region of interest definition). For example, the radioactivity concentration in small foci of tracer uptake, those smaller than three times the reconstructed resolution of a scanner, (i.e., 2.5–3 cm), is typically underestimated due to resolution losses (often called partial volume errors), in which background tissue is admixed with target tissue within a voxel and thus dilutes the signal. Methods to correct for these effects can introduce large uncertainties due to assumptions of target tissue size, shape, and uniformity (Soret et al., 2007). Therefore, 'truth' regarding bias can rarely be determined in human studies. Careful calibration of scanners with a phantom of known activity concentration and protocol definition and monitoring are the primary methods to assure quantitative accuracy of PET imaging.

There have been several studies of the repeatability of FDG-PET measurements of tumor SUVs in cancer patients. Minn et al. showed that changes in maximum SUV (SUV_{max}) greater than 20% were likely to be significant in single center test/retest studies of untreated lung cancer patients with FDG-avid tumors (Minn et al., 1995). In the multi-center American College of Radiology Imaging Network (ACRIN) trial, lower and upper repeatability coefficients (RC) for SUV_{max} were −28% (95% confidence interval (CI): −35% to 23%) and +39% (95% CI: 31% to 54%). The corresponding RCs from the Merck trial, in which tumors were less FDG-avid, were −35% (95% CI: −42% to −29%) and +53% (95% CI: 41% to 72%) (Weber et al., 2015). The multicenter data suggest that a minimum detection limit for tumor progression or regression in an individual patient requires a fairly substantial change in SUV_{max} of >30%. There is also some data regarding repeatability of metabolic tumor volume and total tumor lesion glycolysis metrics, though these may be

more subject to variance. Given the dynamic nature of BAT volume and activation state, repeatability of PET metrics is not expected to be better for BAT than for tumors.

2. Workshop Panel Recommendations

2.1. Strategies to Reduce Data Variability and Maximize Reproducibility of BAT Measurement

While the experiences of oncologic imaging specialists with FDG PET/CT imaging are instructive for researchers interested in human BAT, there are salient differences in study goals and in the metabolism of the two tissues. The oncological goal for FDG-PET/CT is to find abnormal areas of high FDG uptake and to monitor changes in number, size, biological stage, and signal intensity of these areas and any new foci over time and in response to treatment. Because tumor cells have a fairly consistent rate of glucose uptake over time, SUV_{max} is an accepted marker to monitor such clinically relevant parameters as progression-free survival for some tumors (Bengtsson et al., 2015). In contrast, the goal for BAT studies is to measure the presence, mass and activation state of BAT, estimate the contribution of the entire BAT organ to energy expenditure and/or substrate metabolism, and identify any changes in these parameters that occur in response to interventions. The amount of FDG uptake by BAT under activating conditions such as cold exposure estimates overall BAT metabolic activity. Therefore standards developed for FDG-PET studies in oncology must be modified for use in studies of BAT.

The PET Response Criteria in Solid Tumors (PERCIST) 1.0 (Wahl et al., 2009) are minimum standards for study conduct and reporting, to allow well-founded and consistent comparison of oncology FDG-PET clinical study quality and patient response to treatment. We believe a similar approach should be used to standardize the FDG-PET measurement of human BAT mass and activity, which we designate 'Brown Adipose Reporting Criteria in Imaging STudies' (BARCIST 1.0). BARCIST 1.0 includes a consistent method of patient preparation with cold stimulation as one of several approaches, which must be documented fully. In addition, details of the image acquisition parameters and fundamental analytical approaches are proposed. Compliance with BARCIST 1.0 criteria would not preclude additional analyses that are appropriate for the specific study. They would however provide a baseline for reporting in the BAT literature to help allow greater inter-study comparability. These reporting criteria will almost certainly need to be updated as we learn more about BAT biology.

2.2. Participant Characteristics

Considerable effort to determine the incidence of BAT has resulted in differences of opinion concerning the effect of sex, age, body weight and body composition. Therefore, it is important to document study participant characteristics as completely as possible (Table 1). If possible, lean and fat body mass and the technique used to measure or estimate them should be reported along with body weight, height, and BMI.

Diet is likely to impact BAT detection (Vosselman et al., 2013, Williams and Kolodny, 2008) and is difficult to account for, but fasting for a minimum of 6 hrs prior to study can help

reduce FDG uptake variability. Significant changes in weight are known to affect BAT volume and activity in obese mice (Wu et al., 2014) and obese human subjects (Vijgen et al., 2012, Orava et al., 2013), so unless weight change is dictated by the study design, investigators could consider excluding human participants if there is greater than a 5% change in weight in the preceding three months based on metabolic studies (Tuomilehto et al., 2001). Several classes of drugs are known to modulate BAT function, most notably sympathomimetics and sympatholytics, so these drugs should be in the exclusion criteria unless they are used in the study design (Parysow et al., 2007; Cypess et al., 2015). All other prescription or non-prescription medications should be reported. Participants should not be habitual tobacco users, since nicotine can modulate sympathetic nervous system output (Baba et al., 2007b), and regular smokers who temporarily cease their tobacco intake are at risk for withdrawal symptoms. Unless specified by the study, caffeine and capsinoids should be avoided for 48 hours prior to BAT imaging (Yoneshiro et al., 2013). If fasting blood glucose is >11 mM during a pre-scan screening visit, steps are needed to reduce it prior to the day of study (Graham et al., 2015). It is not yet known whether the phase of the menstrual cycle has clinically significant effects on BAT activity in women, but it is suggested it be recorded. Table 1 includes factors that should be considered in the exclusion criteria.

2.3. Climate

Outdoor temperature can affect BAT volume as well as activation potential (Huttunen et al., 1981; Cohade et al., 2003b; Vosselman et al., 2013). To foster the ability to compare studies across populations, the geographic location, season of the year, and outdoor temperature range during the study should be reported. If participants must undergo a series of imaging studies with an intervention (other than temperature) that is expected to alter BAT volume, the investigator might consider steps to control for seasonal temperature variation. One suggestion is to complete the study of any one subject within six weeks, although this presents a challenge. A more reasonable approach would be to include suitable control groups.

2.4. Subject Preparation Prior to PET/CT Scan

BAT is unique among tissues in that glucose (and FDG) uptake is stimulated acutely by both norepinephrine and insulin (Orava et al., 2011; Figure 3 in van der Lans et al., 2014). During cold activation, norepinephrine stimulates thermogenesis, which drives glucose uptake by BAT. Post-prandial insulin release stimulates glucose uptake without increasing thermogenesis and must be avoided in any study where the goal is to estimate increased BAT thermogenesis due to cold stimulation. In addition, insulin-induced glucose uptake into other tissues increases the background FDG-PET signal and reduces the ability to distinguish BAT from skeletal muscle. BAT signal intensity may even be reduced due to insulin-activated tissues (e.g., skeletal muscles) ‘stealing’ FDG from BAT (Vosselman et al., 2013). Participants should therefore fast for at least 6 hours prior to the injection of FDG, and drink only room temperature water during that time. Evidence shows that fatty foods can suppress BAT glucose uptake (Williams and Kolodny, 2008), likely in favor of fatty acid and triglyceride consumption, so participants could be asked to avoid high-fat meals within 24 hours of the FDG-PET/CT study.

Hyperglycemia may reduce FDG-PET signal intensity in activated BAT. Therefore, plasma glucose should be measured within three hours of the radiotracer injection, and its value should be reported. Glucose should ideally be <7 mM, and must be <11 mM. If blood glucose is >11 mM, the study should be postponed or the participant excluded (Graham et al., 2015). These considerations are particularly important for participants who have diabetes or pre-diabetes. Insulin injections can substantially alter the biodistribution of FDG, and participants with diabetes should not have received regular insulin just before scanning, in order to avoid confounding from insulin-induced FDG uptake into BAT. Alternatively, a tracer other than FDG could be used. For instance, [18F]fluoro-thiaheptadecanoic acid uptake during cold exposure was unaffected by diabetes (Blondin et al., 2015a). However, quantitative PET imaging may not be reliable in participants with insulin-requiring diabetes and is an area requiring further study.

Unintended environmental cold exposure can activate BAT, and should therefore be minimized prior to the experiment. Participants should stay warm while traveling to the research site. During cold weather investigators should house their participants overnight in a warm room prior to the study, if possible. Ideally, participants should remain at thermoneutrality for at least 12 hours prior to BAT stimulation (cold or pharmacological agents). While the precise ranges of thermoneutrality in heterogeneous human subjects remain to be defined, it is advisable to keep the subjects dressed in sufficient clothing and housed in a comfortably warm room temperature (such as 24 °C with sweat pants, T-shirt, and socks) without inducing piloerection (goose bumps), shivering, or sweating. One quantitative approach to assess thermoneutrality is to target a zero finger-underarm temperature gradient (Sessler, 2003). Exposure of a study participant to temperatures below thermoneutrality for the purpose of BAT activation may cause fat oxidation and reduction in BAT fat content that is detectable as a change in CT radiodensity. The recovery of BAT fat content over the subsequent hours is likely to be accompanied by increased glucose uptake, which could impact tracer uptake in an FDG-PET scan. Therefore, cold and warm exposure interventions should be separated by at least 48 hours. For similar reasons, intensive exercise should be avoided during the 24 hours preceding a measurement of BAT activity. Experimental data are needed to determine whether shorter periods of cold and exercise avoidance, or shorter time between temperature interventions could be used without compromising the outcome of the experiments.

2.5. Cold-Exposure – BAT Activation Paradigm

Clothing impacts the experience of cold and can alter the required temperature for BAT activation. All participants, male and female, should wear light clothing with the same degree of thickness and coverage. Several convenient options exist, such as hospital scrub suits. The thermal insulation value of clothing (clo) should be reported (International Standard, 2004).

There are two principal approaches for cooling participants to stimulate BAT activity: exposure to a fixed, defined temperature, or a personalized cooling protocol that cools to a specific physiological response (Ouellet et al., 2012; Cypess et al., 2012; van der Lans et al., 2014). A third consists of placing one or more limbs on ice, but this is not recommended

since it stimulates the sympathetically-mediated response to pain, which may confound the effects of cooling on BAT and other organs. The workshop panel agreed that the two approaches were each suited to different questions concerning BAT epidemiology or physiology. The fixed temperature method appears to be suitable for comparing cold-induced physiological responses between similar individuals at a standard environmental condition, while the personalized approach is likely needed for longitudinal intervention studies expected to alter cold tolerance, and for comparing between groups of dissimilar participants. Other sources of variability include age, different fat distributions such as those that differ between men and women, disease state, or recent weight change. However, even similar participants often differ in their sensitivity to cold. The personalized protocol is likely most often more appropriate, and is recommended by the workshop panel for maximizing BAT activation.

The fixed temperature method involves choosing a specific temperature for the cooling intervention, whether it is air temperature or that of water in a cooling vest, suit or blanket (Ouellet et al., 2012). The fixed temperature method is straightforward to use because the temperature target and duration are consistent across participants. These parameters must be chosen to achieve BAT activation while minimizing shivering. As an example from one group, for air cooling the thermoneutral temperature was about 24 °C , and effective cooling without shivering took place at 16 °C for young, lean men w earing jogging shirts and pants (clo=0.71) (van Marken Lichtenbelt et al., 2002). In comparison, another group used air cooling of 19 °C vs. 24 °C in young healthy men and women wearing ho spital scrubs (clo=0.55) (Chen et al., 2013). In a study in women with anorexia nervosa and normal weight controls, the room temperature was 19 °C and the cooling vest was cool ed to 17 °C (Bredella et al., 2012). Of note, the extent of cooling is affected by both the temperature of the room air and the water circulating in the vest or suit, so both parameters should be kept constant throughout a given study, and the specific values should be reported.

In the personalized protocol using a cooling vest/suit, the coolant ideally begins at or near skin temperature and the coolant temperature is decreased in defined increments until participants subjectively report shivering, confirmed by continuous measurement of muscle activity by surface electromyography (EMG) combined with subjective and/or observed shivering. While surface EMG can measure muscle electrical activities, deeper shivering muscles (e.g., scalenes, psoas) may not accessible to surface electrodes. Moreover, EMG signals are highly sensitive to position, orientation, skin conditions, and thus have to be carefully normalized to either resting/non-shivering conditions or to isometric maximum voluntary contractions. The latter can be difficult for some muscle groups to measure. Of note, both fixed and personalized cooling assumes that there is limited variability within a subject in (a) the extent of BAT activation at any specific temperature, and (b) the temperature at which maximum BAT activation is achieved. Neither of these two parameters is currently known and determining them is an important area of future investigation. Of note, the level of cooling in the personalized protocol is dependent on the cold sensitivity of the participant, which can be altered by cold acclimation (Hanssen et al., 2015). Thus, in repeat experiments done on the same participant for studying the potential effects of an intervention, the conditions and timing of cooling should ideally be the same before and after the intervention. When a cooling vest/suit is used, the coolant temperature displays on

the controller may be different than what is the actual temperature at the vest/suit-to-skin contact surface. We recommend measuring skin temperature changes.

Shivering should always be monitored and minimized. It substantially increases the measured amount of energy expenditure in tissues besides BAT, and if shivering occurs after FDG has been injected, the increase in skeletal muscle activity may lead to increased glucose uptake and signal background, and have the effect of diverting the circulating FDG from BAT, thus reduce the FDG-PET signal intensity and underestimate BAT activity. It is possible that BAT activity per se can be altered by the onset of shivering (Lee et al., 2014b). Therefore, shivering should be minimized for 60 minutes before and after the injection of FDG.

While there are no published studies that specifically establish the optimal cooling period, the workshop panel recommended cooling for a minimum of 60 minutes prior to intravenous FDG injection. Following the injection, BAT studies have used the standard practice established in other applications of FDG-PET, of waiting 60 minutes (55 to 75 minutes acceptable) after injecting FDG before imaging, during which cold exposure must continue and it is recommended that study participants remain quiet in a resting state with minimum physical activity. It is likely not necessary , and indeed may be logistically impractical, to continue cold exposure once the subject is in the PET/CT scanner, because FDG is trapped as FDG-6-phosphate once it enters a cell and uptake is largely completed after 55 minutes. This highly useful biochemistry does not necessarily occur with other radiotracers (e.g. [11C]acetate), with Magnetic Resonance-based methods, or when dynamic FDG-PET analyses is applied, and participants must be kept cool during imaging with these approaches (Blondin et al., 2014).

It is now known that besides mild cold exposure, drugs in the class of β3-adrenergic receptor agonists can also stimulate human BAT glucose uptake (Cypess et al., 2015). At this point in time, additional experience with these drugs is needed before recommendations could be constructed for standard protocols that employ these drugs.

2.6. FDG-PET/CT Examination

Table 2 lists the scanning parameters likely to impact the ability to compare data among studies and which therefore should be reported. In addition, many hormones and metabolites exhibit a circadian rhythm, and the time of day may therefore be important in regulation of BAT activity. It is likely a good idea to study all participants at the same time within each study and report the scan time in publications.

Most groups use static FDG-PET/CT for imaging BAT activity due to its simplicity and reproducible tracer uptake (at least as shown in tumor imaging studies) when performed under standardized conditions. An alternative design collects the entire dynamic time-activity curve of tissue FDG and generates additional information about BAT function including a more quantitative measure of the rate of tissue glucose metabolism. However, a limited number of research groups have the technical expertise and additional image analysis software to do this experiment, and the workshop panel therefore did not consider additional standards for dynamic imaging experiments. This subject can be addressed in the future as

more dynamic FDG experiments are performed for the purpose of measuring BAT mass and activity.

A perceived risk to research participants undergoing FDG-PET/CT is due to the radiation exposure from both the PET tracer and the ionizing radiation from the CT scanner. Although risks from a standard PET/CT scan are in general minimal (AAPM, 2011), concerns about risk may adversely affect patient recruitment. The workshop panel recommends using the lowest possible FDG dose that results in statistically adequate, interpretable data. A reduced FDG dose can generally be compensated with increased acquisition time, although it is recommended that scan time be minimized for participant comfort, preferably 60 min or less. The optimal injected dose for a heterogeneous group of participants depends on experiment design. For a single study, a higher dose may be used, whereas for test/retest studies, a lower dose may be more suitable. The choice is particularly difficult and important in studies involving participants with a high BMI or a wide range of BMI (Geismer et al., 2015). Informed by specific guidelines in the EANM/SNMMI Procedure Guidelines for Tumor PET/CT Imaging (Boellard et al., 2015) and the UPICT protocol (Graham et al., 2015), the workshop panel feels that 0.1 mCi/kg may be reasonable for many studies. While reduced radiation from CT is an important goal, reduced exposure must be balanced against photon starvation artifacts, particularly in regions of high attenuation, such as the shoulders where the principal supraclavicular BAT depots are found. Photon starvation artifacts will impair BAT activity quantification strategies that utilize both FDG-PET SUV and CT radiodensity (HU). Some groups position participants with their arms up to reduce the mass of bony structures directly in line with supraclavicular BAT (Cypess et al., 2015).

Based on autopsy and whole-body FDG-PET/CT data, the principal regions of BAT activity are found between the base of the skull and the lower abdomen. Therefore, although CT imaging protocols often continue past the umbilicus, it appears there is little additional knowledge gained from scanning into the pelvis with concomitant exposure of the reproductive organs to external ionizing radiation. In practice, the majority of BAT activity lies within the cervical-supraclavicular-axillary depot, so when the study goals focus only on that region, radiation exposure from CT will be minimized. These considerations become more important when the study design requires FDG-PET/CT scans at several time points, and multiple adjustments to the standard clinical scanning protocol may be necessary to reduce radiation exposure.

2.7. Data Analysis and Quantification of BAT Volume and Activity

With current technology, it is not yet possible to non-invasively measure the total mass of brown adipocytes in an individual. Also, as with WAT, BAT is a mixture of several cell types in varying proportions. Therefore, the term BAT is commonly used to refer to regions of adipose tissue with enough FDG uptake to be distinct from the other WAT depots. The overarching goals of the FDG-PET/CT approach are to measure the total volume of BAT in the whole human body or a given anatomical region, its maximal thermogenic impact in units of heat produced (or energy expended), and its actual activation state in response to a given stimulus. Ideally, BAT volume and activity should be independently measured, and PET and CT seem to be an outstanding combination of modalities since CT provides

information about fat depot volume and fat fraction within that volume, and PET provides signal related to BAT substrate consumption and thermogenesis. In practice, it is difficult to unequivocally identify BAT with FDG PET/CT in the inactive state, and more reliable estimates of volume and activity can best be made under activating conditions by combining PET and CT data, and choosing appropriate thresholds for SUV and HU to signify the presence of BAT. Thresholds that are too stringent will result in an underestimation of measured BAT volume and activity, while thresholds that are too low will yield overestimated BAT volumes. At this point in time there are no unequivocal means to validate a specific choice of HU thresholds for BAT volume analysis in people, and because of variation in CT scanner settings, a given threshold may not be suitable for every dataset. In order to best serve the research focused on understanding the incidence and physiology of human BAT, the workshop participants recommend the use of relatively low SUV and reasonably wide HU thresholds that maximize the ability to detect human BAT, and therefore accept the risk of overestimated BAT volume and total calculated activity (Table 1).

The panel recommends a threshold of $SUV_{bm} \geq 1.5$ as a starting point to define the volume of activated BAT within an ROI, based on collected experience (Blondin et al., 2014; Cypess et al., 2015). However, there is a potential for ‘overdetection’ of BAT if this threshold is used in obese subjects, where normal organ SUV can be 25–30% higher than in lean people (Tahari et al., 2014). Therefore, just as appropriate normalization of the FDG-PET signal increases the chances of consistently quantifying a tumor, SUV as an estimate of BAT thermogenesis can likely be improved by using an appropriate index of body mass. Given that FDG uptake is much lower for typical white fat than for lean tissue, a measure of LBM is needed for optimal interpretation of PET data, especially for metabolic studies. LBM can be used to calculate SUV_{lean} ($SUV_{lean} = SUV_{bm} \times LBM/BM$), and for lean young men, LBM/BM ~ 0.80, which yields an absolute lowest threshold of $SUV_{lean} \geq 1.5 \times 0.8$ or $SUV_{lean} \geq 1.2$. It is recognized that use of SUV_{lean} and a fixed threshold is not a perfect solution for analysis of PET data, as WAT does take up some FDG, and 1.2 may be too low a threshold to distinguish activated BAT from WAT or blood pool , for some participants such as those that are obese. Another choice is to select an individualized threshold for SUV_{bm} based on body composition, defined as the minimal threshold ($SUV_{lean} \geq 1.2$) divided by actual LBM/BM for that participant. For a person where LBM/BM = 0.6, the threshold would be $SUV_{bm} \geq 1.2/0.6 = 2.0$. It is further recommended that LBM be measured directly using a reliable method such as dual-energy X-ray absorptiometry (DXA). Values to be reported (for both SUV_{bm} and SUV_{lean}) include SUV_{max} defined as the signal in the most FDG-avid voxel within the BAT anatomical region, and SUV_{mean}, which is the average SUV within an entire volume denoted as BAT. While an additional parameter gleaned from the oncology field, SUV_{peak}, may have limited utility to describe activation state in small BAT depots, it may also be less variable and less subject to noise than SUV_{max}, and thereby facilitate cross-study comparison. SUV_{peak} is defined as the average SUV in a 1 cm volume sphere centered within the most active BAT in the body.

Radiodensity, as represented by X-ray attenuation coefficients and measured in CT as HU, provides useful anatomical information and signal contrast between bone, lean and adipose tissues. The distinct difference between HU of lean (i.e. positive HU values) and adipose

tissues (i.e. negative HU values) facilitates tissue identification and in particular adipose tissue segmentation. Current data suggests that for a properly calibrated CT system where pure water has a HU representation of 0±4 HU, the inclusive range for adipose tissue is roughly −190 to −10 HU unless substantially admixed with lean tissue. Within this negative range, the specific HU values with which BAT can be represented can vary, dependent on the proportion of white adipocytes within the BAT depot, the amount of lipids stored within BAT adipocytes, the level of intra- and extra-cellular water present, the degree of blood flow (i.e. perfusion) to the tissue, and the extent of BAT activity. Furthermore, the fraction of white adipocytes in BAT and the intracellular fat content of BAT are both altered subsequent to BAT activation. BAT mean HU values are higher than those for WAT, and the HU of BAT in adults with high FDG uptake is significantly higher (−71.6 ± 18.0) than in those with low FDG uptake (−104.4 ± 16.8, Baba et al., 2010). A narrower mean HU range has been reported in BAT of children (−75 to −55, Hu et al., 2011). Therefore, while HU of activated BAT will most commonly be negative, some individual voxels that are FDG-avid and contiguous may have a positive HU value. It is suggested that these voxels be included in estimates of total BAT volume, as they may represent admixed BAT and muscle, be due to the inferior resolution of PET than CT, and/ or be due to minor misregistrations between PET and CT. Even for the same tissue the measured attenuation will vary with the CT acquisition peak kilovoltage (kVp) setting and scanner model, due to changes in the x-ray energy spectrum. Therefore future studies are needed to determine the differences of including adjoining areas of high FDG uptake. The panel suggests using a CT tube setting of 120 ± 10 kVp and adequate x-ray current (mA) to quantify fat HU. In children, lower kVp and current are often used to reduce radiation dose, and this may affect the ability to quantify BAT HU. The panel recognized that the most appropriate negative HU cutoff values for BAT need to be determined on a study-by-study basis and consistently reported in the subsequent literature publications.

The use of a fixed volumetric ROI (VOI) based solely upon the CT HU measurements to estimate BAT volume has several additional limitations. A study designed using repeated measures of BAT mass and activity in response to an intervention presents an especially difficult problem as BAT mass can change, yet the chosen VOI presumably should be constant for all scans and employ the same volume in the same anatomical region across time so as to reduce bias. If the VOI has been defined in the control condition (e.g., the warm temperature state), it may underestimate BAT volume after an intervention that results in browning. On the other hand the method has advantages in intervention studies for within-subject comparisons when BAT FDG uptake is lower than the recommended SUV threshold, and secondly for comparison of glucose uptake between different tissues (van der Lans et al., 2013; Hanssen et al., 2015).

As stated above, a better estimate of BAT volume can be made by selecting those voxels where thresholds are met for both HU and SUV. Care must first be taken to ensure that CT and PET images are spatially well registered (Gifford et al., 2015). Still, this approach can lead to inaccurate estimations of BAT activity since FDG-PET signal tends to spread out beyond the regions defined by HU due to partial volume effects or where BAT is mixed with considerably lean tissue, and if this signal is not counted, BAT activity is underestimated. One potential remedy (that would require validation) is to first define potential BAT using a

fixedVOI or ‘mantle’ that is larger than that defined as BAT in the CT image, to account for signal spillover outside the voxels of origin, but devoid of likely false-positive areas such as glands, blood vessels, skeletal muscles, and air spaces. Next, BAT volume can be further defined as the summed area of voxels found within the VOI where SUV is above a given threshold (Chen et al., 2013), or that meet both SUV and HU criteria (Lee et al., 2014c). Volume multiplied by the SUV_{mean} yields an estimate of total BAT activity in the VOI. Such an approach may be appropriate for studies that employ repeated measures of BAT over time and/or compare within-subject differences, especially with interventions that alter BAT volume and activation potential such as long term exposure to different temperatures. Just as VOIs that are too small lead to an underestimation of BAT activity, BAT volume is likely to be overestimated if large VOIs are chosen, again due to partial volume effects for PET signal and the tendency to include non-BAT tissues that fall within the VOI. Therefore, special care must be taken to avoid including activated muscle tissue or large blood vessels. No automated software exists to aid in establishing an appropriate mantle VOI. If this approach is chosen, the details of the protocol used to define the VOI should be described, and the total volumes and location should be reported.

In some instances, such as when reliable CT data are not available, it may be useful to use tracer uptake in a reference tissue to establish the threshold for BAT activity, as recommended in the PERCIST criteria for tumor detection (Wahl et al., 2009). This approach requires the assumption that intense FDG uptake in expected locations of BAT reflects actual BAT activity. Care must be taken that FDG uptake in the reference tissue is stable. For example, liver SUV_{lean} can change when exposed to sympathomimetic drugs. Other tissues commonly used as an internal standard include the aorta blood, large skeletal muscles such as the erector spinae, or cerebellum (Vosselman et al., 2013). The use of a reference tissue in FDG-PET studies of BAT has yet to be validated. However, the workshop panel suggests that SUV in liver (measured in a 3 cm spherical VOI placed in the right lobe of normal liver as defined in PERCIST (Wahl et al., 2009) and descending aorta be reported to facilitate comparison among studies and provide the relationship between background activity and the SUV threshold used to define BAT. As an extreme example to indicate why this data is useful, if the blood SUV is higher than the BAT SUV threshold, BAT volume and activity will likely be overestimated.

“Total body BAT glucose metabolism” is the FDG-PET/CT metric that may best reflect total BAT energy expenditure upon cold exposure, and is defined as FDG uptake summed over the entire body for all PET image voxels located in BAT. Total body BAT glucose metabolism is, however, technically very difficult to measure. The location and mass of BAT are highly variable among participants, highly elastic within an individual over time, and can be spread over many small depots in the neck, spine and peri-organ regions. Even the biological definition of BAT volume is less than clear. BAT depots tend to be highly heterogeneous with a large variation in white adipocyte fraction, and so the volume of the tissue depot defined either by PET or CT will likely not equal the brown adipocyte cell volume. Therefore total 'BAT metabolic volume' calculated using FDG-PET/CT data ($BMV_{PET/CT}$, defined as ml of tissue consistent with BAT, where the SUV and HU thresholds are met) is likely to underestimate the true volume of BAT. Similar issues exist for the measurement of BAT activity and its contribution to whole body energy expenditure.

Although BAT FDG uptake correlates with whole body non-shivering thermogenesis in many studies, there remains a large amount of variation that is unexplained. One reason may be that fatty acids, not glucose, contribute the bulk of substrate for brown adipocyte thermogenesis, and another may be variable levels of insulin action. The relation therefore depends on the physiological state and the presence of other nutrients in addition to measurement inaccuracies in both parameters and potential contribution of other tissues (Ouellet et al., 2012; Blondin et al., 2015b). Hopefully other techniques will arise that can yield independent measures of BAT volume and thermogenesis.

3. CONCLUSIONS

We propose BARCIST 1.0 criteria (Table 1) as current minimum suggested guidance for performance, analysis and reporting of FDG-PET imaging studies of BAT in humans. FDG-PET/CT is nominally a quantitative technique, but there are many assumptions and choices made during subject preparation, data acquisition and data analysis that impact on the final calculated total BAT volume, FDG uptake, and the use of these numbers to estimate total tissue energy expenditure. It is therefore imperative that certain of these assumptions and choices be recorded in published reports as detailed in Table 1.

There are still relatively few published FDG-PET/CT studies of human BAT, and therefore there is no widely acceptable, optimized, validated approach to data analysis or data reporting. All recommendations noted here are therefore merely meant to provide consistency so that data from many studies can be directly compared, but in no way prevents investigators from using other additional suitable methods of data analysis in addition.

The BARCIST 1.0 recommendations leverage the experience to date, but are not yet fully validated. There are several activities that could help in validation. For instance, considerable FDG-PET/CT data on BAT in humans exists and perhaps could be mined in order to improve the recommendations. This approach could help identify the optimum SUV normalization strategies, the SUV and HU threshold values, and stable internal standard tissues across many experimental conditions. PET studies of human BAT would be greatly facilitated with a defined automated process for quantifying BAT metabolic activity that employs validated algorithms to measure BAT volume that exclude bone, air, lung, and skeletal muscle. Tools and recommendations should be tested in several labs on shared de-identified data sets. Finally, the authors are happy to improve BARCIST recommendations using community input. Researchers are encouraged to send suggestions to the authors at barcist1.0@gmail.com.

Acknowledgments

This research was supported in part by the Intramural Research Program of the NIH, The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the National Cancer Institute (NCI).

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Box 1

Elements of BARCIST 1.0 Criteria

- Standardized report of participant characteristics
- Standardize and report patient preparation, including cooling protocol
- Standardize and report PET protocol (time from tracer injection to imaging, injected radioactivity dose, etc.)
- Methods of acquisition consistent with UPICT (Graham et al., 2015) or EANM (Boellaard et al., 2014) standards
- Standardized experimental criteria for scanner performance, in order to compare across sites
- Normalize tracer uptake values to lean or fat-free body mass (SUV_{lean}) as well as total body mass (SUV_{bm})
- Report active BAT volume based on SUV_{lean} and HU ranges and maximum BAT SUV_{lean} peak.
- Report presence and level of BAT activity within a specified anatomical field of view (FOV) to include at a minimum the neck, shoulders and clavicular region
- Defined criteria for minimum calculated BAT mass and metabolic activity

Box 2

Lexicon

BARCIST: **B**rown **A**dipose **R**eporting **C**riteria in **I**maging **ST**udies, derived from PERCIST for use with FDG-PET/CT studies of BAT.

BAT: brown adipose tissue

BM: total body mass

BMV_{PET+CT}: BAT metabolic volume derived from CT and PET, defined as the summed volume of all voxels where FDG-PET $SUV_{bm} > 1.5$ and CT HU is between −190 and −10, in expected areas for BAT (neck, subclavicular, mediastinal, etc.). It can also be normalized for body composition as suggested in Table 1.

CT: x-ray Computed Tomography

FDG: [18F]fluorodeoxyglucose, a radiotracer of the first steps in glucose metabolism in the body that can be monitored with PET. FDG is transported into tissues and retained there due to phosphorylation by hexokinase, but it is not further metabolized. Only tissues with glucose-6-phosphatase (principally liver and kidney) can dephosphorylate and release FDG back into the blood. FDG is avidly taken up and substantially retained in activated BAT.

LBM: lean body mass

PERCIST: Positron Emission Response Criteria in Solid Tumors, a formalized method for assessing treatment response with FDG-PET/CT, which includes methodological details for study performance and analysis.

PET: Positron Emission Tomography

ROI: region of interest (2D)

SUV_{bm}: Standardized Uptake Value = (radiotracer uptake in μCi/ml tissue as determined from PET image) / (total injected radiotracer activity in μCi/g body mass)

SUV_{lean}: SUV normalized to lean (fat free) body mass instead of total body mass. Lean body mass ideally should be measured directly with a validated and reproducible method, but can be estimated using the Janahasastian Formula if no direct measure is available (Tahari et al, 2014).

$SUV_{lean/liver}$: $SUV_{lean\ mean}$ in 3 cm sphere in the mid right lobe of the normal liver

$SUV_{lean/max}$: SUV_{lean} in the single most FDG-avid voxel in anatomical regions of BAT

$SUV_{lean/mean}$: average SUV_{lean} in any volume of BAT

$SUV_{lean/peak}$: average SUV_{lean} in 1 cm volume sphere of the most active BAT in the body.

VOI: volume of interest (3D)

WAT: white adipose tissue

Brown adipose tissue was recently discovered to be a functional organ in adult humans, with great therapeutic potential. To better advance the field through standardization of experimental methods and reporting, Chen et al. provide recommendations (“BARCIST 1.0”) by an expert panel for conducting human clinical studies using FDG-PET/CT.

Table 1

Checklist and recommendations for human FDG-PET/CT experiments of BAT.

Participant Characteristics	**Recommendation**
Age, sex, ethnicity/race, height, weight, BMI	Report
Lean (fat free) and fat body mass	Report (including method of determination)
Prescription and over counter medications	Report
β-blockers, β-adrenergic agonists	Exclusion criterion
Weight change of >5% within 3 months	Exclusion criterion (if weight change prior to the study is expected as part of the study design, consider using dynamic PET/CT FDG or the use of another tracer in combination with FDG)
Habitual tobacco use	Exclusion criterion
Habitual excessive alcohol use	Exclusion criterion
Menstrual cycle phase, hormone replacement therapy use	Report Recommend that participants be studied at same phase if possible
Pregnancy	Exclusion criterion
Plasma Glucose	Exclude or control if >11mM
Subject Preparation	
Meals 24 hours before scan	Avoid high fat foods
Caffeine 24 hours before scan	Not recommended
Fast duration before scan	>6 hours
Pharmaceuticals	Report
Fasting plasma glucose (within 3 hours of tracer injection)	Report Should be <7mM. Do not proceed with experiment if >11mM
Strenuous activity within 48 hours of scan	Not recommended
Clothing during scan	Report thermal “R” insulation value (CLO) Examples of acceptable clothing: hospital gown, scrubs, tee shirt and shorts
Environmental (room) temperature	Report if subject was exposed to cool temperatures within 12 hours of cooling period or scan
BAT Activation / Cooling Protocol	
Fixed or personalized cooling paradigm	Report Recommend personalized paradigm if the study population is heterogeneous or if an intervention is used that is expected to change BAT volume or activation potential. Exposure conditions should be the same for repeated tests on a participant.
Cooling device	Report Recommend room air; water in cooling vest, suit, or blanket
Air or Coolant temperature at shivering (if any)	Report Room air or water in cooling vest, suit, or blanket.
Coolant temperature during cool period	Report both room air temperature and water temperature in cooling device, if used
Total duration at cool temperature	Report

Participant Characteristics	Recommendation
	Recommend minimum of 60 min. (after any incidence of shivering) prior to injection and ~60 min. after injection (until scan)
Warm temperature (if applicable)	Report
Duration at warm temperature (if applicable)	Report
Method used to monitor skin temperature	Recommend surface temperature probes at multiple sites for continuous recordings.
Method used to monitor shivering	Report Recommend EMG, observation, and/or self-report (in order of decreasing preference) Shivering should be minimized for 60 min. before and after tracer injection.
PET/CT Examination	
Manufacturer, model of PET/CT machine	Report Recommend using the same scanner for all scans within a study, especially for test/re-test in same participant
Data acquisition	Methods should be consistent with UPICT, QIBA and/or EANM standards.
Reconstruction algorithms, reconstruction parameters and reconstruction software version used	Report Record software version number if possible, and recommend using the same software version for all images within a study
FDG dose, site of injection	Report Recommend using a dose as low as possible for statistically valid imaging, with consideration for total dosage in repeat studies.
Method used to normalize FDG dose	Report Recommend using lean (fat free) body mass, Measured directly via densitometry, DEXA, or other validated method. If no direct measure is available, it can be estimated with Janmahasatian Formula
Time between FDG injection and PET/CT scan (at cold temperature if cooling is used)	Report Recommend target 60 minutes with 55–70 minutes range
Time of day for scan	Report using 24-hour notation Recommend that all scans within a study be done at approximately the same time of day, if possible
Geographic location, time of year, outdoor temperature range	Report (latitude and longitude) For longitudinal interventional studies, recommend completing all scans within a single season or using an appropriate control group
Volume of water intake between injection and scan	Report Recommend drinking water be lukewarm such that participant perceives no difference between water and room temperature
Duration of PET scan	Report Recommend less than 60 min
PET acquired voxel sizes and Field of View	Report Recommend that PET FOV to include the base of skull through inferior margin of liver, if possible
CT scan parameters, including kVp, acquired voxel sizes, tube power and Kv used, and Field of View	Report Recommend kVp = 120 ± 10 Recommend excluding pelvis to minimize

Participant Characteristics	**Recommendation**
	radiation dose
Extent and Duration of CT scan	Recommend base of skull to umbilicus, or as small as possible for study
Data Analysis and Report	
SUV and CT radiodensity scales	Continuous intensity scales should be used. SUV should be reported to 2 decimal places
SUV normalization	Recommend using lean (fat free) body mass to calculate SUV_{lean} (measured directly (i.e., DEXA). If no direct measure is available, can be estimated using Janmahasatian Formulation)
Minimum BAT metabolic activity threshold for calculation of BAT volume	$SUV_{lean} \geq 1.2$ If only the SUV_{bm} is available, convert to $SUV_{bm} \geq 1.2$ / (LBM/BM). (This approximates $SUV_{lean} \geq 1.2$ for lean young men of 20% body fat). Use measured LBM; if not available, use population-estimated LBM.
Hounsfield Unit range used to define adipose tissue (WAT and BAT)	Report Recommend −190 to −10 for all fat (WAT + BAT). VOI should not include obvious non-fat tissues within this range such as lung
BAT metabolic activity	Report $SUV_{bm/max}$ for the hottest single voxel in a VOI within BAT region, and $SUV_{bm/mean}$ for all voxels within BAT region. Report $SUV_{lean/max}$ for the hottest voxel within BAT region and $SUV_{lean/mean}$ for all voxels within BAT region. Report $SUV_{bm/peak}$ and $SUV_{lean/peak}$ for the hottest VOI within BAT region, for comparison between studies as this parameter is expected to vary less than SUV_{max}. Recommend reporting up to six VOI (hottest VOI in left and right supraclavicular region, left and right neck, left and right mediastinal)
BAT metabolic volume (BMV)	At a minimum, report BAT metabolic volume as the sum of all voxel volumes within suspected BAT region where $SUV_{bm} \geq 1.5$ and HU is between −190 and −10. It is recommended that a correction be made for body composition. Therefore, BMV should also be reported in one of two additional ways: **a)** the sum of all voxel volumes within suspected BAT region where $SUV_{lean} \geq 1.2$ and HU is between −190 and −10 or **b)** the sum of all voxel volumes within suspected BAT region where $SUV_{bm} \geq 1.2$ / (LBM/BM) and HU is between −190 and −10. Option b) is suggested for obese participants, although has not been validated. If a fixed volume or ‘mantle’ is used, describe the procedure for selecting it, as well as the volume size and location.
Reference tissue	Report reference tissue if used. To facilitate comparison among studies, recommend reporting mean normal tissue SUV in blood (descending aorta), 3 cm sphere in right lobe of liver (per PERCIST), and cerebellum if included in field of view.
Other data analysis as needed to assess experimental outcomes	Report

Table 2

Experimental factors that can affect quantitative measures of BAT activity and volume from FDG-PET/CT

Factors affecting SUV_{lean} or BMV	**Effects**
PET/CT manufacturer and model, system calibration, and data reconstruction algorithms	Source of variability
Resolution of scanner, image field of view, and matrix size for PET image reconstruction	Poor scanner resolution reduces SUV_{max} especially for small BAT depots Small voxels for image reconstruction result in higher SUV_{max} than larger voxels.
Injected dose of FDG	Lower injected dose increases noise in image and may raise SUV_{max}
Time from injection of FDG to PET imaging	Shorter or longer times from injection can lower or raise SUV
Total PET scan duration	Longer scan times reduce image noise
SUV_{lean} threshold (lower limit) for definition of BAT	Higher threshold will result in lower BAT volume
ROI volume and method of determination	SUV_{mean} is typically reduced as ROI volume is increased
CT acquisition kVp setting	Can change HU values for adipose tissue
CT HU range	Narrow range may result in a smaller volume of BAT being defined