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Brown adipose tissue in humans: detection and functional analysis using PET (Positron Emission Tomography), MRI (Magnetic Resonance Imaging), and DECT (Dual Energy Computed Tomography)

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Abstract

Research with the aim to translate findings of the beneficial effects induced by brown adipose tissue (BAT) on metabolism, as seen in various non-human experimental systems to also include human metabolism requires tools that accurately measure how BAT influences human metabolism. This review sets out to discuss such techniques, how they can be used, what they can measure and also some of their limitations. The focus is on detection and functional analysis of human BAT and how this can be facilitated by applying advanced imaging technology such as: PET (Positron Emission Tomography), MRI (Magnetic Resonance Imaging), and DECT (Dual Energy Computed Tomography).

Key words: Human, Brown adipose tissue, PET (Positron Emission Tomography), MRI (Magnetic Resonance Imaging), DECT (Dual Energy Computed Tomography)

Introduction

The newly established role of brown adipose tissue (BAT) in humans – as a metabolically active tissue – is based on the fact that we and other groups have

identified brown adipose tissue at paracervical and supra-clavicular locations in healthy adults using positron emission tomography (PET) (Virtanen et al. 2009, van Marken Lichtenbelt et al. 2009, Cypess et al. 2009, Saito et al. 2009). These studies showed a several-fold increase in uptake of the glucose analog ^{18}F -fluoro-deoxy-glucose (^{18}F -FDG) in response to cold temperatures. Biopsy specimens of identified tissues were used to assess mRNA and protein levels, which together with typical morphology confirmed BAT identity and made it clear that adult humans have metabolically active BAT (Virtanen et al. 2009). Interestingly, human BAT activity was significantly lower in obese as compared to lean subjects and also significantly negatively correlated to body mass index (BMI) and percent body fat (van Marken Lichtenbelt et al. 2009, Cypess et al. 2009). Moreover, BAT was positively related to resting metabolic rate. It was also noted that women had larger BAT depots and ^{18}F -FDG uptake activity, and that BAT mass positively correlated with younger age. As an example, one of the subjects studied had an estimated supraclavicular BAT depot of approximately 60 grams. It was concluded that, if fully activated, this would burn an amount of energy equivalent to approximately 4.1 kg of WAT over the course of a year (Virtanen et al. 2009). Thus, it is likely that in humans, activated BAT has the potential to contribute substantially to energy expenditure.

Although we know what ultimately causes obesity – it results when energy intake exceeds energy expenditure – today, the only proven therapy is bariatric surgery. This is a major abdominal procedure that, for reasons that remain largely unknown and which cannot be explained solely by a reduction in ventricular volume, significantly reduces energy intake. Due to cost and limited availability, however, this procedure will most likely be reserved for only a small fraction of those who stand to gain from effective anti-obesity treatment. Clearly, alternative ways to treat obesity are needed. Another way to combat excessive accumulation of WAT would be to increase energy expenditure. Rodents, hibernators, and humans all have a specialized tissue – BAT – with the unique capacity to regulate energy expenditure by a process called adaptive thermogenesis. This process depends on the expression of uncoupling protein 1 (UCP1), which is a unique marker for BAT. UCP1 is an inner mitochondrial membrane protein that short-circuits the mitochondrial proton gradient, so that oxygen consumption is no longer coupled to ATP synthesis. As a consequence, heat is generated. It has been demonstrated that mice lacking Ucp1 are severely compromised in their ability to maintain normal body temperature when acutely exposed to cold and they are also prone to become obese. Moreover, it is also known that, in mice, BAT protects against diet-induced obesity, insulin resistance, and type-2 diabetes. This is, at least in part, based on prevention of excessive accumulation of triglyceride in non-adipose tissues like muscle and liver. Ectopic triglyceride storage at these locations is associated with initiation of insulin resistance and, ultimately, development of type-2 diabetes. To collect evidence of a similar role for BAT in humans we need new methods to measure both the *mass* and *metabolic activity* of this tissue enabling accurate assessments of energy turnover of this tissue both under normal and stimulated conditions. This review sets out to give an overview of some of the new ways in which modern imaging technology can be used for this purpose.

PET

Introduction

PET is an imaging technique that enables direct and quantitative observation of tissue radioactivity over time *in vivo*. An almost unlimited number of natural substrates (e.g. glucose, fatty acid), substrate analogs, and drugs can be labeled for use with PET. In general, PET makes it possible to isolate the organ of interest from surrounding tissues and, by mathematical modeling, the quantification of metabolic processes within the target tissue. PET is non-invasive, and allows imaging of several organs during the same session in humans. PET combined with tracer kinetic models measures blood flow, membrane transport and metabolism noninvasively and quantitatively. Thus, PET appears to provide comprehensive insights into molecular processes occurring in tissues *in vivo*.

Tracers for BAT metabolic imaging and principles of modeling

PET is based on the use of short-lived positron emitting radioisotopes (Table 1). Nearly any substrate and drug can be labeled without changing their physicochemical or pharmacological properties (Phelps et al. 1979). Due to rapid decay of the positron emitters most PET tracers have to be synthesized on site. The tracer is injected intravenously to the subject or inhaled as a gas after which it distributes throughout the body via the bloodstream and enters into tissues and organs. Positrons emitted from the nucleus collide with their counterparts, electrons, and the masses of the two particles are converted into two photons emitted simultaneously in opposite directions. PET imaging is based on the detection of these paired photons in coincidence. Physiological and pharmacological phenomena and biological parameters can be estimated *in vivo* by mathematical modeling of the tissue and blood time-activity curves obtained. PET is a highly sensitive and specific imaging tool for studies of BAT physiology (Table 1).

Table 1. PET tracers used in human studies of BAT with quantitative modelling

	Tracer	Half-life (min)
Perfusion (Orava et al. 2011, Muzik et al. 2012)	[¹⁵ O]H ₂ O	2
Glucose uptake	[¹⁸ F]FDG	109

(Virtanen et al. 2009, Orava et al. 2011)

Free fatty acid metabolism	[¹⁸ F]FTHA	109
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(Ouellet et al. 2012)

Oxidative metabolism	[¹⁵ O]O ₂	2
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(Muzik et al. 2012)

(Ouellet et al. 2012)	[¹¹ C]acetate	20
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[¹⁸F]FDG, fluorine-18-labelled 2-fluoro-2-deoxy-D-glucose;
 [¹⁸F]FTHA, fluorine-18-labelled 6-thia-hepta-decanoic acid.

PET is currently the only technique that permits non-invasive quantification of regional tissue perfusion, glucose and fatty acid metabolism, protein synthesis, and oxygen consumption. To this purpose, sequential images are acquired after tracer injection to follow the distribution of the tracer in the system of interest. Regions are drawn over the organ of interest to obtain tissue-specific time-activity curves. These curves, together with corresponding radioactivity levels in plasma (input to the system), are used in tracer kinetic modelling.

The glucose tracer [¹⁸F]FDG is a fluorine-18 labelled glucose analogue, which is transported into the cell and phosphorylated (Sokoloff et al. 1977, Phelps et al. 1979). In contrast to glucose, it cannot enter glycolysis, being trapped for the duration of most PET studies; thus, initial steps in glucose uptake and metabolism can be traced with this radio-pharmaceutical. [¹⁸F]FTHA is a long-chain fatty acid analogue and inhibitor of fatty acid metabolism; after transport into the mitochondria it undergoes initial steps of β -oxidation and is thereafter trapped in the cell (DeGrado et al. 1991, Ebert et al. 1994).

Graphical methods have the advantage of being independent of compartment number and configuration. Gjedde-Patlak analysis was first developed for the assessment of irreversible tracer transfer (Patlak and Blasberg 1985). A graph is generated by plotting:

$$C_t(t) / C_p(t) \quad \text{vs} \quad \int_0^t C_p(t)dt / C_p(t)$$

where C_t is tissue radioactivity at each sampling time point (t) and C_p is plasma radioactivity. When irreversible influx of tracer occurs, the two variables describe a linear relationship after a few minutes of equilibration. The influx constant is then given by the slope of the linear fit of the data, excluding the first few values. If reversible uptake occurs, metabolite loss can be corrected for by using the linearisation method described by Patlak and Blasberg (Patlak and Blasberg 1985). The quantification of the fate of a natural compound, e.g., glucose by use of its analogue, FDG, requires the

introduction of a conversion term, denominated lumped constant, representing the ratio of the metabolic rates of the tracer and the tracee (Sokoloff et al. 1977). Relative to FDG, this numerical value has been shown to approximate unity in skeletal muscle (Peltoniemi et al. 2000), myocardium (Ng et al. 1998) and adipose tissue (Virtanen et al. 2001), and to hold constant across metabolic disease states. Therefore it has been assumed to be 1.0 also for human BAT (Virtanen 2009).

[¹⁵O]H₂O is an ideal tracer for the measurement of blood flow as it is chemically inert and freely diffusible. The kinetics of the perfusion tracer [¹⁵O]H₂O have been validated experimentally and clinically (Iida et al. 1988, Ruotsalainen et al. 1997) and applied to the study of BAT as well (Orava et al. 2011, Muzik et al. 2012). Oxygen consumption may be determined directly in BAT using ¹⁵O₂-PET (Muzik et al. 2012). This tracer is given by inhalation and requires on-site blood radioactivity measurement or the use of image-derived input function for the modeling.

Discovery of BAT in adult humans using PET

The association between the prevalence of supraclavicular ¹⁸FDG accumulation and cold outdoor temperatures was reported (Cohade et al. 2003). This tracer accumulation during diagnostic clinical PET studies was problematic for clinicians. When hybrid PET/computed tomography (CT) scanners became available, the precise localization of FDG accumulation became possible and gave further evidence for active BAT in humans (Nedergaard et al. 2007). However, confirmation and direct evidence of BAT in adults took place after functional imaging was combined with biopsies taken from the active hot spots in 2009 (Virtanen et al. 2009, van Marken Lichtenbelt et al. 2009).

Since 2009, many research and clinical laboratories have actively studied BAT using ¹⁸FDG-PET or PET/CT, but results have been discordant. Although the graphical method is validly used for the assessment of glucose metabolic rate, BAT activity has often been estimated using standard uptake values (SUV) and static late scanning or simply using counts per time unit. The main drawbacks with these semiquantitative methods are that inter-individual differences between tracer distribution volume and changes in its relative distribution and the current plasma glucose concentration are not taken into account. If an obese subject is studied, the distribution of the tracer between different tissues is not similar as compared to a lean one although the dose is adjusted with total body weight. Mathematical modeling becomes mandatory when the effects of hormones and nutrients on brown fat activity are studied. During insulin stimulation muscle glucose uptake is up to six-fold enhanced and covers 60-70% of the whole body glucose utilization (Nuutila et al. 1992). Therefore ¹⁸FDG is cleared from the plasma into muscles, its availability in plasma is decreased and 'counts' are lower in BAT. However, by modeling FDG uptake over time, we were able to demonstrate that insulin is a potent stimulator of BAT activity (Orava et al. 2011). Only dynamic data analysis with input information gives reliable values of BAT activity for comparisons between groups or subjects in interventions.

- FIGURE 1 APPROX. HERE -

Cold activation enhances hBAT perfusion significantly when studied using ^{15}O -H $_2$ O-PET (Orava et al. 2011, Muzik et al. 2012). Insulin stimulation during euglycemia increases BAT glucose uptake five-fold in line with whole body insulin sensitivity but without any increment in perfusion (Orava et al. 2011). When Muzik and co-workers used $^{15}\text{O}_2$ to assess directly oxygen consumption during cold stimulation, MRO_2 values were shown to increase (Muzik et al. 2012). Based on this, the authors estimated that thermogenesis of supraclavicular BAT depots account less than 20 kcal/day. However, they found significant differences in total energy expenditure between BAT+ and BAT- subjects (Muzik et al. 2012). This might suggest that even smaller BAT depots need to be included in the analyses to properly estimate human BAT activity. Although PET and PET/CT or PET/MRI scanners are ideal for functional imaging, they are not updated for the optimal assessment of tiny regions of BAT, which are widely spread over the body. The resolution of PET scanners has improved but it is still between 1 to 4 mm. Also the concept of brite or beige adipose tissue means that we are analyzing data that contains a mixture of white and brown adipocytes.

MRI

Introduction

While PET detects the metabolic activity of BAT, Magnetic Resonance Imaging (MRI) and Dual Energy Computed Tomography (DECT) can be used for imaging the morphology of the BAT organ itself. Importantly, BAT that is not necessarily metabolically active can also be imaged. Furthermore, since MRI does not use ionizing radiation, as opposed to PET and CT, it is more suitable for large prospective studies on healthy volunteers.

A number of different contrast mechanisms have been used for detecting BAT with MRI, for example fat-water fraction determined by chemical shift imaging (Lunati et al. 1999); fat-water fraction in combination with T1 relaxation time (Hamilton et al. 2011); or intermolecular zero-quantum coherence (Branca and Warren 2011). The contrast mechanism used here is similar to that used by Lunati et al. and also in the recent work by Hu et al. (Hu et al. 2010, Hu et al. 2012, Hu et al. 2013) and is based on the higher water content in BAT compared to White Adipose Tissue (WAT). The composition of WAT is typically around 90 % lipid, whereas BAT has a significantly lower lipid fraction. By using multi-echo chemical shift imaging (Dixon 1984), the fat and water signals can be separated based on the difference in their resonance frequencies. Hence, the relative fat fraction can be estimated, and used for detecting BAT. The fat-water ratio itself is, however, not a reliable measure since this ratio is very noise sensitive in areas of low signal strength, e.g. in air. In order to only consider anatomically relevant voxels, we use an absolute image intensity scale where WAT is used as internal reference. Using these calibrated fat images, adipose tissue with any given fat content can be detected.

Multi-echo chemical shift imaging also enables detection of T2* relaxation time of the water and lipid signals (Yu et al. 2007, O'Regan et al. 2008). T2* quantification enables correction of signal loss due to T2* relaxation and also provides an additional parameter for characterization of BAT tissue (Hu et al. 2013). Khanna et al. showed in a rat model the potential of using T2* sensitive imaging to detect BAT activity. In this study a well-localized signal drop was observed in activated BAT caused by a strong increase in

oxygen consumption and consequent increase in blood deoxyhemoglobin levels leading to a drop of the T2* relaxation time (Khanna and Branca. 2012).

Below follows a more detailed description of the different steps in the proposed imaging method.

Image Acquisition

Images were acquired using an Ingenia 3.0 T MRI scanner (Philips) from the upper part of thorax planned axially with the lower end of the image stack at the level of the aortic arch. Phased array coils were used for signal reception. The subjects were asked to breath shallow to minimize respiratory motion in the upper part of the thorax, thereby enabling free breathing image acquisition. This allows longer acquisition times with simultaneous coverage of supraclavicular and intrascapular BAT with high resolution and high signal to noise ratio (SNR). A spoiled three-dimensional gradient echo sequence was used with a twelve-echo read out. The first echo was recorded at 1.15 ms and the following using 1.15 ms echo spacing. The repetition time was 26 ms and the flip angle was 10°. The obtained voxel dimension was 1.76*1.76*1.75 mm³ with a field of view (FOV) of 380*302*138 mm³. Parallel imaging acceleration, using a sensitivity-encoding (SENSE) factor of 2.5, resulted in a scan time of 1 min and 51 sec.

If image acquisition with volume coverage in the lower thorax is desired, a lower number of echoes and repetition time and/or a reduced FOV coverage may be used to decrease the scan time and thereby enabling breath-hold acquisition to avoid motion artifacts.

Phase Sensitive Reconstruction

Fat and water image volumes were reconstructed using the 3D Inverse Gradient method (Rydell et al. 2007, Rydell et al. 2008) using the complex-valued image volumes from the first two echoes. As opposed to region-growing methods, which are essentially one-dimensional algorithms acting in two or three dimensions, the Inverse Gradient Method is a true multi-dimensional method, which can benefit from the connectivity in three-dimensional data. The Inverse Gradient method generates a pair of phase-unwrapped real-valued in-phase (IP) and opposed-phase (OP) images. To resolve the fat-water classification problem, i.e. to decide the correct sign of the OP image, the method described in (Romu et al. 2011) was used.

The images were corrected for the T2*-decay effect by fitting T2*-decay parameters for fat and water signals respectively using a model similar to that described by O'Regan et al. (2008).

Intensity Inhomogeneity Correction and Image Calibration

In order to correct for image intensity inhomogeneities caused by various physical imperfections in the imaging system, such as variations in B0 field and coil sensitivity, WAT was used as internal reference (Leinhard et al. 2008). Voxels representing pure WAT were identified using thresholding of the quotient between the fat image and the in-phase image at 0.9. Then a bias field was interpolated from the identified pure WAT voxels using a multi-scale interpolation technique (Romu, Borga and Leinhard, 2011).

The image volumes were corrected using the inverse of this estimated bias field. The voxel values in these calibrated fat images show the *Relative Fat Content* (RFC), which is one in WAT and zero in voxels with no adipose tissue. Note that the RFC is different from the more commonly used “Fat Fraction” (Reeder, Hu and Sirlin, 2012).

Visualization

Since there is no narrow fat-water fraction interval that is generally applicable for BAT, we use a color coding for the fat fraction that is overlaid on the fat image, as shown in Figure 1. Hence, the hue represents the fat fraction and the brightness represents the fat signal. Finally the water image and the colored fat image are added in order to supply more anatomical information. In the resulting image, yellow represents WAT; absence of color represents absence of fat, and a reddish color represent possible BAT. Examples of this visualization are shown in Figures 2-3. In these figures, yellow indicates subcutaneous WAT, while red indicate possible BAT in the intrascapular and supraclavicular depots.

- FIGURES 2-3 APPROX. HERE -

DECT

Introduction

In Computed Tomography (CT) imaging, materials having different chemical compositions can be represented by the same, or very similar, Hounsfield Units (HU), making the differentiation and classification of different types of tissues extremely challenging (Macovski et al. 1976). In addition the accuracy with which material concentration can be measured is degraded by the presence of multiple tissue types (Alvarez and Macovski 1976). The reason for these difficulties in differentiating and quantifying tissue types is that the measured HU of a voxel is related to the linear attenuation coefficient $\mu(E)$, which is not unique for any given material but is a function of the material composition, the photon energies interacting with the material, and the mass density of the material. In dual energy CT (DECT), an additional attenuation measurement is obtained at a second energy, allowing the differentiation of two materials (Figure 4). Although medical x-ray tubes generate polychromatic spectra, the general principle remains valid. Thus, DECT can be defined as the use of attenuation values acquired with different energy spectra, and the known changes in attenuation between the two spectra, in order to differentiate and classify tissue composition.

- FIGURE 4 APPROX HERE -

Projection-Based Two Material Decomposition

Dual energy methods for CT were first investigated by Alvarez and Macovski in 1976 (Macovski et al. 1976; Kalender, Klotz and Suess 1987). They demonstrated that even with polychromatic x-ray spectra, one can still separate the measured attenuation

coefficients into their contributions from the photoelectric effect and Compton scattering processes. In the 1980s, a modified commercial CT system (Siemens DR) used fast tube voltage switching to allow alternate projection measurements at the low and high tube potentials (Alvarez and Macovski 1976). However, the tube current could not be increased quickly enough for the low tube potential setting to achieve comparable noise levels in both the low and high kV datasets. Recently, the simultaneous acquisition of dual-energy data has been introduced using multi detector CT with two X-ray tubes and rapid peak kilovoltage (kVp) switching (gemstone spectral imaging, GSI) (Flohr et al. 2006; Johnson et al. 2007; Petersilka et al. 2008; Marin et al. 2009; Ho et al. 2009). Two major advantages of DECT are material decomposition and the elimination of misregistration artifacts. The simplest method for combining the dual energy image data for the purpose of material differentiation is to perform a linearly weighted image subtraction. The low tube potential images (typically 80 kV) are multiplied by a weighting factor and subtracted from the high voltage images (140 kV) to suppress or enhance a specific material (Kalender, Klotz and Kostaridou 1988). Reconstructed CT images acquired using the two different tube potentials can be processed with a three-material decomposition algorithm (Johnson et al. 2007). The principle behind this technique is illustrated in Figure 5, where the typical HU of three materials of known densities are plotted on a graph where the y-axis is the HU at 140 kV and the x-axis is the HU at 80 kV. Ideally, the three materials should be sufficiently different as to create a triangle in this plot. Thereafter, corresponding HU pairs from the low- and high-energy images are mapped onto the calibration diagram. Depending on their position in the diagram, the composition of a certain material is determined. The voxels can be color coded according to the relative composition of certain materials, or specific materials can be either suppressed or enhanced, depending on the desired clinical application.

- FIGURE 5 APPROX HERE -

BAT Characterization

Recent studies have showed that the CT HUs of BAT are significantly greater under activated conditions than under non-activated conditions in both patients and animal models (Baba et al. 2010; Hu et al. 2011). Several reasons could explain the greater X-ray attenuation. BAT has unique cytological characteristics comprised of multiple lipid droplets surrounded by significant amounts of intracellular water. Brown adipocytes are also densely packed with mitochondria and are heavily vascularized and innervated. The greater proportion of non-lipid components in metabolically active BAT versus any other inactive adipose tissues (e.g. WAT) is the cause for its greater tissue density and more positive HUs. Due to significant overlap of HU values, it currently does not seem possible to separate metabolically inactive BAT from inert WAT based on HUs alone. To overcome this problem, and to distinguish different types of fat tissues from each other, some physical property that differs between the tissues needs to be measured. Conventional attenuation measurements based on a single photon energy spectrum have limited value in this respect. When the attenuation is measured at two energies, however, their values are not exactly proportional to each other and BAT can be separated from WAT (Zachrisson et al. 2010), (Fig. 6).

- FIGURE 6 APPROX HERE -

CT Scanner Protocol for BAT

Currently, three systems are capable of the nearly simultaneous acquisition of dual-energy data during a single breath hold (Flohr et al. 2006; Petersilka et al. 2008; Marin et al. 2009): 64-slice dual-source CT (Definition, Siemens Medical Systems; Erlangen, Germany), 128-slice dual-source CT (Definition Flash, Siemens Medical Systems) and high-definition 64-MDCT (Discovery 750 HD, GE Healthcare; Milwaukee, Wisconsin, USA). In the two systems available from Siemens, the two tubes (tubes A and B) use different kVp (80 and 140 kVp), and in high-definition 64-MDCT, the kVp switches from 80 to 140 kVp in less than 0.5ms. Below follows a more detailed description of the different steps in the proposed imaging method using a 128-slice dual-source CT (Definition Flash, Siemens Medical Systems). Typical Dual energy scanner settings to acquire DECT data for fat characterisation are: 140 sn and 80 kVp simultaneous acquisition, ref. mAs 461 for BMI <20. 140 sn and 100 kVp simultaneous acquisition, ref. mAs 230 for BMI >20. Collimation 32x0.6-mm. Pitch 0.7, rotation time 0.5 sec. Slice thickness 0.6 mm, reconstruction interval 0.6 mm, kernel Q30f medium soft, field of view <33.6 cm, depending on the patient size. DECT examinations should be avoided for individuals with BMI >30.

Post Processing

Post processing of DECT scan data can be performed either directly in the CT scanner or in a dedicated workstation with software designed for managing raw data or DECT image data. An example of such post processing is shown in Figure 8.

- FIGURES 7 AND 8 APPROX HERE -

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Figures

Fig 1. An example PET/MR image of a young male with significant activation of supraclavicular adipose tissue during cold exposure. The light colour indicates high uptake of ^{18}F FDG.

Fig 2. Relative fat content (RFC) color map used on the fat images.

Figure. 3. Coronal section from an adult male subject with indications of supraclavicular BAT.

Figure 4. Axial and coronal sections from a male subject with indication of intra-scapular BAT.

Figure. 5. Assuming the use of mono energetic x-rays, at approximately 100 keV, the same linear attenuation coefficients are measured for bone and iodine. Data acquired at approximately 40 keV allows the differentiation of the two materials, regardless of their respective densities.

Figure. 6. Calibration diagram based on WAT, BAT and water.

*Fig 7. CT examination of a one year old child with biopsy-verified BAT *. Single energy dataset (120kVp). BAT measures 10HUs higher than WAT indicating activated BAT (Baba et al. 2010). Using this approach, it is not possible to visualize the BAT organ.*

Fig. 8. The same child as in Figure 7 Datasets acquired at 80 and 140kVp were loaded into a Siemens multimodality workstation with volume rendering 3D software (Inspace) in merged mode. For each dataset, voxels were mapped with green color for BAT and black color for WAT. Settings; WAT: 140 kVp, -50HU, 80 kVp, -75HU and for BAT: 140 kVp, -20HU, 80kVp, -35HU. BAT can be separated from WAT.