The various colours of type 2 diabetes: Pathogenesis and epidemiology in different ethnic groups
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Combining $^{123}$I-metaiodobenzylguanidine SPECT-CT and $^{18}$F-fluorodeoxyglucose PET-CT for the assessment of brown adipose tissue activity in humans during cold exposure

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Abstract

Background

Brown adipose tissue (BAT) has become a focus of research in the hope of finding a new target to fight obesity. Metabolic BAT activity can be visualized with $^{18}$F-fluoro-deoxyglucose ($^{18}$F-FDG) positron-emission-tomography (PET) computed-tomography (CT). Furthermore, the sympathetic innervation of BAT can be visualized with the radiolabeled norepinephrine-analogue $^{123}$I-metaiodobenzylguanidine ($^{123}$I-MIBG).

We aimed to determine whether $^{123}$I-MIBG single-photon-emission-CT (SPECT-CT) and $^{18}$F-FDG-PET-CT identify the same anatomical regions as active BAT in adult humans. Furthermore, we investigated whether the magnitude of BAT activity measured by these techniques correlated. Finally, we tried to establish the optimal time-interval between $^{123}$I-MIBG administration and subsequent SPECT-CT acquisition to visualize sympathetic stimulation of BAT.

Methods

Ten lean (BMI 19-25 kg/m$^2$), healthy, Caucasian males (18-32 years) underwent one $^{18}$F-FDG PET-CT and two $^{123}$I-MIBG-SPECT-CT’s within a 2-week interval. On two separate occasions, the subjects were exposed to mild cold (17°C) for 2 hours after an overnight fast. After one hour of cold-exposure, $^{18}$F-FDG (one occasion) or $^{123}$I-MIBG (other occasion) was administered. The $^{18}$F-FDG-PET-CT was performed 1 hour after $^{18}$F-FDG administration, whereas $^{123}$I-MIBG-SPECT-CT’s were performed 4 and 24 hours after $^{123}$I-MIBG injection.

Results

$^{18}$F-FDG uptake in BAT was observed in 8 out of 10 subjects, whereas $^{123}$I-MIBG uptake was observed in 7 out of 10 subjects in both the SPECT-CT scans acquired 4 and 24 hours after $^{123}$I-MIBG administration. All subjects that showed $^{123}$I-MIBG uptake in BAT, also showed $^{18}$F-FDG uptake in BAT.

There was no statistically significant correlation between SUVmax of $^{18}$F-FDG and semi-quantitative-uptake of $^{123}$I-MIBG 4 hours after administration. However, a positive correlation was found between SUVmax of $^{18}$F-FDG and semi-quantitative-uptake of $^{123}$I-MIBG 24 hours after administration ($r=0.64$, $p=0.04$)

Conclusions

$^{123}$I-MIBG-SPECT-CT, as a marker of sympathetic activity and $^{18}$F-FDG-PET-CT, as a marker of metabolic activity, identified the same anatomical regions as active BAT. Moreover, when the $^{123}$I-MIBG-SPECT-CT was performed 24 hours after $^{123}$I-MIBG administration, the magnitude of BAT activity measured with these techniques correlated strongly. This not only supports that BAT activity in humans is sympathetically influenced, but also identifies $^{123}$I-MIBG SPECT-CT, when performed 24 hours after $^{123}$I-MIBG injection, as a method to visualize and quantify sympathetic stimulation of BAT.
Introduction

Given its high capacity to dissipate excess energy, brown adipose tissue (BAT) has become a focus of research in the hope that activation of BAT may be a new target to fight obesity (1-4). However, knowledge of mechanisms regulating BAT activity in humans is still limited. BAT can be assessed with various radiolabeled metabolic substrates, of which 18F-fluorodeoxyglucose (18F-FDG) positron-emission-tomography (PET) computed-tomography (CT) is most commonly used, typically under conditions of mild cold exposure (1-5).

Based on animal data and observational studies in humans, BAT is likely to be activated by the sympathetic nervous system (2). 123I-metaiodobenzylguanidine (123I-MIBG), a radiolabeled norepinephrine analogue, is commonly used for scintigraphic assessment of neuroendocrine tumors and cardiac sympathetic activity (6-8). Uptake of 123I-MIBG does not always correspond to tumor localization and is known to correspond with the typical distribution pattern of BAT (9). 123I-MIBG scintigraphy has already been used specifically to localize BAT in rats (10).

The aim of this study was to determine whether 123I-MIBG single-photon-emission-CT (SPECT-CT), as a measure of sympathetic stimulation/activation, and 18F-FDG PET-CT, as a marker of metabolic activity, identify the same anatomical location of BAT in adult lean humans. Furthermore, we investigated whether the magnitude of BAT activity measured by these two techniques correlated.

Finally, we tried to establish the optimal time interval between 123I-MIBG administration and subsequent acquisition to visualize and quantify sympathetic stimulation/activation of BAT. In clinical practice, imaging of the cardiac sympathetic nerves is commonly performed 4 hours after 123I-MIBG injection, whereas a time-interval of 24 hours post-injection is used to visualize tumors from neuroendocrine origin (11,12). Therefore, we compared 123I-MIBG-SPECT-CT images obtained 4 and 24 hours after 123I-MIBG administration and determined which time-interval resulted in an optimal correlation with 18F-FDG PET-CT assessed BAT activity.

Materials and Methods

We studied a group of 10 healthy, lean, Caucasian male volunteers (18-32 years; body-mass-index [BMI] 19-25 kg/m²). The institutional ethics committee of the Academic Medical Center approved the study protocol and all subjects provided written informed consent. Subjects were recruited through public advertisements. All underwent a physical examination and a fasting blood sample was drawn. Each of the 10 subjects underwent one 18F-FDG PET-CT and two 123I-MIBG SPECT-CT’s (i.e. 4 and 24 hours after administration of 123I-MIBG).
interval between the $^{18}$F-FDG-PET-CT and the $^{123}$I-MIBG scintigraphies was set between 1 and 2 weeks. To minimize possibility of order bias five subjects first underwent $^{18}$F-FDG-PET-CT followed by $^{123}$I-MIBG-SPECT-CT and in the other five subjects the order was reversed. All subjects were scanned after an overnight fast.

**Anthropometric and Laboratory Measurements**

Weight was recorded in light clothing on a SECA mechanical scale to the nearest 100 grams. Height was recorded to the nearest 0.01 meter. Blood pressure was measured in seated position (Omron-M5-1). Furthermore, fasting plasma glucose (FPG) levels were assessed.

**$^{18}$F-FDG-PET-CT: Scanning Protocol**

All subjects were exposed to mild cold ($-17^\circ$C, controlled by use of an airco/ventilated system) for the duration of 2 hours. Shivering was neither reported by subjects nor noticed by research staff. After 1 hour of cold-exposure, approximately 200 MBq of $^{18}$F-FDG was administered intravenously and exposure to cold was continued for another hour. Upper-body (from the base of the skull till the groins) static PET acquisition was performed 60 minutes after $^{18}$F-FDG injection.

PET-CT images were acquired with the use of a Gemini time of flight multidetector helical PET-CT-scanner (2 minutes/bed position) (PHILIPS Medical Systems, the Netherlands). In areas where uptake of $^{18}$F-FDG was identified by PET and presence of fat was identified by CT (Hounsfield units between -250 and -50), the maximal standardized uptake values (SUVmax), defined as activity in Becquerel per milliliter within the region of interest divided by injected dose in Becquerel per gram of body weight, were determined (Hybrid Viewer, HERMES Medical Solutions, Sweden). Anatomical regions of interest were cervical, supraclavicular, and superior mediastinal depots. In these areas a SUVmax of $^{18}$F-FDG of at least 2.0 g/ml was considered to indicate BAT (1).

**$^{123}$I-MIBG-SPECT CT: Scanning Protocol**

All subjects were pretreated with potassium-iodide to block thyroid uptake of $^{123}$I-MIBG. Again, the subjects were exposed to mild cold for the duration of 2 hours. After 1 hour of cold-exposure, approximately 185 MBq of $^{123}$I-MIBG was administered intravenously and exposure to cold was continued for another hour. Thereafter, the subjects resided in a thermo-neutral environment for another 3 hours. Subsequently, an upper-body SPECT-CT acquisition was performed (i.e 4 hours after $^{123}$I-MIBG injection). The next day, 24 hours after administration of $^{123}$I-MIBG, a second SPECT-CT scan was performed of the same anatomical region. The SPECT-CT images were acquired with use of Infinia SPECT-CT (General Electric, USA) with a medium-energy all-purpose collimator and a 128x128 matrix. A fifteen percent window was set for the main energy peak of $^{123}$I (159 keV). SPECT images were iteratively recon-
structed (OSEM) and corrected for attenuation using low-dose CT (no intravenous contrast). In areas where uptake of $^{123}$I-MIBG was identified by SPECT and the presence of fat was identified by CT, semi-quantitative uptake of $^{123}$I-MIBG was calculated as the maximum (decay corrected) count per voxel in the volumes of interest (VOI) divided by the mean count per voxel in a reference region (i.e. the mediastinum) (Hybrid-Viewer, HERMES Medical Solutions, Sweden).

Alignment of $^{18}$F-FDG PET-CT and $^{123}$I-MIBG-SPECT CT
The $^{18}$F-FDG-PET-CT and $^{123}$I-MIBG-SPECT-CT were aligned using the CT images with Hybrid-Viewer (HERMES Medical Solutions, Sweden). The results of this automated nonrigid registration algorithm were visually validated. The specific VOI's on the anatomical images of $^{18}$F-FDG-PET-CT in which metabolically active BAT was present (i.e. an SUVmax of $^{18}$F-FDG $\geq$2.0 g/ml) were copied to the aligned $^{123}$I-MIBG-SPECT-CT images. Subsequently, the semi-quantitative uptake of $^{123}$I-MIBG in these VOI's was calculated.

Statistical Analysis
Depending on distribution of the data, the characteristics of study subjects are reported as mean with standard-deviation (SD) or median with interquartile-range (IQR). P-values for differences in semi-quantitative uptake of $^{123}$I-MIBG between 4 and 24 hour acquisitions were determined with a paired sample t-test. The correlation between semi-quantitative uptake of $^{123}$I-MIBG (4 and 24 hours after administration) and SUVmax of $^{18}$F-FDG was determined with a Pearson correlation coefficient. Data analysis was performed using SPSS software 16.0 (Chicago, Illinois). P-values <0.05 were considered statistically significant.

Results
Table 1 shows the characteristics of the 10 volunteers. Median age of the participants was 22.5 (interquartile-range 21.2-25.1) years and mean BMI was 22.2 (SD 1.2) kg/m$^2$. $^{18}$F-FDG uptake in BAT was visually observed in 8 out of 10 subjects, whereas $^{123}$I-MIBG uptake in BAT was observed in 7 out of 10 subjects in both the SPECT-CT scans acquired 4 hour and 24 hours after $^{123}$I-MIBG administration. The mean semi-quantitative uptake value of $^{123}$I-MIBG was higher 24 hours after administration than it was 4 hours after administration (3.11 [1.05] mean counts per voxel versus 1.8 [0.51] mean counts per voxel, p=0.002). This was the result of a relatively lower $^{123}$I-MIBG uptake in the reference region, and not of a higher $^{123}$I-MIBG uptake in BAT itself (Table 1).
All subjects that showed $^{123}$I-MIBG uptake in BAT, also showed $^{18}$F-FDG uptake in BAT in the same anatomical location (Figures 1 and 2).
The SUVmax of $^{18}$F-FDG and semi quantitative uptake values of $^{123}$I-MIBG 4 and 24 hours after administration were normally distributed. There was no statistically significant correlation between SUVmax of $^{18}$F-FDG and the semi-quantitative uptake of $^{123}$I-MIBG 4 hours after administration ($r=0.35$, $p=0.33$). However, a strong, positive correlation was found between SUVmax of $^{18}$F-FDG and semi-quantitative uptake of $^{123}$I-MIBG 24 hours after administration ($r=0.64$, $p=0.04$) (Figure 3). Semi-quantitative uptake of $^{123}$I-MIBG 24 hours after administration in BAT explained approximately 40% of the variance in SUVmax of $^{18}$F-FDG in BAT ($R^2 = 0.407$).

**Discussion**

$^{123}$I-MIBG-SPECT-CT, as a marker of sympathetic activity, and $^{18}$F-FDG-PET-CT, as a marker of metabolic activity, identified the same anatomical regions as active BAT. Moreover, when the $^{123}$I-MIBG-SPECT-CT was performed 24 hours after administration of $^{123}$I-MIBG, the correlation between these two techniques was strongly positive. These findings support the notion that metabolic BAT activity in humans is influenced by the sympathetic nervous system.

The ability to visualize metabolically active BAT in humans with $^{18}$F-FDG PET-CT under conditions of mild cold exposure has been reported in several studies (1-5). In our study, metabolically active BAT was found in 80% of the subjects after 2 hours of cold exposure (16-18°C) with $^{18}$F-FDG PET-CT, which is in line with previous publications (3).

The opportunity to identify BAT with $^{123}$I-MIBG scintigraphy has previously been described in rats(10,13). Okuyama et al. retrospectively investigated $^{123}$I-MIBG scans performed in 266 pediatric patients who had been treated for, or who were suspected of, having neuroendocrine tumors (9). In 22 of these patients, they observed $^{123}$I-MIBG accumulation in the nape-of-the-neck region not corresponding to tumour tissue. As all of these 22 scans were performed during winter, this accumulation of $^{123}$I-MIBG was deemed to be related to active BAT (9). As these images were not combined with CT no definite conclusion on the presence of BAT could be made. In another study, Hadi et al. retrospectively reviewed images of 83 patients evaluated with $^{18}$F-FDG PET/CT for known/suspected pheochromocytoma, of which 10 had undergone a $^{123}$I-MIBG SPECT scan as well (13). In three of these 10 patients, BAT was observed on both $^{123}$I-MIBG and $^{18}$F-FDG images. In the remaining 7 patients, BAT was either detected with $^{18}$F-FDG-PET-CT only (n=3), $^{123}$I-MIBG only (n=1), or with neither modality (n=2).

There is an inherent problem with retrospective studies on BAT activity, as the reported BAT in retrospective studies is only the incidentally detected BAT (14). As mentioned earlier, BAT
is optimally visualized during cold exposure. Inactive BAT will not be visible on PET scans (14). As the patients included in the retrospective studies were not specifically exposed to cold, it is likely to assume that BAT, while present, was not detected in some of these patients.

To our knowledge, this study is the first to visualize sympathetic activity of BAT with \(^{123}\text{I}-\text{MIBG}\) after cold exposure in adult humans. Furthermore, we do not know of any studies that (semi-quantitatively) compared BAT activity measured by \(^{123}\text{I}-\text{MIBG}\) SPECT-CT and \(^{18}\text{F}-\text{FDG}\) PET-CT that were performed in the same period in the same individuals.

In our study, the SPECT-CT’s performed 4 hours and 24 hours after administration of \(^{123}\text{I}-\text{MIBG}\) both identified the same anatomical regions as active BAT as the \(^{18}\text{F}-\text{FDG}\) PET-CT. However, semi-quantitative uptake value of \(^{123}\text{I}-\text{MIBG}\) was higher after 24 hours than after 4 hours. As mentioned earlier, this was the result of a relatively lower \(^{123}\text{I}-\text{MIBG}\) uptake in the reference region (i.e. lower background activity), and not of a higher \(^{123}\text{I}-\text{MIBG}\) uptake in BAT itself (i.e. higher absolute uptake of \(^{123}\text{I}-\text{MIBG}\)). We only found a strongly positive correlation between \(^{18}\text{F}-\text{FDG}\) SUVmax and the semi-quantitative uptake of \(^{123}\text{I}-\text{MIBG}\) after 24 hours (and not after 4 hours). This finding suggest that the higher signal-to-noise-ratio in \(^{123}\text{I}-\text{MIBG}\)-SPECT-CT images obtained after 24 hours, when compared to images obtained after 4 hours, results in a more accurate assessment of sympathetic stimulation of BAT.

A limitation of the study may be the relatively small sample size. For this reason, caution must be applied, when extrapolating these results to the broader community. However, despite this small sample size, we still found a statistically significant and strongly positive correlation between the SUVmax of \(^{18}\text{F}-\text{FDG}\) and the semi-quantitative uptake of \(^{123}\text{I}-\text{MIBG}\) after 24 hours.

In our study, \(^{18}\text{F}-\text{FDG}\) and \(^{123}\text{I}-\text{MIBG}\) were not administered simultaneously, as we did not want the \(^{123}\text{I}-\text{MIBG}\) SPECT-CT performed 4 hours after administration to be influenced by the still radioactive \(^{18}\text{F}-\text{FDG}\), which has a half-life of 109.8 minutes (15). Although cold-exposure of the subjects was performed in exactly the same way, the fact that the subjects were studied on separate days might have influenced comparability of the \(^{18}\text{F}-\text{FDG}\) PET-CT and the \(^{123}\text{I}-\text{MIBG}\) SPECT CT images. Since we demonstrated that a time interval of 24 hours between \(^{123}\text{I}-\text{MIBG}\) administration and SPECT-CT is preferable over a 4 hour-interval in order to visualize sympathetic BAT activity, \(^{18}\text{F}-\text{FDG}\) and \(^{123}\text{I}-\text{MIBG}\) can be administered simultaneously in future studies, thereby limiting the cold-exposure of subjects to only once and reducing subject related sources of variability.

As mentioned earlier, BAT has become a focus of research in the hope that activation of BAT may be a new target to fight obesity (1-5). Several studies have shown that BAT activ-
ity is much lower in obese people, when compared to their lean peers (1,3,16). The ability to quantify sympathetic stimulation of BAT in humans makes it possible to investigate the extent to which metabolic BAT activity is attributable to central activation, as opposed to peripheral factors (such as hormones or intrinsic cellular factors, i.e. sensitivity of BAT to sympathetic stimulation). In future studies, we will simultaneously visualize and quantify sympathetic and metabolic activation to elucidate the mechanisms behind a diminished BAT activity in obese people.

Conclusion

123I-MIBG SPECT-CT and 18F-FDG PET-CT identify the same anatomical regions as active BAT. Moreover, the magnitude of BAT activity measured with these techniques correlates strongly. This not only supports the notion that BAT activity in humans is influenced by the sympathetic nervous system, but also identifies 123I-MIBG SPECT-CT as a method to visualize and to quantify the sympathetic stimulation of BAT.
Combining 123I-metaiodobenzylguanidine SPECT-CT and 18F-fluorodeoxyglucose PET-CT for the assessment of brown adipose tissue activity in humans during cold exposure

Reference List


### Table 1. Characteristics of the male volunteers*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study population (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>22.5 [21.2-25.1]</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2±1.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>117±7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 [72-72]</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>Presence of BAT based on $^{18}$F-FDG-PET-CT (n)</td>
<td>8</td>
</tr>
<tr>
<td>SUVmax of $^{18}$F-FDG (g/l) **</td>
<td>5.8±4.58</td>
</tr>
<tr>
<td>Presence of BAT based on 4hr $^{123}$I-MIBG SPECT CT (n) †</td>
<td>7</td>
</tr>
<tr>
<td>Uptake of $^{123}$I-MIBG in reference region (mean cnts/vox) after 4 hrs ‡</td>
<td>21.0 [5.6]</td>
</tr>
<tr>
<td>Absolute uptake of $^{123}$I-MIBG in BAT after 4 hrs (mean max cnts/VOI) ¶</td>
<td>37.4 [14.1]</td>
</tr>
<tr>
<td>Semi-quantitative uptake of $^{123}$I-MIBG after 4hrs §</td>
<td>1.8±0.51</td>
</tr>
<tr>
<td>Presence of BAT based on 24hr $^{123}$I-MIBG SPECT CT (n)**</td>
<td>7</td>
</tr>
<tr>
<td>Uptake of $^{123}$I-MIBG in reference region (mean cnts/vox) after 24 hrs ‡</td>
<td>3.2 [0.5]</td>
</tr>
<tr>
<td>Absolute uptake of $^{123}$I-MIBG in BAT after 24 hrs (mean max cnts/VOI) ¶</td>
<td>9.8 [3.10]</td>
</tr>
<tr>
<td>Semi-quantitative uptake of $^{123}$I-MIBG after 24 hrs ¶</td>
<td>3.1±1.1</td>
</tr>
</tbody>
</table>

BMI, body mass index; BAT, brown adipose tissue; SUVmax, maximal standard uptake value; FDG, fluorodeoxyglucose; MIBG, metaiodobenzylguanidine; VOI, volume of interest

*Data are presented as n, mean [standard deviation] (SD) or median [interquartile range]  
** SUVmax of $^{18}$F-FDG, defined as activity in Becquerel per milliliter within region of interest divided by injected dose in Becquerel per gram of body weight  
† Presence of BAT was determined visually  
‡ Uptake of $^{123}$I-MIBG in reference region (i.e. mediastinum) was calculated as mean counts per voxel in this reference region  
¶ Uptake of $^{123}$I-MIBG in BAT was calculated as maximum count per voxel in volumes of interest (VOI)  
§ Semi-quantitative uptake of $^{123}$I-MIBG was calculated as maximum count in anatomical volumes of interest divided by mean count per voxel in reference region (i.e. the mediastinum)  
ǁ decay corrected
Figure Legends

Figure 1. Brown adipose tissue visualized with $^{123}$I-MIBG - SPECT and $^{18}$F-FDG - FDG-PET

There is a clear association between $^{18}$F-FDG uptake in brown adipose tissue (BAT) and $^{123}$I-MIBG (panel A and B), both showing increased uptake in BAT (maximum intensity projection images).

Figure 2. Brown adipose tissue visualized with $^{123}$I-MIBG - SPECT-CT and $^{18}$FDG - FDG-PET-CT

$^{18}$F-FDG and $^{123}$I-MIBG uptake on corresponding transversal PET and SPECT images is suggestive of BAT and is superimposed on adipose tissue on the correlated transversal CT images (panels A, B, C and D). Panels A and B represent $^{18}$F-FDG-CT and panels C and D represent $^{123}$I-MIBG-CT.
Figure 3. Correlation between SUVmax of $^{18}$F-FDG* and semi-quantitative uptake of $^{123}$I-MIBG 24 hours after administration**

Correlations between SUVmax of $^{18}$F-FDG and semi-quantitative uptake of $^{123}$I-MIBG with corresponding p-values were determined with Pearson’s correlation-coefficient.

* SUVmax of $^{18}$F-FDG was defined as the activity in Becquerel per milliliter within region of interest divided by the injected dose in Becquerel per gram of body weight.

** Semi-quantitative uptake of $^{123}$I-MIBG was calculated as maximum count in anatomical volumes of interest divided by mean count per voxel in reference region (i.e. mediastinum).