Supraclavicular Brown adipose tissue FDG uptake and cardiovascular disease

Short title: BAT FDG uptake and CVD

Richard A. P. Takx, MD MSc1,2; Amorina Ishai, MD1; Quyhn A. Truong, MD MPH3; Meghan H. MacNabb, BA1; Marielle Scherrer-Crosbie, MD PhD4; Ahmed Tawakol, MD1,4

1Cardiac MR PET CT Program, Massachusetts General Hospital and Harvard Medical School, Boston, MA
2Department of Radiology, University Medical Center Utrecht, Utrecht, the Netherlands
3Department of Radiology, Weill Cornell College of Medicine, New York, NY
4Cardiology Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA

ADDRESS FOR CORRESPONDENCE:
Ahmed Tawakol, MD
Massachusetts General Hospital
165 Cambridge Street, Suite 400
Boston, MA 02114-2750
Phone: 617-726-0791
Fax: 617-724-4152
Email: atawakol@partners.org
ADDRESS FOR FIRST AUTHOR
Richard A.P Takx (research fellow)
Massachusetts General Hospital
165 Cambridge Street, Suite 400
Boston, MA 02114-2750
Phone: 617-726-3745
Fax: 617-724-4152
Email: rtakx@mgh.harvard.edu

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ABSTRACT

Rationale
Pre-clinical data suggests a negative correlation between brown adipose tissue (BAT) and the degree of coronary atherosclerosis. We sought to evaluate the relationship between 18F-FDG uptake in supraclavicular BAT in relation to: 1) arterial inflammation and 2) subsequent cardiovascular disease (CVD) events in humans.

Methods
Individuals who underwent 18F-FDG PET/CT for clinical indications, but who did not have either cancer or known atherosclerotic disease at time of imaging were included. While blinded to clinical data, 18F-FDG uptake was measured within BAT (in supraclavicular region) as well as in subcutaneous adipose tissues (SAT). Tissue density was evaluated using CT (Hounsfield units). Arterial inflammation was assessed by measuring of arterial FDG uptake and calculating target to background ratio (TBR). Cardiovascular disease (CVD) events were independently adjudicated by blinded cardiologists. Thereafter, the relationship between BAT activity and CVD events was evaluated.

Results
A total of 443 patients (age, 43% male, body mass index (BMI) 26 (23-31)) were included and 34 experienced a cardiovascular event during a median follow-up of 4 years. BAT activity negatively correlated with arterial inflammation (r=-0.178, p<0.01), a relationship that persisted after correcting for age and BMI (r=-0.147, p<0.01). Using
either high sensitivity or high accuracy thresholds (from receiver operating curve analyses) to define elevated BAT, high BAT was associated with a reduced risk of CVD events (p=0.048) even after correcting for age (p=0.037).

Conclusion

Our results suggest that increased supraclavicular BAT activity is inversely associated with arterial inflammation, independently of age and BMI. Additionally, increased BAT may be associated with fewer cardiovascular events.

Key words

Brown adipose tissue; Positron-emission tomography; Arterial FDG uptake; Cardiovascular disease

Abbreviations

BAT= Brown adipose tissue
BMI= Body mass index
CVD= Cardiovascular disease
FDG= Fluorodeoxyglucose
HU= Hounsfield units
PET/CT= Positron-emission tomography and computed tomography
SAT= Subcutaneous adipose tissue
SUV= Standardized uptake values
TBR= Target to background ratio
INTRODUCTION

Brown adipose tissue (BAT) is primarily observed in young children and regresses with increasing age (1). Several studies found a high incidence of metabolically active BAT in the supraclavicular and paraspinal regions in adults, an observation that is accentuated after cold activation (2-7). Studies furthermore suggest that BAT activation is associated with improved regulation of energy expenditure; Further evidence for a role of BAT in energy metabolism is that BAT activation is associated with improved whole-body glucose homeostasis and heightened insulin sensitivity (8). BAT activity may also have beneficial impact on atherosclerosis, which is a chronic inflammatory condition (9). A postmortem study in humans (10) demonstrated a negative correlation between BAT and coronary atherosclerosis (as luminal narrowing). Thus we hypothesized that supraclavicular BAT might play an important protective role against atherosclerosis.

The combination of positron-emission tomography and computed tomography (PET/CT) allows for the identification of BAT, since BAT demonstrates high fluorodeoxyglucose (FDG) uptake on PET and CT allows for the identification of fat tissue on the basis of Hounsfield units (HU) (11, 12). Moreover, FDG uptake in the aortic wall can be used as a reliable imaging biomarker of inflammation in atherosclerotic plaques (13). Hence, the aim of this study was to evaluate the relation between 18F-FDG uptake in supraclavicular BAT and arterial inflammation in humans using PET/CT imaging. As a secondary aim we sought to evaluate the relation between supraclavicular BAT uptake and subsequent cardiovascular events.
MATERIALS AND METHODS

In this retrospective observational study, participants who underwent PET/CT imaging for clinical reasons at the Massachusetts General Hospital between 2005 and 2008 were included. Pre-defined inclusion criteria were: 1) absence of prior cancer diagnosis or clinical remission of cancer throughout the follow-up period, 2) age 30 years or older, 3) absence of known cardiovascular disease at the time of imaging, 4) absence of inflammatory or autoimmune disease or use of chronic anti-inflammatory therapy. Additionally, in order to ensure adequacy of follow-up clinical information, the study participants had to have at least three subsequent medical visits spanning >1 year. The study protocol was approved by the local IRB; informed consent was waived because of the study’s retrospective nature.

FDG-PET/CT imaging

FDG-PET/CT imaging was performed using previously described and validated methods (14). All subjects fasted for at least 8 hours prior to intravenous FDG injection (approximately 370 MBq for a 70 kg patient). PET/CT image acquisition was performed approx. 60-90 minutes after FDG injection, on a Biograph 64 (Siemens Healthcare, Forchheim, Germany).

FDG-PET/CT image analysis

The images were analyzed by blinded readers at a core laboratory using TrueD (MMWP VE40A, Siemens Healthcare, Forchheim, Germany), using previously described methods (14). FDG uptake was measured within the wall of the ascending aorta in the
axial plane from 1 cm above the origin of coronary vessels and up to the bottom of the aortic arch in 5 mm increments. Arterial FDG uptake was recorded as the mean of maximum standardized uptake values \( (SUV_{max}) \) of all slices. For background correction mean SUV were measured in the superior vena cava. Arterial target to background ratio (TBR) was calculated as aortic \( SUV_{max} \) divided by venous blood SUV. The predictive role of arterial inflammation in this cohort was evaluated in a previous publication (15).

BAT FDG activity was quantified by an experienced radiologist within the supraclavicular region as previously described by Cohade et al. (11). First, the right and left supraclavicular fossae were identified and the supraclavicular fat depots (wherein BAT resides) were identified using CT density information (-250 to -50 HU, Fig. 1) (16). Subsequently, a region of interest was placed within the supraclavicular fossae adipose depots, the SUV data were recorded. The average of the mean BAT SUV was calculated as the average of the bilateral recorded BAT SUV\( _{mean} \). The average HU values for supraclavicular BAT were recorded simultaneously with the SUV values. As a control fat tissue FDG uptake was measured in the subcutaneous adipose tissue (SAT SUV).

In adult humans imaged under ambient conditions, the incidence of BAT activation, if defined using the classic criteria of SUV\( \geq 2 \), is low (<2% of cases) (17). Since such a definition of activated BAT would severely limit power for analyses, we sought alternate methods to describe relative BAT activation in our population. Thus, we defined relative BAT activation using two main methods. The first method to define relative BAT activation employed measures of absolute BAT SUV. The second method to define relative BAT activation used a ratio of BAT SUV to SAT SUV. For each method, we identified two separate thresholds to identify high BAT activity: 1) a
threshold derived using the receiver operating curve (ROC) to determine the lowest value associated with 100% sensitivity to detect CVD events, and 2) a threshold derived using the ROC curve to determine the value associated with highest accuracy. Out of the four resultant thresholds to define “activated BAT”, we prospectively chose the absolute BAT SUV value associated with 100% sensitivity for the primary analysis.

**Cardiovascular events**

Cardiovascular disease (CVD) events were adjudicated using medical records by 2 independent cardiologists who were blinded to all imaging data. Incident cardiovascular events included ischemic stroke or transient ischemic attack, acute coronary syndrome, revascularization (coronary, carotid, or peripheral), unstable angina pectoris, heart failure, and cardiovascular death. For the exact definition of events we refer to the online supplement of a previous publication (18).

**Statistical analysis**

Continuous variables were expressed as mean (± standard deviation) and non-normally distributed variables were expressed as median (P25, P75). Quantile-quantile (QQ) plots were evaluated to determine normal distribution. Categorical variables were expressed as frequencies and percentages. To assess for statistically significant differences in continuous variables the independent-samples Student T-test was applied. Levene's test was used to assess the equality of variances for a variable. In case of non-normal distribution the Mann-Whitney U test was used. Univariate associations were evaluated using Pearson correlation coefficients. ROC curves and areas under the curve
(AUC) were generated to determine cut-points. Kaplan-Meier estimates of free cardiovascular events of patients were calculated and log rank test was applied to test for statistical significance. P-values were two-sided and a P-value of 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 22 (IBM, Armonk, New York, USA) and R version 3.12 combined with the package survival (R Foundation for Statistical Computing, Vienna, Austria).
RESULTS

We were able to measure BAT activity in 443 subjects, patient demographics are shown in Table 1.

Cardiovascular outcomes

The median follow-up period was 3.7 years and 30 patients experienced a incidental cardiovascular event. Nine developed acute coronary syndrome, 4 underwent coronary revascularization, 6 experienced a stroke, 2 had a transient ischemic attack, 1 underwent carotid revascularization, 5 had new-onset angina pectoris, 2 were diagnosed with peripheral artery disease and underwent peripheral revascularization, and 1 cardiovascular death.

Brown adipose tissue metabolic activity vs. arterial inflammation

BAT metabolic activity (SUV) negatively correlated with arterial inflammation (arterial TBR), age and body mass index (BMI) (Figs. 2A-2C). Further, BAT SUV was significantly higher in females compared to males (Fig. 3). The relationship between BAT SUV and arterial inflammation remained significant after controlling for age and BMI (r=-0.147, p=0.002), as well as for gender (r=-0.148, p=0.002). Additionally, BAT SUV modestly correlated with BAT CT density (HU, Fig. 2D). Generally similar correlation were observed when the BAT/SAT ratio was substituted for the absolute BAT SUV (see Supplemental Table 1).
**BAT metabolic activity vs. CVD events**

BAT metabolic activity (SUV) was lower in individuals who subsequently experienced a cardiovascular event (0.65±0.55 vs. 0.51±0.14, p<0.001, Table 2). Similarly, BAT/SAT activity ratio was also lower in who subsequently experienced a cardiovascular event (3.89±3.41 vs. 3.04±1.29, p=0.004) In contrast, there was no difference in SAT SUV between patients with and without a CVD event (Table 2).

**BAT uptake and events free survival**

The relationship between BAT activation and events was evaluated using 4 separate thresholds to define activated BAT (Table 3). The prospectively defined primary analysis defined “activated BAT” according to the lowest BAT SUV value that identified 100% of individuals who experienced a CVD event (a BAT SUV of >1.0) (Fig. 4). Using that threshold, we observed significantly greater arterial inflammation in those with lower BAT metabolic activity (TBR 2.02±0.29 vs. 1.84±0.20, p<0.001). Moreover, individuals with lower BAT metabolic activity had more CVD events during the subsequent follow-up period (Fig. 5A). These differences in survival remained significant after correcting for BMI, though became non-significant after correcting for age. However, using a high-accuracy threshold to define activated BAT, the inverse relationship between BAT SUV and events remained significant even after correcting for age (Table 3). Similar inverse relationships between BAT activation and events were observed when BAT activity was assessed in relation to SAT activity (using BAT/SAT ratios, see Table 3, Fig. 5B).
DISCUSSION

To our knowledge, our study is the first to evaluate the relation between BAT activity, arterial inflammation and CVD events. We found that BAT was moderately, inversely associated with arterial inflammation independent of age and BMI. Furthermore we observed that cardiovascular were more frequent in patients with low BAT activity. Our data suggests that BAT activity is inversely associated with arterial inflammation and that a high BAT activity may be protective for subsequent CVD events.

BAT is an active metabolic organ and potentially plays an important role in the basal metabolic rate and inflammation (19, 20). Atherosclerosis is a chronic inflammatory disorder characterized by an accumulation of lipids, collagen, foam macrophages, and proliferated smooth muscle cells (21-23). FDG PET allows for the quantification of arterial inflammation and improves CVD risk prediction beyond traditional clinical risk prediction models (e.g. Framingham Risk Score) (15). In this study, we observed a modest inverse relationship between BAT activity and arterial inflammation. Given that PET/CT measures of arterial inflammation have repeatedly been shown to predict CVD events (13, 15, 24), we hypothesized that BAT activation would also be associated with CVD events. Indeed we observed that in individuals who experienced CVD events, arterial FDG uptake was higher while BAT uptake was significantly lower. Further, no events were observed in subjects with high BAT activity, raising the possibility that BAT could have an important role in vasoprotection.

It is important to note that the relationship between BAT activity and CVD events became non-significant in the primary analysis after correcting for age. However, on
secondary analyses, using a different threshold for BAT activation, we found that BAT activity remained significantly inversely associated with CVD events even after adjusting for age. Moreover, we observed a robust inverse association between BAT activity and arterial inflammation, which persisted after correcting for age and other covariates. Taken together, the observations support the hypothesis that BAT activation is cardioprotective.

**Limitation**

In many prior studies, the identification of BAT is further facilitated through cold-stimulation (which provokes BAT thermogenesis and increases BAT FDG uptake) (3, 4, 25). In our study, however, patients were imaged under ambient conditions; cold stimulation was not performed, due to the retrospective design. Hence it is important to consider the results of this study under this light. None-the-less, un-stimulated BAT might yield important information regarding its potential anti-atherogenic impact. Notably, BAT presumably exists primarily in its unstimulated state (in the absence of cold provocation) hence evaluation of BAT under ambient conditions might provide insights into the more typical metabolic state of that tissue. Thus, unstimulated BAT activity may even better-reflect the salutary contributions of that tissue on long-term health. Future studies will need to compare the value of cold-activated compared to ambient BAT measures for predicting its atheroprotective impact. Other limitations also deserve mention. The study consisted primarily of patients who had a prior history of treated cancer, which may hamper the generalizability of our results. Also, we did not adjust for the effect of beta-blockers, which could reduce BAT activity (26).
It is important to note that while the findings of this study underscore a link between BAT activation and better cardiovascular health, the data do not denote causation and indeed several important questions remain. Among them, while BAT activity can be increased pharmacologically (27) or through cold exposure (28), it is not clear if modulation of BAT activity will translate in improved CVD outcomes. Further, consistent with previous research (24), we also found that BAT activity tends to be lower in elderly subjects. It is unknown to what degree BAT activity can be up-regulated in older adults, where its potentially protective effects are needed the most. Consistent with prior research, we observed higher BAT activity in females (2, 16). Though the mechanisms underlying this observed difference is unclear, it is possible that sex hormones play a role (29).

**Conclusion**

The study demonstrates that supraclavicular BAT activity is inversely associated with arterial inflammation (independent of age and BMI). Furthermore, we observed that higher BAT activity is associated with significantly fewer CVD events and that no cardiovascular events occurred in individuals with a high BAT activity. These observations provide support for the concept that BAT activation may be associated with better cardiovascular health.
REFERENCES


<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Full cohort (n=443)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55 (44-66)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>194 (44)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26 (24-31)</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>43 (10)</td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td>37 (8)</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>124 (28)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>151 (34)</td>
</tr>
<tr>
<td>Cardiovascular event (%)</td>
<td>30 (7)</td>
</tr>
</tbody>
</table>

**Framingham Risk Score***

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (10-y risk &lt;10%)</td>
<td>169 (38)</td>
</tr>
<tr>
<td>Medium (10-y risk 10%-20%)</td>
<td>42 (9.5)</td>
</tr>
<tr>
<td>High (10-y risk &gt;20%)</td>
<td>4 (2)</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation), median (P25-P75), or n (%). BMI, body mass index.

* available in 215 patients
Table 2. FDG uptake

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Full cohort (n=443)</th>
<th>No CVD event (N=413)</th>
<th>CVD event (N=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial TBR</td>
<td>2.00±0.29</td>
<td>1.99±0.28</td>
<td>2.18±0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average BAT (SUV)</td>
<td>0.64±0.54</td>
<td>0.65±0.55</td>
<td>0.51±0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average BAT/SAT</td>
<td>3.84±3.31</td>
<td>3.89±3.41</td>
<td>3.04±1.29</td>
<td>0.004</td>
</tr>
<tr>
<td>Average SAT (SUV)</td>
<td>0.17±0.05</td>
<td>0.17±0.05</td>
<td>0.18±0.05</td>
<td>0.554</td>
</tr>
</tbody>
</table>

Mean± standard deviation
Table 3. BAT Activation vs. Events

<table>
<thead>
<tr>
<th>BAT Activity Measure</th>
<th>Method used to define High BAT</th>
<th>Cutoff Value</th>
<th>Significance of Association with Events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Univariate</td>
</tr>
<tr>
<td>BAT SUV</td>
<td>High sensitivity on ROC analysis</td>
<td>1.00</td>
<td>0.048**</td>
</tr>
<tr>
<td></td>
<td>High accuracy on ROC analysis</td>
<td>0.46</td>
<td>0.065*</td>
</tr>
<tr>
<td>BAT/SAT Ratio</td>
<td>High sensitivity on ROC analysis</td>
<td>8.32</td>
<td>0.114</td>
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<tr>
<td></td>
<td>High accuracy on ROC analysis</td>
<td>3.00</td>
<td>0.028**</td>
</tr>
</tbody>
</table>

P<0.05**(significant)

P<0.1*(trend)
FIGURE LEGENDS

Figure 1. Measurement of BAT uptake

Supraclavicular fat was identified using CT density thresholds (HU -50 to -250). BAT FDG uptake was assessed by manually drawing a region of interest (ROI) within the supraclavicular fat in the axial plane. SUVmean was recorded bilaterally.
Figure 2 Scatter plot between Average mean BAT uptake and aortic TBR (A), age (B), BMI (C) and CT assessment (HU) of BAT (D).
Figure 3

Bar plot comparing average mean BAT uptake in females compared to males. Error bars represent +/- standard error.
Figure 4

Scatter plot in average mean BAT SUV and cardiovascular event
Figure 5

KM plot displaying Proportion Free of CVD Events in individuals with vs without BAT activation. In figure 5A, activated BAT was identified using a threshold BAT SUV of 1.0. In figure 5B, activated BAT was identified as a BAT SUV that had 3 times the activity of SAT SUV. Using either definition, individuals with activated BAT (green) had fewer CVD events compared to individuals without activated BAT (blue).
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