Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Associations Between Brain Gray Matter Volumes and Adipose Tissue Metabolism in Healthy Adults

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Objective: Gray matter (GM) volume in different brain loci has been shown to vary in obesity and diabetes, and elevated fasting plasma nonesterified fatty acid (NEFA) levels have been suggested as one potential mechanism. The hypothesis presented in this study is that brown adipose tissue (BAT) activity may correlate with GM volume in areas negatively associated with obesity and diabetes.

Methods: A total of 36 healthy patients (M/F: 12/24, age 39.7 ± 9.4 years, BMI 27.5 ± 5.6 kg/m^2) were imaged with positron emission tomography using fatty acid analog [^{18}F]FTHA to measure NEFA uptake and with [^{15}O]H_2O to measure perfusion during cold exposure, at room temperature during fasting, or during a postprandial state. A 2-hour hyperinsulinemic euglycemic clamp was performed to measure whole-body insulin sensitivity (M value, mean 7.6 ± 3.9 mg/kg/min). T1-weighted magnetic resonance imaging at 1.5 T was performed on all patients.

Results: BAT NEFA uptake was associated directly with GM volume in anterior cerebellum and occipital lobe ($P \leq 0.04$) when adjusted for age, gender, and intra-abdominal fat volume and with anterior cerebellum, limbic lobe, and temporal lobe GM volumes when adjusted for M value.

Conclusions: BAT NEFA metabolism may participate in protection from cognitive degeneration associated with cardiometabolic risk factors, such as central obesity and insulin resistance. Potential causal relationships between BAT activity and GM volumes remain to be examined.

Obesity (2021) **29**, 543-549.

Study Importance

What is already known

- Gray matter (GM) volume in different brain loci has been shown to vary in obesity and diabetes.
- Elevated fasting plasma nonesterified fatty acid (NEFA) levels have been suggested as one potential mechanism mediating the negative effects of obesity and diabetes on brain GM volumes.

What does this study add?

- We found that BAT NEFA metabolism associates positively with GM volume in anterior cerebellum and occipital lobe independently of visceral obesity and with GM volume in anterior cerebellum, limbic lobe, and temporal lobe independently of whole-body insulin sensitivity.

How might these results change the direction of research or the focus of clinical practice?

- Higher BAT activity may play a role in protection from cognitive degeneration associated with cardiometabolic risk factors such as central obesity and insulin resistance.
- The clinical significance of our findings remains to be studied because our current study setting cannot examine the causal relationship or the magnitude of the potential association between BAT activity and GM volumes.

Introduction

Human body weight and BMI have been associated with global and regional decreases in brain tissue volumes (1), which can be viewed as worsening brain tissue health. Obesity has been linked to reduced gray matter (GM) volumes in many studies (2-4). The extant studies have reported quite a dispersed set of brain regions associated with increased weight, which implies that changes accompanying increased weight have marked individual variability, part of which may be linked to individual metabolic profiles.

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Received: 21 July 2020; **Accepted:** 12 November 2020; **Published online 2 February 2021. doi:10.1002/oby.23094**

Although the hypothalamus governs the long-term energy balance (5), it has been suggested that brown adipose tissue (BAT) is a peripheral regulator of energy homeostasis in healthy adults (6). BAT activity during cold exposure correlates positively with increased brain glucose uptake in cerebellum, thalamus, cingulate, temporoparietal, lateral frontal, and occipital cortices in lean adults, but this association is not found in individuals with obesity (7), which implies that, in a healthy state, both BAT and brain tissue metabolism are coupled and increased body weight and accompanying changes disturb the balance. No data exist on associations between BAT metabolism and brain GM volume. We hypothesize that BAT activity is inversely associated with obesity-related phenotype in GM volumes.

Development of cognitive degeneration has been linked to accumulation of cardiometabolic risk factors in numerous studies (8), whereas higher BAT activity is known to be associated with healthy weight status and high insulin sensitivity. Therefore, BAT activity might protect against neural degeneration in brain areas affected in cognitive impairment, such as the frontal and temporal cortices. In addition to systemic metabolic health, high BAT activity could even promote brain health and protect against aging (9).

In the current association study, we examined nonesterified fatty acid (NEFA) uptake in supraclavicular BAT depots during fasting, postprandially after a standardized lunch, and during cold exposure with positron emission tomography (PET), and their associations with GM volume in different brain loci were measured with 1.5-T magnetic resonance imaging (MRI) and voxel-based morphometry (VBM). Fasting state at room temperature was used as the baseline BAT activity, and cold exposure and postprandial state were used as the stimulated BAT activity. BAT activity provides plasma glucose and fatty acid clearance, which may be one factor that mediates potential neuroprotective effects by better maintaining normoglycemia and normal levels of plasma-free fatty acids in a range of 300 to 600 μmol/L observed in healthy populations (10). Our primary hypothesis was that remaining levels of BAT activity would associate positively with brain GM volumes that have negative correlations with obesity-related metabolic indices. Our secondary hypothesis was that the observed associations between BAT activity and GM volumes would be independent of age, obesity, and insulin sensitivity, which would suggest that BAT activity is an independent protective factor or marker against neurodegeneration.

Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Hospital District of South-Western Finland. All participants signed ethical committee–approved informed consent forms prior to measurements. A description of the study visits is displayed in Supporting Information Figure S1. The current study utilizes PET data published in a previous study (11) combined with previously unpublished brain GM volumes measured with MRI.

Participants and metabolic measures

Thirty-six healthy adult patients (M/F 12/24, mean age 39.7 ± 9.4 years, mean BMI 27.5 ± 5.6 kg/m^2) participated in the study. The screening protocol included exclusion of pregnancy, diabetes (prior diagnosis or diagnosed on visit 1 by fasting glucose and a 2-hour oral glucose tolerance test [OGTT]), hypertension (medication for hypertension or untreated previously diagnosed hypertension), and dyslipidemia (medication for dyslipidemia). Patients were excluded from the study if their fasting plasma glucose was ≥7.0 mmol/L or 2-hour OGTT plasma glucose was ≥11.1 mmol/L. No previous data on associations between BAT NEFA uptake and brain GM volumes exist, so power analyses could not be performed.

To compare different BMI groups, the patients were divided into BMI group 1 (BMI < 25 kg/m^2), group 2 (25 ≤ BMI < 30 kg/m^2), and group 3 (BMI ≥ 30 kg/m^2).

PET scans

All patients underwent imaging with 14(R,S)-^{18}F-fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA) (fatty acid analog) and [^{15}O]H_2O during nonshivering cold exposure. The participants underwent a second PET scan at room temperature with the same tracers in either a fasting (N = 18) or postprandial state after a standardized meal (N = 15). Three patients were imaged only during cold exposure.

PET scans were performed using a PET/CT scanner (Discovery 690 PET-CT scanner; General Electric Medical Systems, Milwaukee, Wisconsin). The nonshivering cold exposure started 2 hours before the PET scanning session and continued throughout the scanning session. An adjustable cooling blanket with a closed water circulation was used to induce the nonshivering cooling of the patients (mean temperature during cold exposure 13°C ± 2°C). Patients were attached to a 12-lead electrocardiogram (ECG) to monitor for potential adverse cardiac effects, such as arrhythmias. Shivering was estimated in real time with visual observation by the researcher, reports from the patients, and muscle tremor observed in the ECG, and the cooling temperature was elevated if muscle tremor was observed to avoid heat production in skeletal muscles. Skin temperature was also measured on the right lateral abdominal region with an attachable thermometer.

Imaging at room temperature was performed either following an overnight fast or after a standardized carbohydrate-rich meal. In the postprandial imaging, patients ingested a meal of approximately 542 kcal (58% carbohydrates, 25% fat, and 17% protein). The meal was consumed in approximately 15 minutes, and the PET scanning started approximately 15 minutes after the meal was finished. The mean room temperature during the scans was 22.4°C ± 0.4°C.

BAT perfusion was measured with intravenous [^{15}O]H_2O injection. A dynamic emission scan of the cervicothoracic region was performed (frames: 6 × 5 seconds, 6 × 15 seconds, 8 × 30 seconds). NEFA uptake was measured with a palmitate analog, [^{18}F]FTHA. A dynamic emission scan was performed in the cervicothoracic region after an intravenous [^{18}F]FTHA injection (frames: 1 × 60 seconds, 6 × 30 seconds, 1 × 60 seconds, 3 × 300 seconds, 2 × 600 seconds).

Computerized reconstruction of the acquired imaging data was performed after the scans. Quantitative corrections included detector normalization, dead-time and radioactive decay, randoms, attenuation, and scatter. Image reconstruction was performed using iterative 3D-OSEM (GE Vue Point HD-S; GE Healthcare, Chicago, Illinois) with 24 subsets and 2 iterations. Images underwent filtering with 6.4-mm Gaussian postfilter.

PET data analysis. Volumes of interest (VOIs) were drawn in the dynamic PET-CT images. VOIs for BAT were drawn in the supraclavicular fat depots identified as adipose tissue based on CT

radiodensity. Supraclavicular BAT was identified by the typical anatomical BAT distribution in the supraclavicular fat depot and the observed elevated BAT activation during cold exposure when compared with PET scans in room temperature. VOIs were drawn manually in PET-CT fusion images, and the mean radiodensity of the resulting VOIs in cold exposure scans was −86.0 ± 8.5 HU and −88.0 ± 8.0 HU in room temperature scans. In the drawn VOIs, NEFA uptake was calculated with Gjedde-Patlak plot and tissue perfusion with a one-tissue compartment model as described previously (11,12). PET analyses were performed with Carimas 2.8 PET image analysis software (Turku PET Centre, Turku, Finland).

Measurement of whole-body insulin sensitivity. A 2-hour hyperinsulinemic euglycemic clamp was performed to measure whole-body insulin sensitivity (13). The patients involved in the study came for a third study visit to undergo the clamp study in which an insulin-saline solution infusion was performed at a constant dose rate of 1 mIU/kg/min and a 20% glucose-saline solution was simultaneously infused at an adjusted rate with plasma glucose being measured at 5-minute intervals. The target range of plasma glucose was between 5.0 and 5.5 mmol/L, and the M value depicting the whole-body insulin sensitivity was calculated as described previously (12). The mean steady state plasma insulin level during the clamp was 73.7 ± 18.5 IU/dL.

MR imaging

MRI was performed with a Philips Gyroscan Intera 1.5-T CV Nova Dual scanner (Philips Medical Systems, Amsterdam, the Netherlands) located at the Turku PET Centre. Anatomical images of the brain with 1 mm^3 resolution were acquired using a T1-weighted sequence (repetition time [TR] 25 milliseconds, echo time [TE] 4.6 milliseconds, flip angle 30°, scan time 376 seconds). To assess the volume of intra-abdominal visceral adipose tissue, axial T1-weighted dual fast-field echo images covering the abdominal area were acquired (TE 2.3 and 4.6 milliseconds, TR 120 milliseconds, slice thickness 10 mm without gaps), and the volumes were later defined with sliceOmatic 5.0 software (Tomovision, Quebec, Canada).

Analysis of T1 brain data

Structural images were analyzed with SPM8 software (Wellcome Trust Centre for Neuroimaging, University College London, UK) running on MatLab 2012 (MathWorks, Natick, Massachusetts) and a vbm8 toolbox that enables automated spatial normalization, tissue classification, and radio-frequency bias correction to be combined within the segmentation step (http://dbm.neuro.uni-jena.de/wordpress/vbm/download/). The cutoff of spatial normalization was 25 mm, medium affine regularization 0.01 was used, and other preprocessing options were as per defaults. Coupled to normalization and segmentation into GM and white matter, a modulation step was performed to take into account volume changes caused by spatial normalization. The segmented, normalized, and modulated GM and white matter images were smoothed using a Gaussian kernel of 10 mm full width at half maximum. The images underwent visual quality control before processing as well as after each preprocessing step. We extracted GM VBM eigenvalues according to WFU Pickatlas (the SPM Wake Forest University, http://fmri.wfubmc.edu/software/PickAtlas) from 13 separate brain loci (frontal cortex, parietal cortex, temporal cortex, occipital cortex, limbic lobe, pons, sublobar regions, hypothalamus, and cerebellum) with Marsbar (http://marsbar.sourceforge.net). We chose these gross anatomical regions to explore neuronal tissue health in line with our previous study (7).

Statistical analysis

Whole-brain statistical analyses were included both to verify that the associations for the current study are in line with prior studies (expected negative associations to adiposity and age) and to provide readers an open report of the associations of BAT activity across the brain. However, the main approach in the current study was based on region of interest analysis.

In the whole-brain approach, we used general linear models that were implemented in SPM8 for voxelwise statistical analysis. In this descriptive setting, we entered the explaining variables separately into the model and asked whether negative or positive linear associations exist between the GM volumes and the variable of interest. Explaining variables included: age, BMI, BAT NEFA uptake in cold, BAT perfusion in cold, visceral fat volume measured with MRI, and insulin sensitivity (M value) as defined in the hyperinsulinemic euglycemic clamp measurements. We set the cluster forming threshold to $P < 0.001$, and the results were false discovery rate corrected for multiple comparisons at the cluster level ($P < 0.05$).

Potential associations between brain GM volumes and M value or intra-abdominal fat volume were examined with general linear models to assess whether M value and intra-abdominal fat volume could be used as covariates when examining whether BAT activity is independently associated with GM volumes. We used two different sets of covariates: the first set included age, sex, and intra-abdominal fat volume, whereas the second included age, sex, and M value. By switching intra-abdominal fat volume with M value, we aimed to examine whether BAT activity had associations independent of both of these variables and to avoid using intercorrelating covariates (central obesity and insulin sensitivity) in the same model.

Shapiro-Wilk tests were performed to assess the normality of all data used in this study. Correlations were examined with Spearman correlation. Linear regression model was used to examine associations with brain GM volumes as response variable; BAT PET measurement as explanatory variable; and age, gender, and intra-abdominal fat volume or M value as covariates. ANOVA was used to compare mean levels of normally distributed variables between BMI groups and sex distribution between BMI groups was compared with χ^2 (Table 1). If the variable was not normally distributed in the BMI groups, Kruskal–Wallis test was used instead of ANOVA (Tables 1-2). The lack of normal distribution in some variables in Tables 1-2 was due to the lower group size when the BMI subgroups were analyzed separately instead of using the whole cohort as was done in other analyses. SPSS 23 (IBM, Armonk, New York) was used for statistical analyses.

Results

Characteristics of the study cohort

Patients had a wide range of BAT NEFA (uptake 0.91 ± 0.80 µmol/100 g/min) and perfusion (19.4 ± 18.0 mL/100 g/min) during nonshivering cold activation, which suggests that the participants included subjects with both high and low BAT activity (Table 1). Skin temperature did not change during the 2-hour cold exposure (32.2 ± 1.6 vs. 32.0 ± 2.0, $P = 0.48$).

Whole-body insulin sensitivity (M value, 7.6 ± 3.9 mg/kg/min) and intra-abdominal fat volume (3,747.5 ± 2,418.5 cm^3) varied markedly. Our

TABLE 1 Description of study cohort

Variable	Group 1: BMI < 25 kg/m^2	Group 2: 25 ≤ BMI < 30 kg/m^2	Group 3: BMI ≥ 30 kg/m^2	*P* for difference between groups
Males/females, *N*	3/9	6/9	3/5	0.36
Age (y)	33.9 ± 11.2	38.8 ± 9.8	42.4 ± 8.7	0.15
BMI (kg/m^2)	23.1 ± 1.5	27.1 ± 1.4	33.6 ± 3.4	<0.001
Intra-abdominal fat volume (cm^3)[a]	2,558.3 ± 1,119.0	3,469.5 ± 1,517.4	5,349.8 ± 3,377.2	0.02
M value (mg/kg/min)	7.4 ± 2.7	7.9 ± 3.8	5.4 ± 3.6	0.21
2-hour plasma glucose during OGTT (mmol/L)	5.64 ± 1.09	6.18 ± 2.26	6.07 ± 1.16	0.68
BAT NEFA uptake in cold (μmol/100 g/min)[a,b]	1.48 ± 1.17	0.66 ± 0.24	0.52 ± 0.35	0.02
BAT perfusion in cold (mL/100 g/min)[a,b]	21.8 ± 19.8	18.1 ± 19.2	16.1 ± 15.1	0.51
BAT NEFA uptake in fasting (μmol/100 g/min)[a,b]	0.78 ± 0.60	0.77 ± 0.96	0.20 ± 0.08	0.09
BAT perfusion in fasting (mL/100 g/min)[a,b]	12.51 ± 10.63	12.88 ± 14.57	4.94 ± 3.38	0.51
BAT NEFA uptake in postprandial state (μmol/100 g/min)[a,b]	1.19 ± 0.75	0.26 ± 0.19	0.46 ± 0.49	0.11
BAT perfusion in postprandial state (mL/100 g/min)[b]	35.07 ± 17.40	10.90 ± 11.81	3.45 (*N* = 1)	0.11
Anterior cerebellum GM volume (eigenvalue)	0.528 ± 0.052	0.519 ± 0.048	0511 ± 0.049	0.73
Posterior cerebellum GM volume (eigenvalue)	0.491 ± 0.063	0.490 ± 0.041	0.492 ± 0.066	0.99
Frontal lobe GM volume (eigenvalue)	0.361 ± 0.027	0.344 ± 0.026	0.350 ± 0.033	0.27
Frontotemporal space GM volume (eigenvalue)	0.360 ± 0.044	0.321 ± 0.047	0.338 ± 0.049	0.09
Limbic lobe GM volume (eigenvalue)	0.509 ± 0.027	0.504 ± 0.027	0.506 ± 0.034	0.91
Medulla GM volume (eigenvalue)	0.173 ± 0.031	0.170 ± 0.029	0.171 ± 0.032	0.96
Midbrain GM volume (eigenvalue)	0.176 ± 0.016	0.183 ± 0.022	0.179 ± 0.018	0.66
Occipital lobe GM volume (eigenvalue)	0.397 ± 0.036	0.405 ± 0.036	0.388 ± 0.019	0.45
Parietal lobe GM volume (eigenvalue)	0.430 ± 0.041	0.414 ± 0.032	0.408 ± 0.027	0.26
Pons GM volume (eigenvalue)	0.100 ± 0.012	0.100 ± 0.017	0.095 ± 0.012	0.64
Sublobar lobe GM volume (eigenvalue)	0.310 ± 0.018	0.299 ± 0.020	0.306 ± 0.025	0.34
Temporal lobe GM volume (eigenvalue)	0.463 ± 0.038	0.455 ± 0.030	0.452 ± 0.037	0.72

Values are expressed as *N* or means±SD.
[a]Analyses performed with Kruskal–Wallis test because of non-normal distribution.
[b]Thirty-six patients were scanned during cold exposure, and out of these patients, eighteen were also scanned at room temperature during fasting and thirteen patients were scanned in postprandial state after a standardized meal.

primary BAT activity estimate, BAT NEFA uptake in cold exposure, was significantly different between BMI groups 1 and 2 and between BMI groups 2 and 3 (Table 1). Difference between BAT NEFA uptake in cold exposure and in fasting was nonsignificant ($P = 0.07$) but difference between BAT NEFA uptake in cold and in postprandial state was significant ($P = 0.007$). BAT perfusion was higher during cold exposure than in fasting ($P = 0.005$), but no difference was observed between cold and postprandial state ($P = 0.27$). There was no difference in fasting or postprandial state perfusion ($P = 0.22$) or NEFA uptake ($P = 0.27$). Brain GM volumes in different loci were presented as VBM-derived eigenvalues in Table 1. There were no differences between the BMI groups (always, $P \geq 0.09$).

Plasma levels of glucose and NEFA were similar in the BMI groups, but plasma insulin during cold exposure was higher in the patients with overweight and with obesity than in those with normal weight ($P = 0.006$) (Table 2).

Correlations between BAT activity and brain GM volumes

Our primary hypothesis was that BAT activity is associated with brain GM volumes in healthy adults. In the whole-brain linear regression, GM volume in medial temporal and frontal lobes, insulae, thalamus, and basal ganglions associated positively with BAT NEFA uptake and perfusion during cold exposure. Similar associations were observed in the region of interest data. BAT NEFA uptake during cold exposure correlated directly with GM volumes in anterior cerebellum ($r = 0.411$, $P = 0.014$), occipital lobe ($r = 0.398$, $P = 0.018$), and temporal lobe ($r = 0.408$, $P = 0.015$), whereas BAT perfusion during cold exposure was linked with GM volume in anterior ($r = 0.369$, $P = 0.04$) and posterior cerebellum ($r = 0.367$, $P = 0.04$) as well as midbrain ($r = 0.427$, $P = 0.017$) (Table 2). BAT perfusion in fasting at room temperature was also linked with GM volume in anterior ($r = 0.596$, $P = 0.01$) and posterior cerebellum ($r = 0.547$, $P = 0.02$). Postprandial BAT perfusion correlated with occipital lobe GM volume ($r = 0.767$, $P = 0.02$).

Associations between BAT activity and brain GM volumes adjusted for age, sex, intra-abdominal fat, and whole-body insulin sensitivity

Our secondary hypothesis was that the observed associations between BAT activity and brain GM volumes may be independent of confounding factors. To test this hypothesis, we adjusted the linear regression models for covariates: age, sex, intra-abdominal fat, and

TABLE 2 Plasma glucose, insulin, and NEFA levels during the PET scans

Variable	Group 1: BMI < 25 kg/m^2	Group 2: 25 ≤ BMI < 30 kg/m^2	Group 3: BMI ≥ 30 kg/m^2	*P* for difference between groups
Plasma insulin level after 2-hour cold exposure (mU/L)	3.2 ± 1.2	4.9 ± 2.3	7.6 ± 4.7	0.006
Plasma insulin level at room temperature during fasting (mU/L)	3.4 ± 1.26	6.5 ± 3.8	7.1 ± 4.6	0.06
Plasma insulin level during postprandial state (mU/L)[a]	21.5 ± 9.4	20.6 ± 9.4	41.6 ± 37.0	0.27
Plasma NEFA level after 2-hour cold exposure (μmol/L)	952 ± 265	821 ± 214	799 ± 183	0.19
Plasma NEFA level at room temperature during fasting (μmol/L)	847 ± 206	637 ± 154	752 ± 284	0.19
Plasma NEFA level during postprandial state (μmol/L)[a]	0.256 ± 0.316	0.340 ± 0.239	0.531 ± 0.429	0.41
Plasma glucose level after 2-hour cold exposure (mmol/L)	5.28 ± 0.40	5.18 ± 0.58	5.27 ± 0.63	0.92
Plasma glucose level at room temperature during fasting (mmol/L)	5.17 ± 0.06	5.61 ± 0.43	5.10 ± 0.58	0.77
Plasma glucose level during postprandial state (mmol/L)	7.26 ± 1.54	6.20 ± 0.97	6.85 ± 2.33	0.47

Values are expressed as means ± SD.
[a]Analyses performed with Kruskal–Wallis test because of non-normal distribution.

whole-body insulin sensitivity. Positive associations were found between M values and similar brain regions associated with BAT activity as shown in Figure 1. Furthermore, age and intra-abdominal fat volume was associated negatively with brain volumes in partly overlapping brain regions (Figure 1). These results suggest that M value, age, and intra-abdominal fat—factors associated with BAT activity—may potentially contribute to the observed associations between BAT activity and brain GM volumes and should therefore be used as covariates in the following analyses for our secondary hypothesis.

In the first model, BAT activity was used as the explanatory variable in linear regression models estimating brain GM volumes with age, sex, and intra-abdominal fat volume as covariates. BAT NEFA during cold exposure remained significantly associated with anterior cerebellum (β = 0.460 ± 0.011/μmol/100 g/min, $P = 0.018$) and occipital lobe (β = 0.406 ± 0.007/μmol/100 g/min, $P = 0.04$) GM volume, whereas associations with limbic lobe (β = 0.361 ± 0.006/μmol/100 g/min, $P = 0.07$) and temporal lobe GM volumes (β = 0.363 0.008/μmol/100 g/min, $P = 0.07$) were attenuated after correction for the covariates. Moreover, adjusting for the covariates rendered all associations between BAT perfusion during cold exposure and GM volumes nonsignificant (always $P \geq 0.80$).

In the second model, intra-abdominal fat volume was replaced with M value as a covariate. Associations between BAT NEFA uptake during cold exposure and GM volume in anterior cerebellum (β = 0.571 ± 0.011/μmol/100 g/min, $P = 0.003$), limbic lobe (β = 0.407 ± 0.006/μmol/100 g/min, $P = 0.04$), and temporal lobe (β = 0.444 ± 0.008/μmol/100 g/min, $P = 0.03$) remained significant, whereas association with occipital lobe was attenuated ($P = 0.10$). Associations between BAT perfusion in cold and GM volumes remained nonsignificant (always $P \geq 0.31$). Associations between GM volumes and BAT perfusion in fasting and in postprandial states were nonsignificant in adjusted analyses (always $P \geq 0.19$).

Discussion

We discovered several associations between GM volumes in numerous brain loci and BAT fatty acid metabolism independently of age, gender, and visceral fat volume. Correlations between BAT perfusion and GM volumes were dependent of intra-abdominal fat volume. Therefore, BAT activity in adulthood may possess neuroprotective capabilities or act as a marker of beneficial neuroprotective health without a direct causal effect.

Our hypothesis for this study was that BAT metabolic activity would associate positively with brain GM volumes in areas associated negatively with insulin resistance and obesity. The main potential effect would be the potential neuroprotective effects provided by active BAT, such as higher resistance toward development of obesity and insulin resistance resulting from plasma glucose and fatty acid clearance. Alternatively, BAT activity might act as a marker of metabolic neuroprotective health without a direct neuroprotective effect. However, our study setting cannot test causal relationships and, contrary to our hypothesis, the observed associations between BAT activity and GM volumes may also be due to the actions and behavior regulated by these brain loci that may contribute to higher BAT activity. In the following sections, we discuss the observed associations between BAT activity and brain GM volumes in loci previously associated with obesity, insulin sensitivity, and humoral factors.

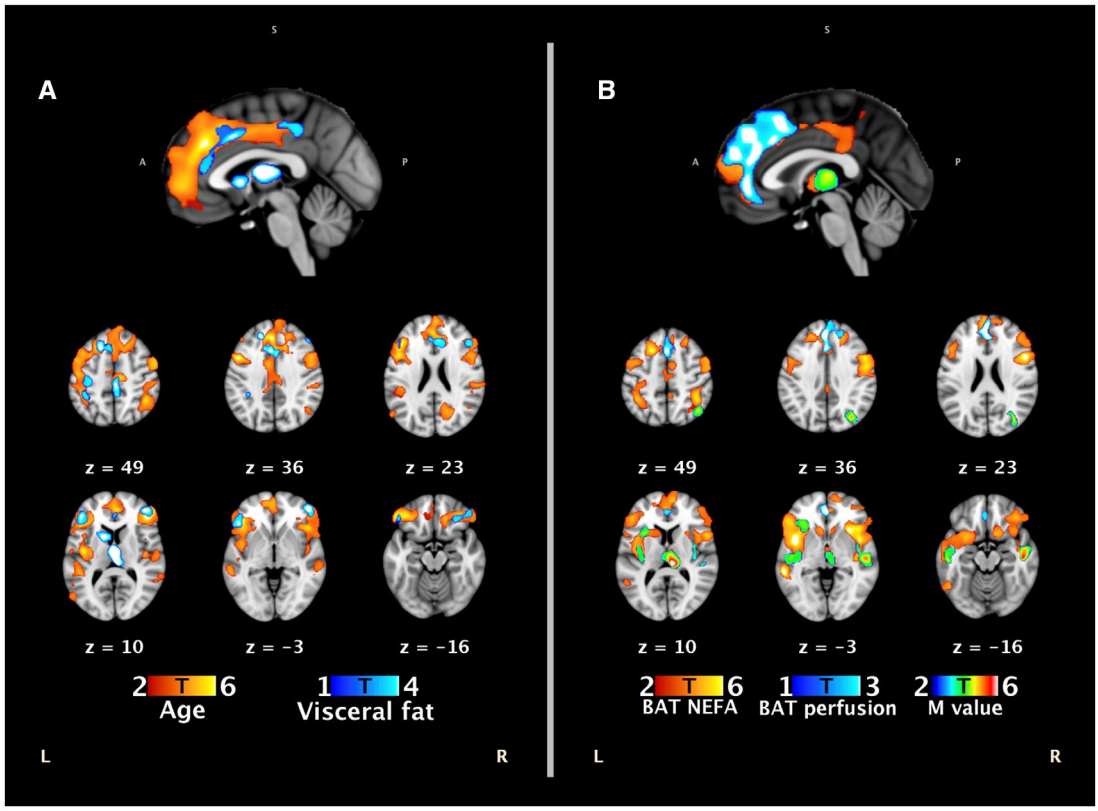

Figure 1 Results from the exploratory whole-brain regression analyses. (**A**) Negative associations between gray matter density and age and visceral fat. (**B**) Positive associations between gray matter density and brown adipose tissue (BAT) perfusion and BAT nonesterified fatty acid (NEFA) uptake and whole-body insulin sensivity (M value) ($N = 36$). All results are thresholded at $P < 0.001$, and false discovery rate corrected for multiple comparisons at $P < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

Obesity

Numerous previous studies have shown negative cross-sectional associations between BMI and GM density in medial temporal lobes, anterior cerebellar lobe, frontal lobe, occipital lobe and midbrain (3,14-26). This association was reflected in our study as was the expected negative association with age (Figure 1). Obesity and plasma leptin levels have been associated with changes in GM volumes in different parts of the brain (27,28). BMI, and especially waist circumference, had negative correlations with GM volume in hypothalamus, prefrontal, anterior temporal and inferior parietal cortices, and cerebellum (29). Visceral fat correlates negatively with cerebellar GM structure and functional connectivity (30), supporting the idea that increased body weight is associated with many plastic features in the brain. Our study showed positive correlations between GM volume in these regions and BAT NEFA uptake and perfusion during cold exposure. When adjusted for age, gender, and intra-abdominal fat volume, associations between BAT NEFA uptake and GM volume in limbic and temporal lobes were attenuated, which suggests that GM atrophy in these regions may be obesity related.

GM volume in anterior cerebellum and occipital lobe was associated with BAT NEFA uptake independent of visceral fat, gender, and age. Associations between GM volumes and BAT perfusion were not independent of obesity. We observed a direct correlation between BAT NEFA uptake and perfusion during cold exposure and anterior cerebellar GM volume. A similar connection was previously found between cerebellar and BAT glucose uptake during cold exposure (7). The cerebellum participates in motor control/proprioception, and GM volumes were reduced even in young adults with obesity and overweight (14). BAT NEFA uptake was associated with anterior cerebellar GM volume independent of obesity. Cerebellum activates and develops during physical exercise, which has been suggested as one factor that induces browning of white adipose tissue (15). Patients who engage in regular exercise are less likely to have obesity, another factor favoring high BAT activity.

Insulin sensitivity

Decreased insulin sensitivity has been associated with GM atrophy in temporal lobes in late-middle-aged adults (21) and the elderly (22), the area affected in Alzheimer disease and other cognition-related neurodegenerative disease. Insulin resistance is directly associated with GM atrophy in the medial temporal cortices in Alzheimer disease and in the parietal lobe in Parkinson's disease (23). Thus, BAT activity in adulthood may act as a protective factor against the development of cognitive impairment even though BAT activity declines with age (31). Patients with type 2 diabetes have cortical GM atrophy, e.g., in the temporal lobe (32), hippocampus, and amygdala (33). In our study, BAT NEFA

uptake was associated with GM volume in anterior cerebellum, limbic lobe, and temporal lobes independently of age, gender, and whole-body insulin sensitivity. Plasma NEFA clearance by BAT may protect GM from adverse fatty acid accumulation and concomitant neuronal degeneration. This would explain why BAT perfusion was not independently associated with GM volumes when adjusted for intra-abdominal fat volume or insulin sensitivity.

Humoral factors

The exact mechanism behind the associations between brain changes and obesity remains unclear but may include adipocytokines and low-grade inflammation (19). Possible contributing factors, which include leptin resistance, are common in obesity, leading to deteriorated regulation of appetite. Animal studies have shown leptin action in dorsomedial hypothalamus to increase sympathetic tone in BAT in spite of systemic leptin resistance (20), which might contribute to the association between BAT activity and GM density independently of obesity or insulin resistance. Furthermore, plasma triglycerides have been associated directly and high-density lipoprotein cholesterol inversely with brain atrophy (24). Active BAT provides plasma triglyceride clearance (25) and correlates with plasma high-density lipoprotein cholesterol levels (26), thus countering dyslipidemia. These may be some of the effects that account for the association between BAT fatty acid metabolism and high GM volume in healthy adults.

PET imaging of tissue fatty acid uptake and perfusion is a nonspecific method of measuring tissue metabolic activity. We can only speculate on the underlying pathways between BAT NEFA uptake and perfusion and GM volumes. Because of the complexity and high scanning costs of our study protocol, the sample size was relatively small; thus, further studies are required to extend and replicate our findings. Our current study setting could not be used to examine either the causal relationship or the magnitude of the potential association between BAT activity and GM volumes.

Our study cohort included patients who had normal weight, overweight, and obesity; because of the relatively small sample size, analyses were not stratified by weight groups. Further studies, including those with only patients with normal weight or obesity, should be performed to observe whether our findings are replicable in a larger set.

Conclusion

We observed several associations between BAT activity and GM volumes in brain loci. Associations between BAT NEFA uptake and GM volume in anterior cerebellum, limbic lobe, and temporal lobe were independent of visceral obesity and insulin sensitivity. BAT may participate in protection from cognitive degeneration associated with cardiometabolic risk factors, such as central obesity and insulin resistance, or it may act as a marker of beneficial neuroprotective health without a direct causal relationship with brain GM volumes.

Funding agencies: This study was supported by Finnish Cultural Foundation, the Hospital District of Southwest Finland, Turku University Foundation, and Finnish Medical Foundation. The funders did not have influence on study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Disclosure: The authors declared no conflict of interest.

Supporting information: Additional Supporting Information may be found in the online version of this article.

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