Overfeeding Over 24 Hours Does Not Activate Brown Adipose Tissue in Humans

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Context: Human brown adipose tissue (BAT) is activated with cold exposure, but it is unknown whether overfeeding activates BAT.

Objective: We determined BAT activation with cold, fasting, and overfeeding and the relationship of BAT activation with future weight change.

Design, Setting, Participants, and Interventions: Sixteen healthy adults were evaluated during energy balance, fasting, and 24 hours of 200% overfeeding. All subjects had a fluorodeoxyglucose-positron emission tomography (PET) scan after exposure to 16°C to determine cold-induced BAT activity (CIBA). The first six subjects had a second PET scan after 36 hours of fasting to establish the lack of BAT activation at 22°C. The other subjects' second PET scan occurred after 24 hours of overfeeding at 22°C but only if they demonstrated CIBA. Twelve subjects returned at 6 months for reassessment of body composition.

Main Outcome Measures: BAT was defined in cool scans as voxels with a standardized uptake value (SUV) of 2.0 or greater and Hounsfield units between −250 and −10. Body composition was assessed by dual-energy x-ray absorptiometry.

Results: Although 75% of the subjects demonstrated visible CIBA, none had visual BAT activity after overfeeding. CIBA was greater than that observed in the same defined BAT voxels after fasting ($n = 6$; 2.9 ± 0.5 vs 1.2 ± 0.2; $\Delta = -1.7$; 95% confidence interval −2.4, −1.0 SUV; $P < .01$). In the second cohort, CIBA was also higher than observed BAT voxel activity after 24 hours overfeeding ($n = 8$; 3.5 ± 0.7 vs 0.9 ± 0.2; $\Delta = -2.6$; 95% confidence interval −3.2, −1.9 SUV; $P < .01$). Baseline CIBA negatively correlated with changes in fat mass after 6 months ($r = -0.72$, $P = .009$).

Conclusions: BAT may be important in weight regulation unrelated to the response to overeating. (*J Clin Endocrinol Metab* 98: E1956–E1960, 2013)

The concept that some individuals have a greater ability to increase energy expenditure (EE) in response to overeating has been appealing since diet-induced thermogenesis (DIT) was linked to the activation of brown adipose tissue (BAT) in rats (1). Because BAT is involved in the nonshivering thermogenic response to cold in both rodents and humans (2–4), it has been hypothesized that BAT may also be activated with excess caloric intake in humans (5), theoretically contributing to obesity resistance in some individuals (6). Human thermograms have demonstrated increased skin temperature over supraclavicular BAT depots after a meal (7). Furthermore, BAT has been shown to be activated on positron emission tomography (PET) scans in the immediate postprandial period

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Abbreviations: BAT, brown adipose tissue; CIBA, cold-induced BAT activity; CT, computed tomography; DIT, diet-induced thermogenesis; EE, energy expenditure; FDG, fluorodeoxyglucose; FFM, fat-free mass; FM, fat mass; PET, positron emission tomography; SUV, standardized uptake value.

after a single, high-calorie, high-carbohydrate meal (8). However, it is not known whether human BAT is activated for an extended period after 24 hours of overfeeding or whether BAT has a relevant role in weight regulation.

Research Design and Methods

Subjects

Between August 2009 and October 2012, 16 healthy volunteers were admitted to the clinical research unit as part of a larger ongoing study (9) (clinicaltrials.gov, number NCT00523627) and placed on a weight-maintaining diet (10) consisting of 50% carbohydrates, 30% fat, and 20% protein. Body composition was assessed by dual-energy x-ray absorptiometry measurement (Lunar Corp) and glucose tolerance by a 75-g oral glucose tolerance test. Study volunteers were invited to return after 6 months for remeasurement of body composition. Prior to participation, all volunteers provided written informed consent. This study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases.

Study design

Two cohorts were recruited for this study, an initial proof-of-concept cohort, and a second, overfeeding cohort (see Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Each participant completed four 24-hour EE measures, two under eucaloric conditions, one while fasting, and one during overfeeding as part of the larger study (9). Details of the EE methods are in the Supplemental Material.

We performed the proof-of-concept study to confirm the absence of BAT activation at 22°C during fasting and that adults residing in the warm climate of the Sonoran Desert have cold-induced BAT activity (CIBA) because environmental temperatures can influence BAT activation (11). In the proof-of-concept study, six subjects had two 15-mCi 18F-fluorodeoxyglucose (FDG) PET/computed tomography (CT) scans in random order, one after an overnight fast and after approximately 2 hours of mild cold exposure (16°C) while wearing standardized clothing of approximately 0.3 clo (which is a unit of measure for the insulating properties of clothing), and the other after 36 hours of fasting under thermoneutral conditions (~22°C) (3).

In the overfeeding cohort, all 10 subjects had an initial PET scan after exposure to 16°C as above. If visible CIBA was observed, then a second thermoneutral PET scan was done after 24 hours of a 60% fat, 20% protein, 20% carbohydrate diet at 200% of energy requirements. This overfeeding diet was chosen because, in the larger study, it induced the greatest increase in sleeping EE (9). To investigate whether BAT is activated as a mechanism of prolonged adaptive thermogenesis or only during the postprandial period, we timed the PET scan in relation to the last overfeeding meal in two different ways. In five subjects, the second PET scan was done 12 hours after the last meal (ie, after an overnight fast). In three subjects, the PET scan was done 4 hours after the 24 hours of overfeeding plus an additional large breakfast individualized to 40% of energy requirements. All efforts were made to minimize unnecessary radiation to healthy research volunteers.

PET image analysis

To minimize CT-related radiation exposure while capturing representative BAT depots (2), CT data were acquired only from the base of the head to the diaphragm. PET and CT images were coregistered and BAT quantified using statistical parametric mapping software (SPM8) package in Matlab (The MathWorks, Inc). CIBA was defined as the collection of voxels with a standardized uptake value (SUV) of 2.0 or greater in the PET image, coinciding with areas in the CT image with Hounsfield units between −250 and −10 (Figure 1A) (2). For each individual, the defined CIBA voxels were then identified in the associated thermoneutral scan (after either fasting or overfeeding), and the average SUV of these same BAT voxels was determined.

Statistical analysis

Alpha was set at .05. See Supplemental Material for sample size explanation. Student's *t* tests were used to assess differences

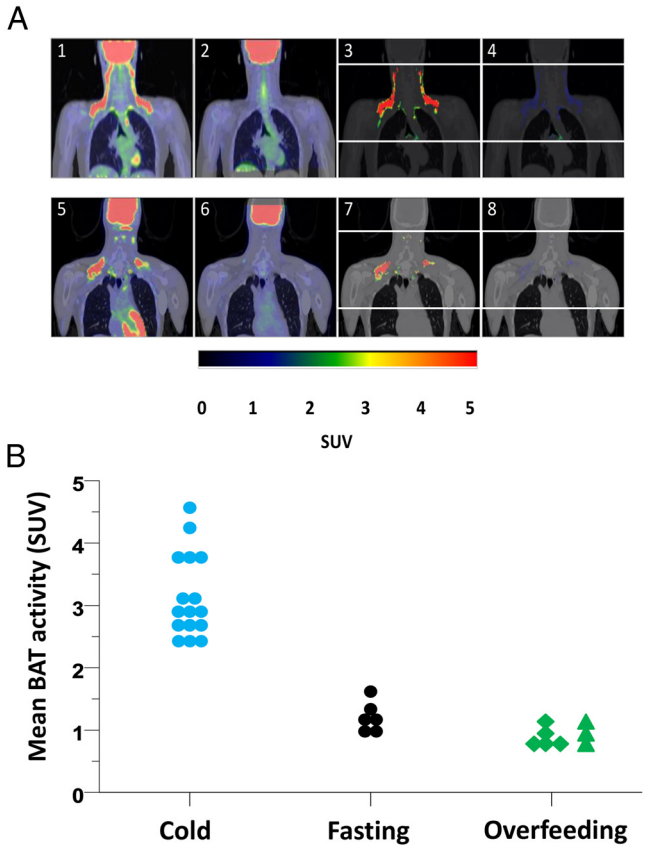

Figure 1. A, Comparison of 18F-FDG PET/CT images for two representative subjects in the cold condition (scans 1 and 5) and either after overfeeding (scan 2) or after fasting (scan 6). In the cold condition, a rectangular mask was drawn from the base of the cerebellum to the apex of the heart (scans 3 and 7). BAT was defined in cool scans (scans 3 and 7) as the collection of voxels with an SUV of 2.0 or greater on PET in areas with Hounsfield units between −250 and −10 on the CT scan. The defined voxels were identified in the associated thermoneutral (scans 4 and 8) scan by coregistering the PET-CT images to the cold-related scan (scans 2 and 6). Images were created in MRIcron (version 7, www.mricro.com,) based on SPM8 output files (The MathWorks, Inc). B, The figure shows mean BAT activity during cold exposure (blue circles) in the entire study group (n = 16), after fasting (black circles, n = 6), 4 hours after the overfeeding period with a high-fat, normal-protein diet (green triangles, n = 3), and 12 hours after the overfeeding period (high fat, normal protein diet, green diamonds, n = 5).

between groups. Paired *t* tests were used to evaluate differences between interventions. Associations between BAT activity and other covariates, including body composition changes at 6 months, were quantified using Spearman (ρ) correlation coefficients. Sensitivity analyses to test the robustness of our findings are described in the Supplemental Material. Statistical analyses were performed using SAS software (SAS E-guide 4.2 and SAS version 9.2; SAS Institute, Inc) and SPSS (version 21, IBM Corp).

Results

Demographics

Of 52 subjects assessed for eligibility, 16 subjects completed the study (see Supplemental Figure 2). Demographics are shown in Table 1. Subjects with visible CIBA (Table 1 and Figure 1A) were younger but otherwise similar. There was no visible BAT in any of the fasting or overfeeding scans.

Proof-of-concept study

CIBA was visible in four of six subjects. Fasting 24-hour EE decreased by −8.3% ± 2.7% ($P = .001$) compared with energy balance. Including scans from all six subjects, CIBA was higher than the measured SUV of BAT after 36 hours of fasting (2.9 ± 0.5 vs 1.2 ± 0.2; Δ−1.7; 95% confidence interval −2.4, −1.0 SUV; $P < .01$) (Figure 1B).

Overfeeding study

CIBA was visible in 8 of 10 subjects. These eight subjects subsequently had a PET scan after overfeeding. Compared with energy balance, 24-hour EE increased by 7.5% ± 5.7% ($P = .008$, n = 8) and EE during sleep by 10.5% ± 10.2% ($P = .02$) during overfeeding. DIT was 280 ± 164 kcal during overfeeding vs 140 ± 116 kcal in energy balance ($P = .009$). Compared with CIBA, the mean SUV of BAT voxels after overfeeding was lower (n = 8; 3.5 ± 0.7 vs 0.9 ± 0.2; Δ−2.6; 95% confidence interval −3.2, −1.9 SUV; $P < .001$) (Figure 1B).

Relationship of cold-induced BAT activity to EE and body composition (all subjects)

CIBA was negatively correlated only with fat-free mass (FFM) ($\rho = -0.61$, $P = .01$) but not with any measures of baseline adiposity (Supplemental Table 1). CIBA was not associated with EE measures during either energy balance or overfeeding (see Supplemental Material). Baseline CIBA was negatively associated with change in fat mass

Table 1. Demographic Characteristics of the Study Group

	All Subjects (n = 16)	Women (n = 7)	Men (n = 9)	No visual CIBA (n = 4)	Visual CIBA (n = 12)
Age, y	30.9 ± 9.9	30.7 ± 8.6	31.1 ± 11.3	39.4 ± 13.2	28.1 ± 7.1[a]
Range	19–50	20–39	19–50	21–51	19–39
Visual BAT					
No visual BAT		2 (29%)	2 (22%)	4 (100%)	0 (0%)
Visual BAT		5 (71%)	7 (78%)	0 (0%)	12 (100%)
Ethnicity					
Blacks	7 (44%)	4 (57%)	3 (34%)	2 (50%)	5 (44%)
Whites	3 (19%)	1 (14%)	2 (22%)	1 (25%)	2 (19%)
Native Americans	4 (25%)	2 (29%)	2 (22%)	1 (25%)	3 (25%)
Hispanic	2 (12%)	0 (0%)	2 (22%)	0 (0%)	2 (12%)
BMI, kg/m^2	26.4 ± 5.5	28.0 ± 6.5	25.1 ± 4.6	29.2 ± 7.1	25.4 ± 4.9
FM, kg	23.0 ± 15.1	32.8 ± 15.6	15.4 ± 9.9[a]	25.4 ± 22.0	22.2 ± 13.3
FFM, kg	55.1 ± 11.4	45.0 ± 3.8	63.0 ± 8.6[a]	58.3 ± 14.7	54.1 ± 10.7
Body fat, %	28.1 ± 14.4	40.1 ± 10.4	18.8 ± 9.2[a]	28.5 ± 20.3	28.0 ± 13.1
Fasting glucose, mg/dL	91.7 ± 5.9	91.9 ± 4.3	91.6 ± 7.2	94.3 ± 10.1	90.8 ± 4.0
2-Hour glucose, mg/dL	104.6 ± 19.3	102.6 ± 16.5	106.2 ± 22.1	112.0 ± 29.6	102.2 ± 15.6
Fasting insulin, mU/L	7.8 ± 5.8	10.1 ± 7.7	6.1 ± 3.2	12.1 ± 10.3	6.4 ± 2.8
2-Hour insulin, mU/L	61.8 ± 48.6	79.0 ± 72.7	50.3 ± 21.2	88.6 ± 94.1	52.0 ± 16.0
24-Hour EE, kcal/d	1984 ± 298	1793 ± 212	2133 ± 276[a]	2094 ± 276	1948 ± 308
Weight change, kg[b]	1.2 ± 4.6				
Range	−5.2 to 8.4				
Weight change, %[b]	1.0 ± 5.3				
Range	−7.2 to 8.6				
FM change, kg[b]	1.2 ± 3.5				
Range	−4.5 to 7.9				
FFM change, kg[b]	0.1 ± 1.9				
Range	−2.6 to 3.9				
BAT volume, cm^3	105.4 ± 84.1	142.0 ± 97.1	76.9 ± 64.0[a]		

[a] $P < .05$ vs women.

[b] Between baseline and the return visit at 6 months.

(FM) ($r = -0.72$, $P = .009$) (Supplemental Figure 3) at the 6-month follow-up visit (n = 12). Results were similar when substituting body weight change or the residual variance of FM change after accounting for age, gender, and initial FM. CIBA was not correlated with change in FFM ($r = -0.11$, $P = .73$). There were no differences in characteristics between subjects with follow-up data and the entire study group.

Discussion

Visible CIBA was evident, even in individuals residing in a warm southwestern US climate. However, we found no evidence of BAT activation after 24 hours of overfeeding with 200% of energy requirements using a high-fat, normal protein diet. In fact, the average SUV of identified BAT was similar after 36 hours of fasting and after 24 hours of overfeeding, at both 4 hours and 12 hours after the last meal. However, CIBA was negatively associated with changes in FM at follow-up.

In rodents, cold exposure activates BAT (12), and caloric excess may also stimulate BAT activity as an adaptation to limit body weight gain (1). Humans also exhibit CIBA (2–4) and increases in human EE with both overfeeding and mild cold exposure are correlated, implying that similar mechanisms may be responsible for both (13). BAT has been shown to be activated in the immediate postprandial period after a single, high-calorie, high-carbohydrate meal (8). However, prolonged BAT activation in humans after 24 hours of overfeeding or with diets of other macronutrient composition has not been clearly demonstrated (14). In lean males, food consumption during cold exposure failed to show increased BAT activation beyond that from cold exposure alone (15). It was unclear whether this was due to lack of activation of BAT with feeding or whether BAT was already maximally activated during cold exposure. Our results indicate that any activation of BAT after excessive caloric consumption does not last beyond 4 hours after the last meal.

Our results did not show BAT activation with overfeeding, leaving the cause of interindividual variance in the EE response to overfeeding unknown. This variance may be due to differences in the efficiency of processing and storing macronutrients or skeletal muscle pathways (16) implicated in the nonshivering thermogenic response to cold (17). Skeletal muscle may be the common factor underlying the increased EE after both cold exposure and overfeeding (13).

Our observed prevalence of CIBA is consistent with previous studies (18). However, we observed an association only with FFM but not with baseline body adiposity or age, as others have (3–4), likely due to our small sample size and young study population (although those with visible CIBA were younger overall). We found that CIBA negatively associated with FM changes at 6 months. It is unclear how CIBA can impact future body weight if BAT is not activated with overfeeding or related to EE. However, other larger studies have found that CIBA correlates with resting metabolic rate (3). Alternatively, BAT ablation in mice resulted in hyperphagia and obesity indicating a potential role for BAT in satiety (19).

Limitations of our study include a small sample size (discussed further in the Supplemental Material). Also, there may have been competitive interaction between consumed glucose and the 18F-FDG in the postoverfeeding scans, obscuring BAT activation. We minimized this possibility by using a low-carbohydrate diet and waiting at least 4 hours after food intake. Although a shorter time period after feeding or a diet of differing macronutrient composition may have revealed BAT activation, our data do not support a sustained BAT response expressly due to overconsumption of kilocalories. The overfeeding diet was 60% fat, a macronutrient reported to have minimal DIT (20). This was partially balanced by dietary protein, which has a high DIT (9, 20) and likely contributed to the increase in sleeping EE after overfeeding. PET scans in the overfeeding portion of the study were not randomized; however, we did have a minimum 4-day interval between scans, and any order effect would be more likely to result in a false-positive, not a false-negative second scan. We did not counsel our volunteers on lifestyle changes, but the knowledge they gained about their health may potentially have influenced the weight change at 6 months.

In conclusion, we have shown that BAT is not activated after 24 hours of overfeeding, but CIBA is negatively associated with changes in FM after 6 months. Any role of BAT in regulating body weight does not occur through a prolonged response to overeating.

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M.S. collected, analyzed, and interpreted the data, contributed to the execution of the study, and wrote the final report. P.P. coregistered PET and CT images, assisted with the interpretation of the data, and provided statistical advice. P.T., K.C., and E.M.R. provided supervisory advice coregistering PET and CT images. C.L. critically reviewed the PET and CT images. J.K. designed the study, contributed to the execution of the study, and assisted with the interpretation of the data. M.S.T. designed the study, collected, analyzed, and interpreted the data, contributed to the execution of the study, provided supervisory statistical advice, and reviewed and approved the final version of the report. All authors critically revised the draft and approved the final report.

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