

ORIGINAL ARTICLE

Cold-Activated Brown Adipose Tissue in Healthy Men

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ABSTRACT

BACKGROUND

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Studies in animals indicate that brown adipose tissue is important in the regulation of body weight, and it is possible that individual variation in adaptive thermogenesis can be attributed to variations in the amount or activity of brown adipose tissue. Until recently, the presence of brown adipose tissue was thought to be relevant only in small mammals and infants, with negligible physiologic relevance in adult humans. We performed a systematic examination of the presence, distribution, and activity of brown adipose tissue in lean and obese men during exposure to cold temperature. Brown-adipose-tissue activity was studied in relation to body composition and energy metabolism.

METHODS

We studied 24 healthy men — 10 who were lean (body-mass index [BMI] [the weight in kilograms divided by the square of the height in meters], <25) and 14 who were overweight or obese (BMI, ≥25) — under thermoneutral conditions (22°C) and during mild cold exposure (16°C). Putative brown-adipose-tissue activity was determined with the use of integrated ¹⁸F-fluorodeoxyglucose positron-emission tomography and computed tomography. Body composition and energy expenditure were measured with the use of dual-energy x-ray absorptiometry and indirect calorimetry.

RESULTS

Brown-adipose-tissue activity was observed in 23 of the 24 subjects (96%) during cold exposure but not under thermoneutral conditions. The activity was significantly lower in the overweight or obese subjects than in the lean subjects (P=0.007). BMI and percentage of body fat both had significant negative correlations with brown adipose tissue, whereas resting metabolic rate had a significant positive correlation.

CONCLUSIONS

The percentage of young men with brown adipose tissue is high, but its activity is reduced in men who are overweight or obese. Brown adipose tissue may be metabolically important in men, and the fact that it is reduced yet present in most overweight or obese subjects may make it a target for the treatment of obesity.

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THERE IS EVIDENCE THAT STIMULATING adaptive thermogenesis, defined as the facultative heat produced in response to cold and diet, might serve as a means of preventing or treating obesity¹; thus, it is of interest to understand the mechanisms underlying adaptive thermogenesis. We previously reported that cold-induced thermogenesis in the absence of shivering accounts for an average of 11.8% of the resting metabolic rate, with high individual variation.² Individual differences in energy expenditure can have large, long-term effects on body weight.³ Several prospective studies have shown that a relatively low energy expenditure predicts a gain in body weight.^{4,5} Hence, adaptive thermogenesis may be an attractive target for antiobesity therapies.

Cold-induced thermogenesis and diet-induced thermogenesis have recently been shown to be related, suggesting that they have a similar underlying mechanism.⁶ In small mammals, brown adipose tissue serves as a thermogenic organ, in which mitochondrial respiration is uncoupled from ATP production (through the action of uncoupling protein 1 [UCP1]) to dissipate energy. Although several early anatomical studies suggested that brown adipose tissue is present in adult humans,⁷⁻⁹ its physiologic relevance was believed to be marginal for most.^{9,10} In the past few years, however, studies conducted for other reasons that made use of integrated positron-emission tomography and computed tomography (PET-CT) indicated that brown adipose tissue is occasionally present and active in adult humans,¹¹⁻¹⁴ with the prevalence ranging from 2.5%¹¹ to 45%.¹⁵ Some studies indicate that brown adipose tissue is related to the body-mass index (BMI),^{15,16} and others do not.^{12,17}

We systematically examined the presence, distribution, and activity of brown adipose tissue in healthy volunteers in relation to body composition and energy metabolism. The activity of brown adipose tissue was assessed with the use of PET-CT scanning with ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG), body composition with dual-energy x-ray absorptiometry (DEXA), and energy expenditure with indirect calorimetry. Brown adipose tissue was activated by a standardized cold-exposure test.

METHODS

SUBJECTS AND PROCEDURES

Between October 2007 and December 2008, we studied 24 healthy male volunteers; 10 were clas-

sified as lean (BMI [the weight in kilograms divided by the square of the height in meters], <25), and 14 as overweight or obese (BMI, ≥25) (Table 1). The ethics committee of Maastricht University approved the protocol. All subjects provided written informed consent. In addition, a female patient provided written informed consent for the removal of samples of brown adipose tissue and white adipose tissue during surgery for multinodular goiter.

Subjects were studied in the morning, from approximately 9 a.m. to 1 p.m., after an overnight fast beginning at 10 p.m. the night before. During the experiment, all subjects wore standardized clothing (0.49 clo, which is a unit of measure for the insulating properties of clothing). In a climate chamber, the subjects rested in a supine position under thermoneutral conditions (22°C) for 1 hour and were then exposed to mild cold (16°C) for 2 hours. After 1 hour of exposure to cold, the PET tracer ¹⁸F-FDG was administered intravenously, and scanning was performed after the second hour of exposure to cold. Three subjects with relatively high levels of brown-adipose-tissue activity underwent an additional PET study in thermoneutral conditions in order to determine whether brown adipose tissue was activated only after cold exposure.

The PET-CT scanning protocol (Gemini TF PET-CT, Philips) included confirmation of the serum glucose level and the intravenous injection of 2 mCi (74 MBq) of ¹⁸F-FDG. Subjects rested comfortably, with the head, neck, and shoulders supported from the outset of the experiment until imaging began. Imaging was performed in three-dimensional mode, with emission scans of 6 minutes per bed position. Six to seven bed positions per subject were needed to cover the areas where brown adipose tissue is usually found. Imaging started with a low-dose CT scan (30 mAs), immediately followed by a PET scan. The scanner was equipped with time-of-flight electronics, which allows the use of a relatively low amount of radioactivity (2 mCi). The resulting total radiation dose from the low-dose CT scan and the injected radioactive tracer was approximately 2.8 mSv. The CT scan was used for attenuation correction and localization of the ¹⁸F-FDG uptake sites. Both image sets were reconstructed in transaxial, coronal, and sagittal images with a slice thickness of 4 mm. Two nuclear-medicine physicians interpreted the PET-CT images using PMOD soft-

Table 1. Characteristics of the Subjects.*

Characteristic	Lean Subjects (N=10)	Overweight or Obese Subjects (N=14)	P Value†
Age (yr)			0.58
Mean	24.3±3.6	23.5±3.4	
Range	20–32	18–30	
Body mass (kg)			<0.001
Mean	78.1±9.2	102.0±17.5	
Range	66.0–95.3	76.5–136.5	
Height (m)			0.99
Mean	1.83±0.09	1.83±0.06	
Range	1.75–2.02	1.74–1.94	
BMI			<0.001
Mean	23.2±1.2	30.3±4.2	
Range	21.3–24.5	25.4–38.7	
Body fat (%)‡			<0.001
Mean	16.2±5.1	27.3±7.4	
Range	9.4–25.1	16.9–41.8	

* Plus-minus values are means ±SD. BMI denotes body-mass index, calculated as the weight in kilograms divided by the square of the height in meters.

† P values are based on unpaired t-tests.

‡ The percentage of body fat was determined with the use of dual-energy x-ray absorptiometry.

ware, version 2.85 (PMOD Technologies, May 2007). Brown-adipose-tissue activity (measured in kilobecquerels) and brown-adipose-tissue volume (measured in cubic centimeters) were quantified in the region of interest by autocontouring the brown-adipose-tissue areas with a set threshold.

Energy expenditure was measured by means of a respiratory gas analyzer with the use of a ventilated hood system (Oxycon, Jaeger). The resting metabolic rate was corrected for fat-free mass by means of linear regression, since 80% of individual differences in energy expenditure can be explained by fat-free mass.¹⁸ The residuals of the regression between energy expenditure and fat-free mass were added to the average energy expenditure. Skin temperatures were measured continually at 16 positions by means of iButtons (type DS1921H, Maxim).¹⁹ The mean skin temperature was calculated with a 14-point method (ISO-9886).²⁰ Underarm-fingertip temperature gradients were calculated to obtain an estimate of blood flow in the fingers.²¹ Core temperature

was measured in the small intestine with the use of a telemetric pill (HQ). In three subjects (two lean and one overweight), the core-temperature measurement failed.

The protocol for temperature (mildly cold, 16°C) was validated earlier.²² Every 15 minutes, each subject was asked whether he had experienced shivering. Body composition was measured with the use of a DEXA whole-body scanner (QDR 4500A, Hologic).

To ascertain whether brown adipose fat in the supraclavicular region could be identified with classic microscopy and an immunofluorescence assay for UCP1, samples were obtained during surgery for a multinodular goiter in a 46-year-old woman with normal thyroid function (body mass, 72 kg; height, 1.72 m; thyroid-stimulating hormone, 1.7 mU per milliliter; and free thyroxine, 12.8 pmol per liter [1.0 ng per deciliter]). Biopsy samples of both brown and white adipose tissues were obtained from the supraclavicular region on the right side. In this area, both fat types were observed in patches measuring approximately 0.5 cm³. Sections (5 μm thick) were fixed for 15 minutes with 3.7% formaldehyde in phosphate-buffered saline, and UCP1 was detected with the use of rabbit polyclonal human UCP1 antiserum and with goat antirabbit fluorescein isothiocyanate as the secondary antibody, together with the nuclei-staining dye 4',6-diamidino-2-phenylindole (0.5 mg per milliliter).

STATISTICAL ANALYSIS

Data are reported as means ±SD. Data analysis was performed with the use of SPSS software, version 11, and Statview SE for Macintosh (SAS Institute). Comparisons were made by means of a paired t-test (for findings under thermoneutral conditions as compared with mild cold exposure) and an unpaired t-test (for findings in lean men as compared with overweight or obese men). Linear regression analyses were conducted to identify correlations between variables. For the relation between brown-adipose-tissue activity and body composition, exponential curve fitting was applied; therefore, log (brown-adipose-tissue activity) was plotted against body composition (BMI or percentage of body fat). Cook's distance values that were larger than 0.5 and Studentized deleted residuals larger than 4.0 were considered to be outliers. P values of less than 0.05 were considered to indicate statistical significance.

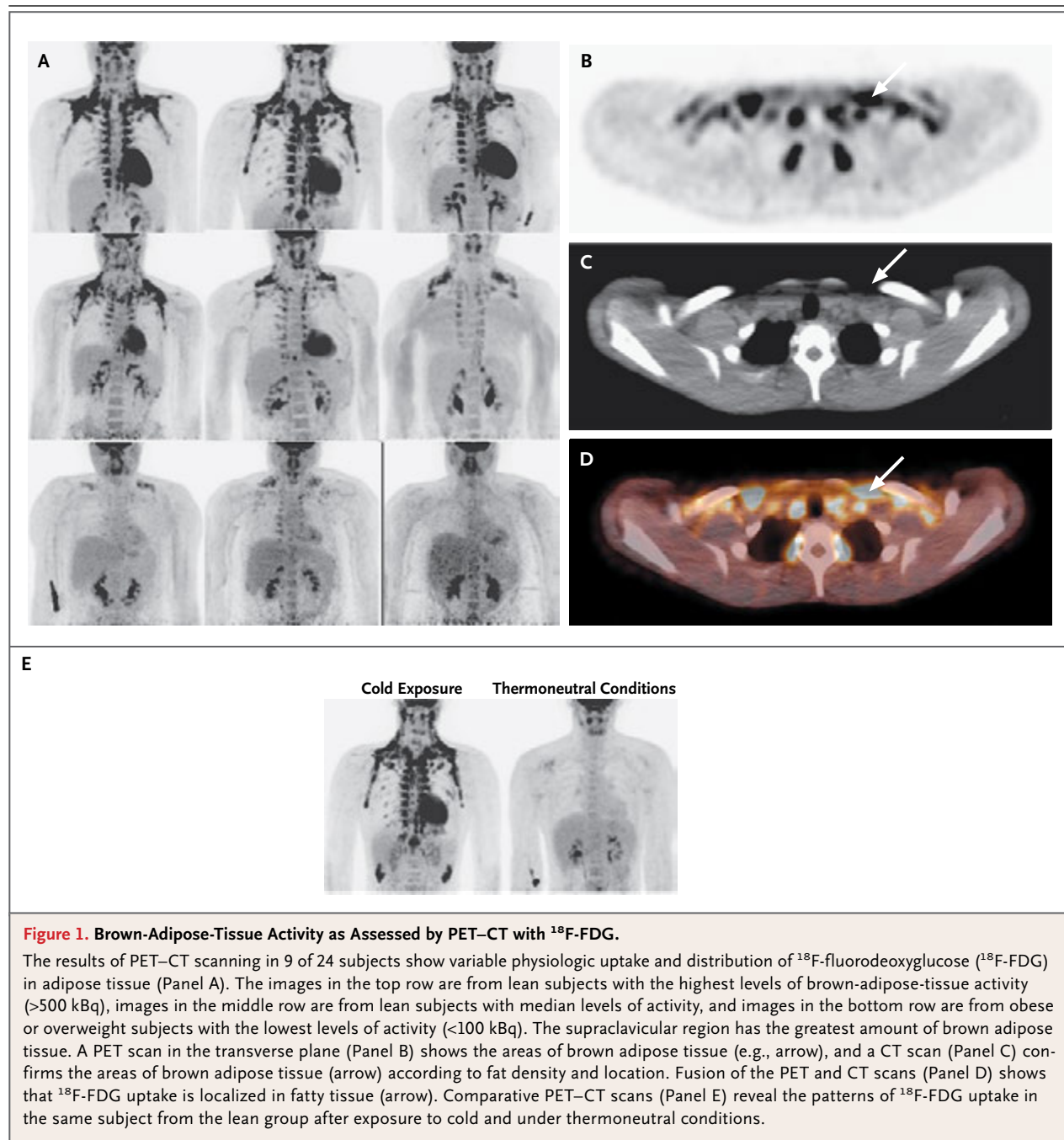


Figure 1. Brown-Adipose-Tissue Activity as Assessed by PET-CT with ^{18}F -FDG.

The results of PET-CT scanning in 9 of 24 subjects show variable physiologic uptake and distribution of ^{18}F -fluorodeoxyglucose (^{18}F -FDG) in adipose tissue (Panel A). The images in the top row are from lean subjects with the highest levels of brown-adipose-tissue activity (>500 kBq), images in the middle row are from lean subjects with median levels of activity, and images in the bottom row are from obese or overweight subjects with the lowest levels of activity (<100 kBq). The supraclavicular region has the greatest amount of brown adipose tissue. A PET scan in the transverse plane (Panel B) shows the areas of brown adipose tissue (e.g., arrow), and a CT scan (Panel C) confirms the areas of brown adipose tissue (arrow) according to fat density and location. Fusion of the PET and CT scans (Panel D) shows that ^{18}F -FDG uptake is localized in fatty tissue (arrow). Comparative PET-CT scans (Panel E) reveal the patterns of ^{18}F -FDG uptake in the same subject from the lean group after exposure to cold and under thermoneutral conditions.

RESULTS

Twenty-three of the 24 subjects had definite, albeit highly variable, amounts of ^{18}F -FDG activity in the neck, supraclavicular region, chest, and abdomen (Fig. 1A). On the fused PET-CT images, the activity was located not in muscle tissue but in fat tissue, which was therefore considered to

be brown adipose tissue (Fig. 1B through 1D). All lean subjects and 13 of the 14 overweight or obese subjects had evidence of brown-adipose-tissue activity in response to cold exposure. Mean brown-adipose-tissue activity was significantly lower in the overweight or obese subjects (102 ± 93 kBq) than in the lean subjects (428 ± 394 kBq) ($P=0.007$) (Table 2). The volume of brown adipose

Table 2. Activity and Volume of Brown Adipose Tissue, Resting Metabolic Rate Adjusted for Fat-free Mass and Body Temperature under Thermoneutral Conditions (22°C) and after 2 Hours of Cold Exposure (16°C).*

Variable	Lean Group (N=10)	Overweight or Obese Group (N=14)	P Value, Lean vs. Obese	P Value, 22°C vs. 16°C	
				Lean Subjects	Overweight or Obese Subjects
BAT activity (kBq) †	428±394	102±93	0.007		
BAT volume (cm ³) †	130±98	77±69	0.14		
Resting metabolic rate (MJ/24 hr)				<0.001	<0.001
Thermoneutral conditions	8.46±0.93	8.16±0.29	0.27		
Cold exposure	9.62±1.36	9.56±0.66	0.89		
Respiratory quotient (VCO ₂ /VO ₂) ‡				0.009	<0.001
Thermoneutral conditions	0.832±0.039	0.833±0.049	0.94		
Cold exposure	0.798±0.027	0.789±0.031	0.46		
Body temperature (°C)					
Core				0.06	0.02
Thermoneutral conditions	36.7±0.3	36.6±0.2	0.49		
Cold exposure	36.8±0.3	36.8±0.2	0.80		
Skin				<0.001	<0.001
Thermoneutral conditions	32.5±0.6	32.4±0.4	0.64		
Cold exposure	29.2±0.8	28.7±0.6	0.06		
Gradient, core and skin				<0.001	<0.001
Thermoneutral conditions	4.2±0.6	4.3±0.4	0.86		
Cold exposure	7.5±0.8	8.1±0.7	0.08		
Gradient, arm–finger				<0.001	<0.001
Thermoneutral conditions	-1.9±1.4	-3.5±1.7	0.03		
Cold exposure	3.4±2.8	4.6±2.8	0.29		

* Plus-minus values are means ±SD. BAT denotes brown adipose tissue, VCO₂ volume of carbon dioxide produced, and VO₂ volume of oxygen consumed.

† BAT activity and BAT volume were measured after cold exposure.

‡ Both VCO₂ and VO₂ are measured in liters per minute.

tissue tended to be lower in the overweight or obese subjects than in the lean subjects, but the difference was not significant ($P=0.14$). Only one subject had no ¹⁸F-FDG uptake in areas of adipose tissue. This subject had the highest BMI (38.7) and percentage of body fat (41.8%). In all subjects with brown-adipose-tissue activity, the area with the most evidence of activity was the supraclavicular region (Fig. 1). However, other sites also had substantial putative brown-adipose-tissue activity.

There was no detectable brown-adipose-tissue activity in the three subjects who were retested under thermoneutral conditions (Fig. 1E). Brown-adipose-tissue activity was recorded in Subject 2 (Fig. 1A) at 856.5 kBq during exposure to cold

and 0 kBq in thermoneutral conditions, in Subject 3 at 587.2 kBq during cold exposure and 0 kBq in thermoneutral conditions, and in Subject 4 at 250.0 kBq during cold exposure and 0 kBq thermoneutral conditions.

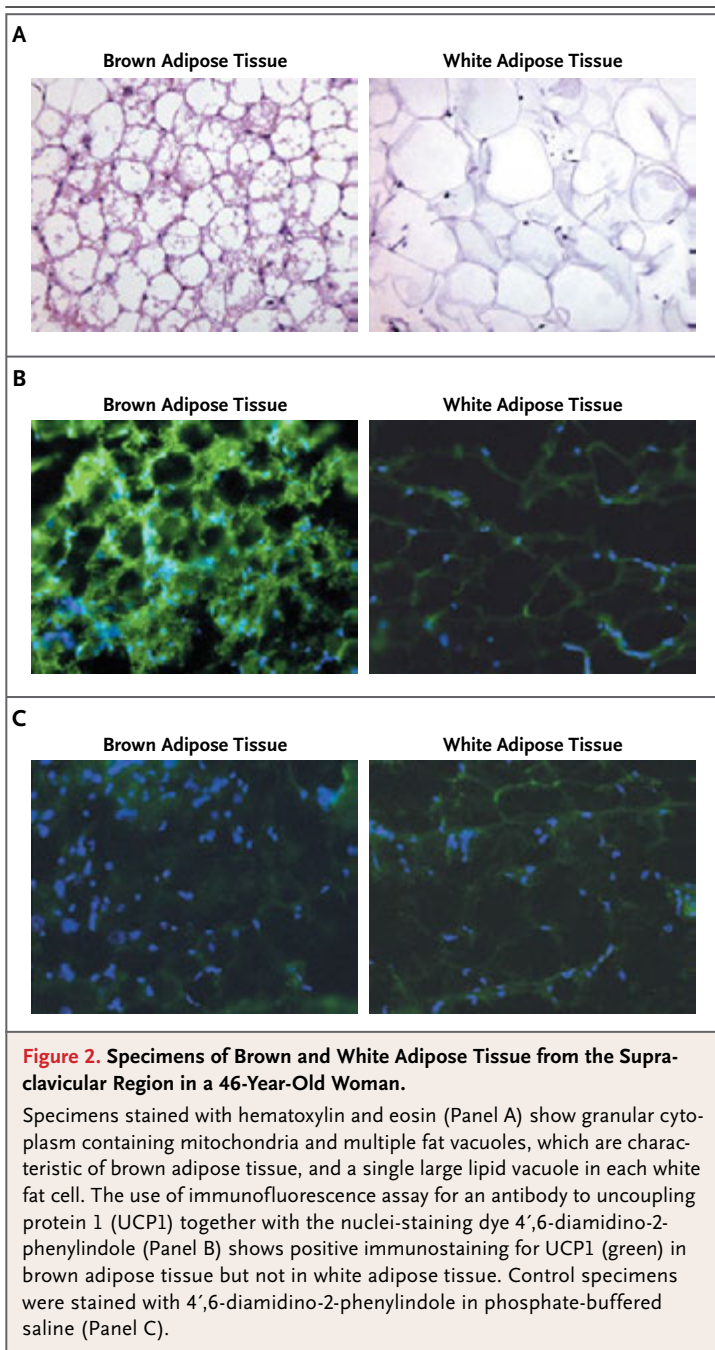
Studies of brown adipose tissue obtained from the supraclavicular region during surgery in the patient with multinodular goiter revealed the presence of UCP1 (Fig. 2). The protein was not detected in white adipose tissue taken from the same region in this patient.

On exposure to cold, all subjects had an insulative response (a reduction of skin temperature relative to core temperature) and a metabolic response (an increase in heat production) (Table 2). Body core temperature increased slightly in re-

sponse to cold exposure, with lean subjects having an increase of $0.13 \pm 0.16^\circ\text{C}$ ($P=0.06$ for the comparison with body), and obese or overweight subjects an increase of $0.16 \pm 0.22^\circ\text{C}$ ($P=0.02$). The mean skin temperature dropped significantly in both study groups, by $3.4 \pm 0.9^\circ\text{C}$ in the lean subjects and by $3.7 \pm 0.5^\circ\text{C}$ in the overweight or obese subjects. The core skin temperature gradient increased significantly during cold exposure as compared with the gradient under thermoneutral conditions ($P<0.001$) (Table 2). The underarm-fingertip gradient also increased during cold exposure, indicating vasoconstriction in the skin of the extremities.²¹ The resting metabolic rate adjusted for fat-free mass was 8.46 ± 0.93 MJ per 24 hours in the lean subjects and 8.16 ± 0.29 MJ per 24 hours in the overweight or obese subjects. The rate increased significantly during exposure to cold in both groups ($P<0.001$ for both comparisons) (Table 2), with much individual variation, ranging from 5 to 30%. No shivering was reported.

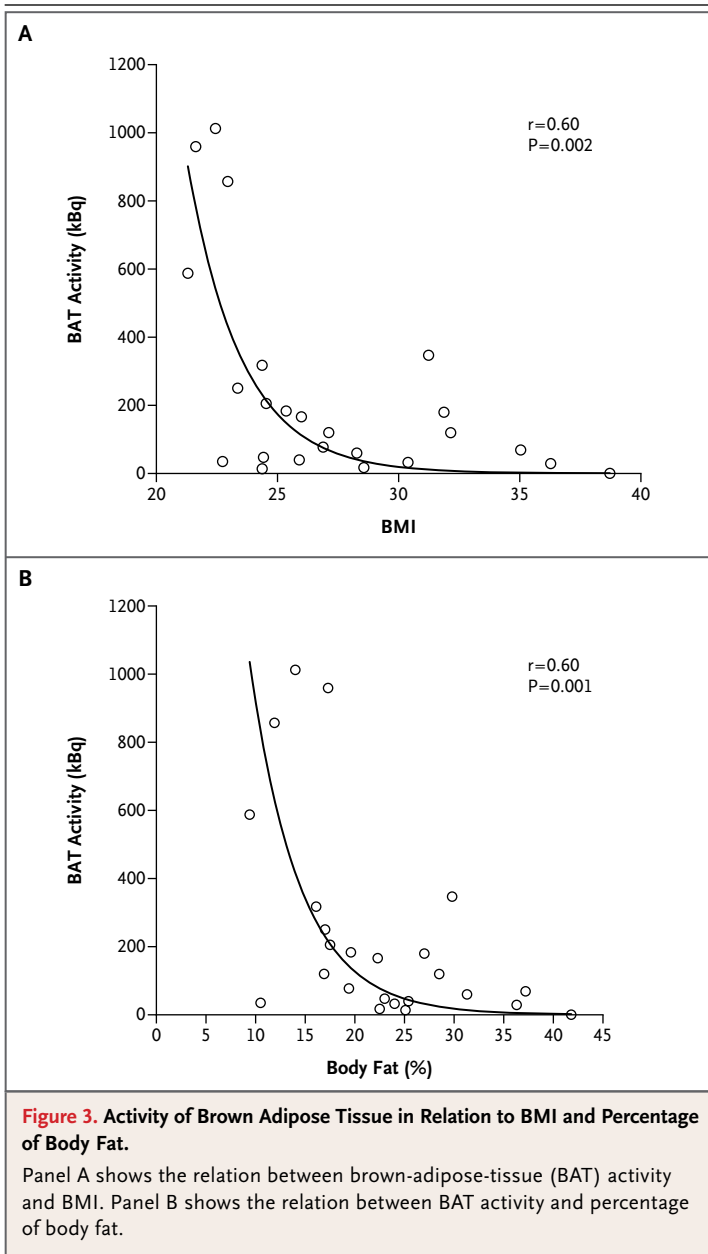
Nonlinear regression analysis showed that the activity of whole-body brown adipose tissue was negatively correlated with BMI ($r=-0.60$, $P=0.002$) and percentage of body fat ($r=-0.60$, $P=0.001$) (Fig. 3A and 3B). There was no correlation between brown-adipose-tissue activity and age, and in a stepwise regression analysis, BMI but not age was significantly associated with brown-adipose-tissue activity.

The change in distal temperature from thermoneutral conditions to cold exposure was related to brown-adipose-tissue activity ($r=0.41$, $P=0.04$), suggesting that a higher level of activity is associated with a smaller decrease in mean skin temperature and that brown-adipose-tissue activity may contribute to the regulation of body temperature by increasing the production of body heat. However, we did not find a significant relation between cold-induced thermogenesis and brown-adipose-tissue activity. The resting metabolic rate was positively correlated with brown-adipose-tissue activity both under thermoneutral conditions ($r=0.56$, $P=0.005$; with one outlier removed, $r=0.64$, $P=0.001$) (Fig. 4) and during cold exposure ($r=0.40$, $P=0.05$; with the same outlier removed, $r=0.46$, $P=0.03$), indicating that brown-adipose-tissue activity is involved in energy metabolism. The core temperature under thermoneutral conditions was also positively correlated with brown-adipose-tissue activity ($r=0.48$, $P=0.03$).



DISCUSSION

We observed a very high rate of occurrence of brown adipose tissue in normal young men (>95%). In tissue samples taken from the supraclavicular area of a surgical patient, an immunofluorescence assay revealed the presence of UCP1, confirming the presence of brown adipose tissue



(the supraclavicular area of the body is the primary location for active brown adipose tissue). Moreover, the results of PET-CT scanning performed just after the subjects had spent 2 hours in thermoneutral conditions revealed no brown-adipose-tissue activity, indicating that brown adipose tissue is activated by cold exposure.

Our study showed that brown-adipose-tissue activity was significantly lower in overweight or obese male subjects than in lean male subjects. There was a significant negative relation between

brown-adipose-tissue activity and BMI as well as between brown-adipose-tissue activity and percentage of body fat. However, only one obese subject had no brown-adipose-tissue activity. This finding indicates that it may be possible to increase brown adipose tissue in most overweight or obese men, and we speculate that methods of increasing brown adipose tissue in such men may be of therapeutic interest. Studies of the effects of regular exposure to cold, weight-loss diets, and increased physical activity on brown-adipose-tissue activity will also be of interest.

In our study, the rate of detection and level of activity of brown adipose tissue were much higher than those in earlier studies. The high detection rate in the current study is most likely due to the use of a standardized test for cold exposure. Previous studies measured brown adipose tissue in patients with cancer and related the occurrence of brown adipose tissue to outside temperatures or the season.²³ One early study used ephedrine rather than cold to stimulate brown-adipose-tissue activity in men.⁹ However, that study focused on perirenal brown adipose tissue, assuming that all depots have the same level of activity.

Our study showed that in all the subjects, the level of brown-adipose-tissue activity was highest in the supraclavicular region. However, other sites with substantial brown-adipose-tissue activity were evident. Most sites have been described in previous autopsy studies⁸ or in studies using PET-CT scanning with ¹⁸F-FDG.¹⁴ A second reason for the high incidence of brown-adipose-tissue activity in our study is the relatively young age of our subjects, ranging from 18 to 32 years. It is likely that the amount of brown adipose tissue decreases with age,¹⁷ although no relation between brown-adipose-tissue activity and age was observed in our study.

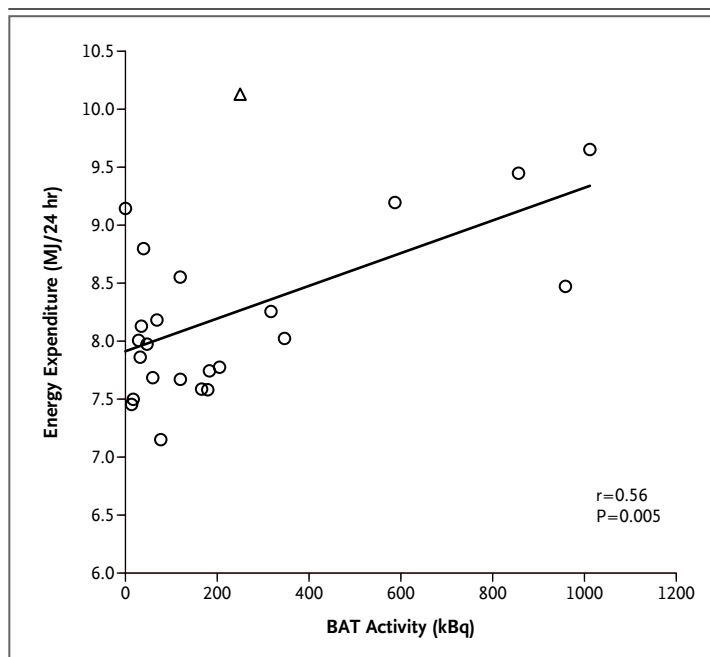
The relation between brown-adipose-tissue activity and BMI observed in our study is similar to the results of previous studies, which showed that the level of brown-adipose-tissue activity was highest in subjects with a low BMI.^{15,16} However, other studies, which were retrospective and were not designed to assess brown-adipose-tissue activity, did not confirm this finding.^{12,17}

Brown-adipose-tissue activity was negatively correlated with a change in distal skin temperature, suggesting that the activity during cold exposure was involved in the immediate response

to cold temperatures. Thus, subjects with the highest level of brown-adipose-tissue activity had the lowest drop in distal temperature, suggesting that these subjects were able to maintain core body temperature through an increase in body heat. However, contrary to our expectation, there was no significant relation between brown-adipose-tissue activity and cold-induced thermogenesis. The most likely reason is that other tissues, such as skeletal muscle,¹ may be involved in the thermogenic response. Indeed, we recently found that mitochondrial uncoupling in skeletal muscle is significantly related to cold-induced adaptive thermogenesis in humans.²⁴ Given the findings of an earlier study, in which subjects were exposed to cold for 1 hour,² we expected less cold-induced thermogenesis in the group of overweight or obese subjects than in the lean group, but the groups did not differ significantly in this respect. Even though the overweight or obese subjects had an increase in thermogenesis on exposure to cold that was similar to the increase in the lean subjects, they had less brown-adipose-tissue activity (or none).

Recent studies conducted at the molecular level have identified a mechanism by which PR domain containing 16 (PRDM16), a zinc-finger protein selectively expressed in brown adipose tissue, can induce gene expression in brown adipose tissue while suppressing it in white adipose tissue, thereby controlling the induction of brown adipose tissue.^{25,26} Moreover, there is recent evidence that brown adipose tissue is more closely related to skeletal muscle than to white adipose tissue.^{27,28} A putative shared lineage between brown adipose tissue and skeletal muscle may provide clues about the origins of stem cells that lead to the formation of brown fat and to adaptive thermogenesis.

Our study suggests that the relatively high level



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