Cold but not sympathomimetics activates human brown adipose tissue in vivo

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As potential activators of brown adipose tissue (BAT), mild cold exposure and sympathomimetic drugs have been considered as treatments for obesity and diabetes, but whether they activate the same pathways is unknown. In 10 healthy human volunteers, we found that the sympathomimetic ephedrine raised blood pressure, heart rate, and energy expenditure, and increased multiple circulating metabolites, including glucose, insulin, and thyroid hormones. Cold exposure also increased blood pressure and energy expenditure, but decreased heart rate and had little effect on metabolites. Importantly, cold increased BAT activity as measured by ^{18}F-fluorodeoxyglucose PET-CT in every volunteer, whereas ephedrine failed to stimulate BAT. Thus, at doses leading to broad activation of the sympathetic nervous system, ephedrine does not stimulate BAT in humans. In contrast, mild cold exposure stimulates BAT energy expenditure with fewer other systemic effects, suggesting that cold activates specific sympathetic pathways. Agents that mimic cold activation of BAT could provide a promising approach to treating obesity while minimizing systemic effects.

metabolism | thermogenesis | respiratory quotient | norepinephrine | white adipose tissue

Brown adipose tissue (BAT) is a type of fat that consumes calories to generate heat. Multiple recent studies have shown that adult humans have functional BAT that can be activated in response to cold exposure in a process called nonshivering thermogenesis (1–4). In both small and large population studies (1, 2, 4, 5), there is an inverse correlation between BAT activity and obesity, suggesting that activating BAT, through pharmacological, environmental, or potentially nutritional interventions, could become a therapeutic means to treat obesity and diabetes. Indeed, human BAT energy expenditure may be a critical counterbalance to the weight gain and metabolic dysregulation caused by excess energy storage in white adipose tissue.

Human BAT has a high density of both nerves and blood vessels (6), providing two general approaches to activate BAT. Based on studies in rodents, it is known that the sensation of cold by the skin and body core sends signals via peripheral neurons to the spinal cord and then up to the preoptic area of the hypothalamus for processing. From the hypothalamus, some signals go to the cerebral cortex for conscious thermal perception and localization, and others go to premotor neurons in the rostral raphe pallidus of the brainstem, projecting to neurons of the peripheral sympathetic nervous system (SNS) (reviewed in ref. 7). Ultimately, postganglionic SNS nerves release norepinephrine to activate BAT via induction of uncoupling protein-1, the tissue-specific protein that allows BAT to generate heat by uncoupling aerobic respiration from the generation of ATP.

Because the endogenous pathways by cold exposure are complex and indirect, an attractive alternative for stimulation of BAT has been the use of pharmacological agents. As norepinephrine itself has too many adverse effects on the cardiovascular system, drugs that bind to the relatively fat-specific β3-adrenergic receptor agonists have been developed in attempt to stimulate BAT, but these have had little success to date in humans (8–11). Given the increasing health burden of the obesity and diabetes pandemics, it is vital to develop novel approaches to increase energy expenditure and induce weight loss through BAT. A critical first phase is to demonstrate the effectiveness of different environmental and pharmacologic methods. In this study we compared the ability of mild cold exposure and the sympathomimetic drug ephedrine to stimulate BAT in 10 healthy volunteers. Ephedrine was chosen because it is comparatively safe yet still achieves broad activation of the SNS; it has been used for decades to increase energy expenditure and achieve weight loss in humans (12); in addition, ephedrine has been shown at very high doses to increase BAT glucose uptake in rodents (13). We find that although these two methods both use the SNS to increase thermogenesis, at similar levels of energy expenditure and cardiovascular response, only cold exposure appreciably stimulates BAT. In fact, the pathways of BAT activation are specific and not mimicked by generalized pharmacological activation of the SNS.

Results

Effects of Ephedrine and Cold Exposure on Vital Signs and Energy Expenditure. For each volunteer (Table 1), we compared the average systolic blood pressure (BP), diastolic BP, and heart rate for two time intervals: during the hour before intervention (four measures) and then from 45 to 90 min afterward (four measures). Paired *t* tests showed that there was no change in any of these parameters after saline injection ($P > 0.05$). Repeated-measures ANOVA showed that treatment condition has a significant effect on vital signs ($P < 0.001$). As expected, ephedrine at 1 mg/kg induced significant increases in systolic BP ($P < 0.001$), diastolic BP ($P = 0.002$), and heart rate ($P = 0.015$). Cold also increased systolic ($P = 0.002$) and diastolic BP ($P < 0.001$), but decreased heart rate ($P = 0.040$) (Fig. 1*A*). Of note, the female volunteers had lower height, body surface area, percent lean mass, and total lean mass, but there was no significant sexual dimorphism seen in any of the other anthropometric parameters, vital signs, laboratory values, bioenergetics, or measures of BAT function.

Neither basal metabolic rate (BMR) nor respiratory quotient (RQ) changed after saline injection ($P > 0.05$). The mean BMR was 1,448 kcal/d. In contrast, the metabolic rate increased by 136 kcal/d, or 9.0%, after ephedrine treatment ($P = 0.01$) and 79 kcal/d, or 5.5%, after cold exposure by ($P = 0.033$). These were not significantly different from each other ($P > 0.05$) (Fig. 1*B*). RQ decreased after both ephedrine treatment ($P = 0.003$) and cold exposure ($P = 0.029$), and again there was no difference between the effects of ephedrine and cold exposure ($P > 0.05$) (Fig. 1*C*).

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Table 1. Clinical characteristics of volunteers

Characteristic (units)	Combined*	Male	Female	*P* value
Sex	4 M/6 F			
Age[†] (y)	27.1 ± 1.7	25.3 ± 2.4	28.3 ± 2.3	0.42
Height (cm)	169 ± 2	176 ± 2	165 ± 2	0.002
Weight (kg)	68.0 ± 4.3	75.6 ± 5.8	62.9 ± 5.3	0.15
Body mass index (kg/m^2)	23.7 ± 1.4	24.3 ± 1.8	23.3 ± 2.1	0.74
Body surface area (m^2)[‡]	1.78 ± 0.06	1.92 ± 0.07	1.68 ± 0.05	0.026
Waist-hip ratio	0.86 ± 0.02	0.89 ± 0.01	0.84 ± 0.04	0.35
Body fat (%)	22.5 ± 2.5	16.8 ± 1.8	26.3 ± 3.3	0.06
Lean body mass (%)	73.9 ± 2.5	79.8 ± 1.7	69.9 ± 3.1	0.042
Lean body mass (kg)	50.3 ± 3.4	60.3 ± 4.1	43.6 ± 2.3	0.005
SAT/VAT[§]	3.8 ± 1.0	2.0 ± 0.3	5.0 ± 1.5	0.15
Systolic BP (mmHg)	110 ± 3	111 ± 1	109 ± 4	0.64
Diastolic BP (mmHg)	68 ± 2	65 ± 1	69 ± 3	0.30
Heart rate (bpm)	61 ± 2	56 ± 4	65 ± 2	0.09

*Values are mean ± SEM.
[†]Age at time of cold exposure.
[‡]Calculated as $0.007184 \times \text{height (cm)}^{0.725} \times \text{weight (kg)}^{0.425}$.
[§]Ratio of the volume of subcutaneous adipose tissue (SAT) to visceral adipose tissue (VAT) at the level of the umbilicus.

Differing Effects of Ephedrine and Cold Exposure on Blood Metabolites. In contrast to the general similarities between the effects of ephedrine and cold exposure on BP and energy expenditure, these two stimuli produced very different effects on the levels of metabolites in the blood (Table 2). With ephedrine, there was extensive stimulation of the SNS, with increased hepatic glucose production and ketogenesis, as demonstrated by the elevations in plasma norepinephrine ($P = 0.010$), glucose ($P < 0.001$), insulin ($P < 0.001$), C-peptide ($P < 0.001$), β-hydroxybutyrate ($P = 0.045$), and nonesterified fatty acids (NEFA) ($P = 0.16$). Anaerobic glycolysis increased as well, as evidenced by elevated plasma lactic acid concentrations ($P < 0.001$). Serum levels of thyroid hormones also went up acutely, with increases in free T4 and total T3 ($P = 0.014$ and $P = 0.026$, respectively). The suppression of appetite seen with adrenergic agonists was reflected by the decrease in ghrelin ($P = 0.007$). There were no significant changes in leptin, glucagon, GLP-1, growth hormone, IGF-1, IL-6, and TNF-α.

Cold exposure led to a much smaller set of metabolic changes. Plasma norepinephrine was higher than after either saline or ephedrine administration ($P = 0.004$ and $P = 0.034$, respectively), and lactic acid was higher than saline control ($P < 0.015$). However, nearly all other metabolites remained unchanged, including plasma glucose, NEFA, insulin, thyroid hormones, and epinephrine.

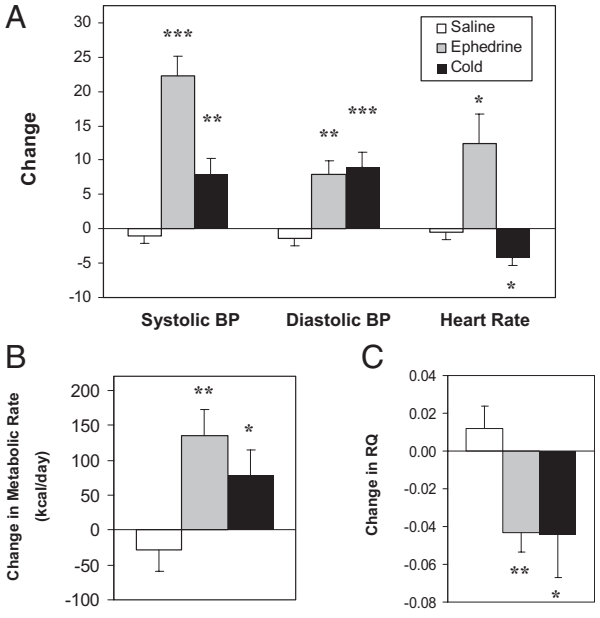

Fig. 1. The effects of cold exposure and ephedrine on autonomic nervous system activity, energy expenditure, and metabolism. Healthy volunteers were treated for 1 h with cold exposure using a cooling vest or given the sympathomimetic ephedrine (1 mg/kg, i.m.) or an equal volume of saline intramuscularly. Values were measured before and after each intervention, as described in *Materials and Methods*. (*A*) SNS activation was monitored through changes in systolic BP (mmHg), diastolic BP (mmHg), and heart rate (beats per minute). (*B*) Metabolic rate. (*C*) Respiratory quotient. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Error bars are SEM.

Cold Exposure, but Not Ephedrine, Increases BAT Activity. The starkest contrast between the effects of ephedrine and cold exposure was seen in their ability to stimulate BAT (Fig. 2). Cold exposure substantially increased the volume and activity of detectable BAT in all 10 volunteers. BAT was found in the cervical and supraclavicular depots, with an estimated median detectable volume after cold exposure of 15 mL. In those with the most detectable BAT, activity was seen in the paraspinal and perirenal depots as well. The increase in ^{18}F-fluorodeoxyglucose (FDG) uptake in response to cold exposure was specific to BAT, because cold exposure caused a relative decrease in maximal standard uptake value (SUVmax) compared with ephedrine in two different subcutaneous WAT depots, the upper humerus ($P = 0.038$) and lower posterior thorax ($P = 0.047$) (Fig. 3*A*). In contrast, ephedrine had no measurable effect on detection of BAT (Fig. 2), as all subjects remained at or below the level of baseline detectable activity (Fig. 3 *B* and *C*).

Discussion

Since the discovery in 2009 that a significant percentage of human adults have functional BAT, there has been considerable interest in using the energetic capacity of BAT to treat obesity and diabetes (14). Two principal limitations, however, are (*i*) reliably measuring BAT volume and activity, and (*ii*) determining the best way to stimulate BAT with the least adverse effects. Although environmental, pharmacological, or even nutritional means are all possibilities, choosing the effective approach will require a more detailed understanding of the physiological processes underlying human BAT activation, as well as the integration of different organ systems that can maximize energy expenditure.

It has been demonstrated in rodent models that BAT is the principal, if not the only, source for nonshivering thermogenesis (15, 16). Multiple studies in humans (2–4, 17), including the present one, demonstrate a role for BAT in that process. The energetic capacity of human BAT when measured in dynamic studies of glucose uptake has been estimated at 14 kcal/g (3) and 5 kcal/g (17), assuming that 10% of the total BAT metabolism is derived from glucose uptake (18). Our finding that cold exposure increased energy expenditure in our volunteers by an average of 79 kcal/d per 15 mL of detectable BAT is of a comparable amount, supporting the position that targeted activation of human BAT could have a physiologically significant effect on whole-body energy balance.

In addition to generating heat via nonshivering thermogenesis, BAT also burns calories in rodents through a process known as diet-induced thermogenesis (19, 20), indicating that despite its relatively small size, BAT could play a prominent role in general metabolic homeostasis. Supporting this possibility are three recent studies in rodents and humans. In cold-acclimatized mice the BAT consumed more than half of ingested lipids and glucose, more than any other individual organ (21). In humans, cold-activated human BAT took up more glucose per gram of tissue than even insulin-

Table 2. Metabolite concentrations after different interventions

Metabolite (units)	Treatment*			*P* value		
	Saline	Ephedrine	Cold	Saline vs. ephedrine	Saline vs. cold	Cold vs. ephedrine
Glucose (mg/dL)	83 ± 2	95 ± 2	83 ± 2	**<0.001**†	0.88	**<0.001**
Lactic acid (mg/dL)	6.9 ± 0.4	11.5 ± 1.1	8.5 ± 0.6	**<0.001**	**0.015**	**0.009**
β-Hydroxybutyrate (mM)	0.16 ± 0.04	0.31 ± 0.09	0.11 ± 0.05	0.045	0.49	0.042
NEFA (mEq/L)	0.59 ± 0.07	0.76 ± 0.12	0.57 ± 0.08	0.16	0.81	0.14
Insulin (μLU/mL)	2.95 ± 0.53	6.79 ± 1.06	3.41 ± 0.61	**<0.001**	0.28	**0.003**
C-peptide (ng/dL)	1.37 ± 0.15	1.98 ± 0.13	1.67 ± 0.14	**<0.001**	**0.005**	**0.003**
Norepinephrine (pg/mL)	120 ± 22	205 ± 38	365 ± 55	**0.010**	**0.004**	0.034
Epinephrine (pg/mL)	12.6 ± 3.4	13.5 ± 3.9	14.9 ± 3.5	0.31	0.48	0.98
Free T4 (ng/dL)	1.13 ± 0.04	1.19 ± 0.05	1.12 ± 0.04	**0.014**	0.62	0.10
Total T3 (ng/dL)	105.5 ± 5.2	114.3 ± 6.0	110.5 ± 5.4	0.026	0.09	0.27
Ghrelin (ng/mL)	117 ± 18	78 ± 17	90 ± 11	**0.007**	0.36	**0.012**

*Values are mean ± SEM.
†Values in bold indicate *P* values < 0.017, the threshold for a Bonferroni correction with three comparisons.

stimulated skeletal muscle (22), and cold exposure increased BAT-mediated uptake of NEFA (17). Our data are consistent with these findings, as most metabolites, including glucose, did not increase when BAT was stimulated by cold via the SNS. Thus, it is possible that human BAT could be used not just for weight control, but for treatment of metabolic dysregulation as well.

The different effects of ephedrine and cold exposure on heart rate, metabolites, and especially BAT underscore how specific the SNS response can be to particular stimuli. In addition to glucose, cold exposure had no significant effect on plasma insulin, thyroid hormones, or even epinephrine, demonstrating that there was not a generalized activation of the classic response to stressors (23). The cold-associated increase in C-peptide without changes in glucose reflects a state of relative insulin resistance that may have been caused by elevated norepinephrine, as previously described (24). The elevated lactic acid levels were likely a result of glycolysis and amino acid metabolism in the BAT itself (18, 25) and possibly also from subclinical, low-grade muscle contraction. Our data support a model in which mild cold exposure activates a specific, sympathetic response to only certain organs, principally BAT and the peripheral vasculature, but not other organs, such as the liver, heart, or adrenal glands. The modest increase in plasma norepinephrine observed after cold exposure is likely a result of leakage from nerve terminals, as much higher concentrations are required to achieve comparable increases in BP when norepinephrine is infused intravenously (26).

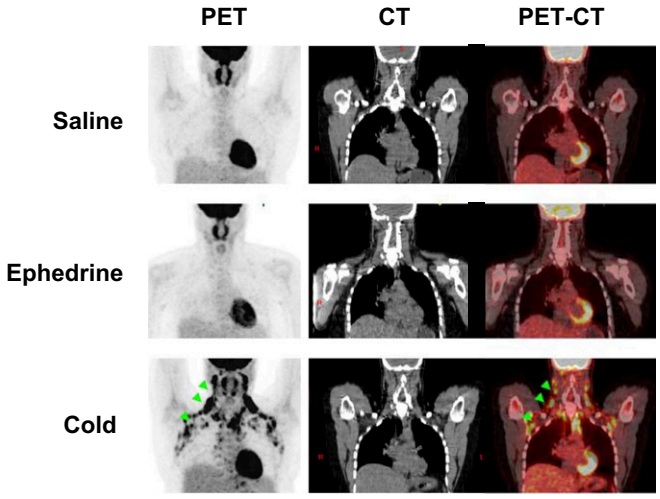

Fig. 2. Detection of BAT after stimulation with cold exposure, ephedrine, and saline. From a representative volunteer, brown fat FDG uptake is illustrated using coronal representations of the PET (*Left*), CT (*Center*), and combined PET–CT (*Right*) that includes the principal cervical, supraclavicular, and thoracic depots of BAT (green arrowheads) after stimulation with saline (*Top*), ephedrine 1 mg/kg (*Middle*), or cold exposure (*Bottom*), as described in *Materials and Methods*.

Ephedrine increases energy expenditure (27) and suppresses appetite (28) through broad activation of the SNS, including direct activation of β-adrenergic receptors, as well as indirectly by causing a release of norepinephrine from sympathetic nerve terminals (29, 30). Because BAT has β-adrenergic receptors and is activated by the SNS, it was reasonable to assume that a moderately high dose of ephedrine could activate BAT in humans. Prior studies using oral doses of ephedrine at 1 mg/kg revealed similar effects on the cardiovascular system, metabolic parameters, and oxygen consumption (27, 31). We now demonstrate that the increase in energy expenditure by ephedrine is not through stimulation of BAT thermogenesis. Rather, other organs, such as skeletal muscle, may be involved (27). The nonspecific β-adrenergic receptor blocker propranolol has been used effectively to reduce BAT activity in patients undergoing ^{18}F-FDG PET/CT scanning at room temperature (32, 33). These observations reinforce the role of the SNS in activating BAT. Nevertheless, our findings with ephedrine indicate that attempting to stimulate BAT through generalized sympathomimetic drugs is not the optimal approach. As in rodents, much higher doses of ephedrine in humans may affect BAT, but they would likely have unacceptable cardiovascular effects to be considered therapeutically. Our findings also raise the question that the potential inefficiency of β3-adreergic receptor agonists to induce weight loss and thermogenesis in previous studies may reflect their failure to stimulate BAT at the doses used. The finding that cold can stimulate BAT while having comparatively few adverse effects on generalized metabolism indicates the potential for using BAT as a therapeutic target.

There are several methodological considerations in this study. First, by using ^{18}F-FDG PET-CT to identify BAT, we were detecting only the tissue that was actively taking up glucose. Any active BAT using a different fuel, or inactive BAT, would not be measured here. Moreover, our definition of BAT was deliberately very specific, using a high level of activity (SUVmax ≥ 2.0) for the threshold and measuring only the principal depot of BAT in humans, found in the cervical-supraclavicular-anterior thoracic regions. The actual volume of BAT in our volunteers is undoubtedly higher than what we calculated, even as we saw detectable BAT in every volunteer. We also did not identify a sexual dimorphism in BAT volume or activity, which is different from what we and others have reported in the larger population studies (1, 5) where BAT is detected more often in women than men. One

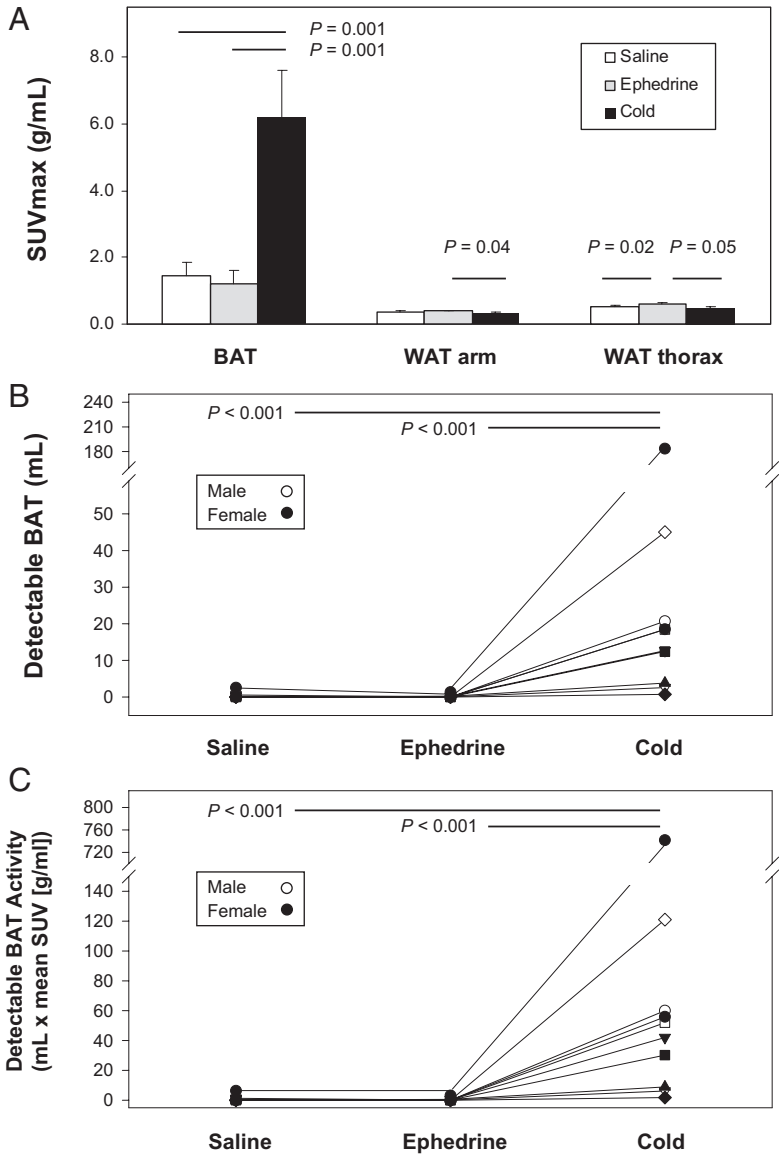

Fig. 3. Comparison of maximal glucose uptake in brown and white adipose tissue and volume and activity of BAT after stimulation with cold exposure, ephedrine, or saline. Volunteers were exposed to cold, given ephedrine 1 mg/kg i.m., or an equal volume of saline intramuscularly, as described in *Materials and Methods*. Open circles are males, and filled circles are females. (*A*) The maximal SUV (SUVmax) of glucose in BAT and the subcutaneous white adipose tissue in the upper arm and posterior thorax. (*B*) Change in the volume of detectable BAT. (*C*) Change in detectable BAT activity.

distinction is that in the retrospective studies, people were categorized only as BAT^+ or BAT^-, but quantification of activity was not done because the patients were not studied under controlled conditions. Given the wide range of BAT activities seen in this and others' prospective studies (2, 17), it is likely that much larger sample sizes will be required to see the effects of sex and other anthropometric parameters on BAT activity. It is also important to note that in more than half (6 of 10) of the volunteers we saw very low levels of BAT activity, even during baseline conditions where they were kept at ambient temperatures ≥23 °C for over 12 h. This finding indicates that human BAT may be metabolically active even in a person who is not exposed to cold, suggesting that simply increasing BAT mass will lead to higher levels of basal energy expenditure.

In summary, we show that we can consistently stimulate BAT in healthy volunteers using a simple, accessible method of cold exposure. In contrast to ephedrine, which even at high doses is unable to appreciably affect BAT, mild cold exposure stimulates a specific response by the SNS to activate BAT and increase energy expenditure with few other metabolic effects. The obesity and diabetes pandemics demand safe and novel treatments, and our findings demonstrate that increasing energy expenditure through BAT is a promising approach.

Materials and Methods

Study Population. This study followed institutional guidelines and was approved by the Human Studies Institutional Review Boards of Beth Israel Deaconess Medical Center (BIDMC) and the Joslin Diabetes Center. Healthy volunteers were recruited through electronic advertisements, and written informed consent was obtained.

Clinical Measurements. BP and heart rate were measured using a SureSigns VS3 vital signs monitor (Philips Healthcare). Metabolic rate and RQ were assessed using a Sensormedics V_{max} Encore 29 (VIASYS Respiratory Care). Body composition was measured via dual-energy X-ray absorptiometry whole-body scanner [Discovery A (SIN 45025); Hologic]. For insulin, C-peptide, norepinephrine, epinephrine, ghrelin, leptin, glucagon, growth hormone, IGF-1, IL-6, and TNF-α, plasma, and serum levels were measured according to procedures defined at the Harvard Catalyst Central Laboratory (http://catalyst.harvard.edu/services/hccl/). NEFA, lactic acid, free T4, total T3, and glucagon were measured using standard procedures at the Laboratory Corporation of America. GLP-1 and β-hydroxybutyrate were measured using the GLP-1 (Active) ELISA kit (Millipore) and β-hydroxybutyrate LiquiColor kit (Stanbio Laboratories), respectively.

Study Day and Imaging Protocol. We recruited 10 healthy volunteers (Table 1) who were studied from February 2010 through March 2011. Each participated in three separate, independent study visits conducted in random order based on a Latin Square design. Given reports showing that BAT activity is affected by different outdoor temperatures (1, 4), we focused on completing the three visits within as short a time span as possible and did not attempt to study the female volunteers at the same phase of their menstrual cycle. The volunteers also were not specifically instructed to have a weight-maintenance diet. However, over the course of their participation, the weights remained stable, with an average coefficient of variation of 1.6%. Volunteers were asked to refrain from caffeine and alcohol intake for 48 h before each study day. The night before the study day, they were admitted to the BIDMC clinical research center and began fasting from 12:00 AM onward. Room temperature was maintained above 23 °C throughout the stay in the clinical research center. Upon waking the next morning, the volunteers put on a standard hospital scrub suit. Vitals signs were recorded every 15 min until the end of the study day. BMR was measured, and depending on the study day, one of three stimuli was given: a single intramuscular dose of ephedrine 1 mg/kg; an equal volume of saline; or the volunteer was transported to a room set to 20 °C and donned a surgeon's cooling vest (Polar Products) with the water temperature set to 14 °C that was monitored by a digital thermometer (Fisher Scientific). This mild cold exposure did not induce shivering, as assessed by questioning of the volunteers. Sixty minutes after the injection of ephedrine, saline, or the initiation of cold exposure, blood was drawn for measurement of metabolite levels, and then an intravenous bolus of 440 MBq (12 mCi) of ^{18}F-FDG was administered. Metabolic rate was measured a second time. Sixty minutes after ^{18}F-FDG injection, images were acquired using a Discovery LS multidetector helical PET-CT scanner (GE Medical Systems). Thus, the volunteers were exposed to a total of 120 min of cold. One volunteer drank cold water after metabolites had been drawn, which led to shivering that lasted for 15 min and then stopped. Her BMR and RQ were not included in those analyses.

PET-CT data were acquired from the level of the eyes to umbilicus. The scan protocol consisted of helical CT, 140 kVp, 120 mAs, and pitch of 1.5 followed by PET acquisition in 2D mode of 8-min for each of four bed-stops (three slice overlap). PET data were reconstructed by an ordered subset-expectation maximization algorithm; both PET and CT images were reconstructed as axial sections at 4.25-mm spacing and then reformatted by the viewer software for analysis in axial sections.

BAT mass and activity were quantified using the PET-CT Viewer shareware (34). BAT in each axial slice was classified on a pixel-by-pixel basis when CT was in the range −250 to −10 Hounsfield Units and when SUVmax ≥ 2.0 as described previously (35) in the cervical, supraclavicular, and anterior thoracic depots from vertebral level C3 to T7. Areas of ^{18}F-FDG uptake on PET colocalizing with regions of fat identified on CT were quantified by their SUVavg: average activity per unit volume within the region of interest divided by the injected dose per body mass in kilograms. Detectable BAT volume was calculated as the sum of pixels meeting this classification criteria multiplied by the pixel volume (64.8 mm^3).

Statistical Analysis. Data were analyzed using JMP Pro-9.0.0 software (SAS Institute). All *P* values presented are two-tailed, and values less than 0.05 were considered to indicate statistical significance. Repeated-measures ANOVA was used to assess the vital signs. For energy expenditure and plasma metabolites, we used individual paired *t* tests and applied Bonferroni's correction for multiple comparisons. BAT SUVmax, volume, and activity were not normally distributed, so the nonparametric Wilcoxon signed-rank test was used.

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1. Cypess AM, et al. (2009) Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 360:1509–1517.
2. van Marken Lichtenbelt WD, et al. (2009) Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 360:1500–1508.
3. Virtanen KA, et al. (2009) Functional brown adipose tissue in healthy adults. *N Engl J Med* 360:1518–1525.
4. Saito M, et al. (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. *Diabetes* 58:1526–1531.
5. Pfannenberg C, et al. (2010) Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. *Diabetes* 59:1789–1793.
6. Zingaretti MC, et al. (2009) The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J* 23:3113–3120.
7. Morrison SF (2011) 2010 Carl Ludwig Distinguished Lectureship of the APS Neural Control and Autonomic Regulation Section: Central neural pathways for thermoregulatory cold defense. *J Appl Physiol* 110:1137–1149.
8. Weyer C, Tataranni PA, Snitker S, Danforth E, Jr., Ravussin E (1998) Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans. *Diabetes* 47:1555–1561.
9. Larsen TM, et al. (2002) Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr* 76:780–788.
10. Arch JR (2008) The discovery of drugs for obesity, the metabolic effects of leptin and variable receptor pharmacology: perspectives from beta3-adrenoceptor agonists. *Naunyn Schmiedebergs Arch Pharmacol* 378:225–240.
11. Redman LM, et al. (2007) Lack of an effect of a novel beta3-adrenoceptor agonist, TAK-677, on energy metabolism in obese individuals: A double-blind, placebo-controlled randomized study. *J Clin Endocrinol Metab* 92:527–531.
12. Shekelle PG, et al. (2003) Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis. *JAMA* 289:1537–1545.
13. Baba S, et al. (2007) Effect of nicotine and ephedrine on the accumulation of 18F-FDG in brown adipose tissue. *J Nucl Med* 48:981–986.
14. Tseng YH, Cypess AM, Kahn CR (2010) Cellular bioenergetics as a target for obesity therapy. *Nat Rev Drug Discov* 9:465–482.
15. Dulloo AG, Young JB, Landsberg L (1988) Sympathetic nervous system responses to cold exposure and diet in rat skeletal muscle. *Am J Physiol* 255:E180–E188.
16. Golozoubova V, et al. (2001) Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J* 15:2048–2050.
17. Ouellet V, et al. (2012) Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* 122:545–552.
18. Ma SW, Foster DO (1986) Uptake of glucose and release of fatty acids and glycerol by rat brown adipose tissue in vivo. *Can J Physiol Pharmacol* 64:609–614.
19. Lowell BB, Spiegelman BM (2000) Towards a molecular understanding of adaptive thermogenesis. *Nature* 404:652–660.
20. Cannon B, Nedergaard J (2011) Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol* 214:242–253.
21. Bartelt A, et al. (2011) Brown adipose tissue activity controls triglyceride clearance. *Nat Med* 17:200–205.
22. Orava J, et al. (2011) Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab* 14:272–279.
23. Wortsman J (2002) Role of epinephrine in acute stress. *Endocrinol Metab Clin North Am* 31:79–106.
24. Celi FS, et al. (2010) Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. *Eur J Endocrinol* 163:863–872.
25. López-Soriano FJ, et al. (1988) Amino acid and glucose uptake by rat brown adipose tissue. Effect of cold-exposure and acclimation. *Biochem J* 252:843–849.
26. Silverberg AB, Shah SD, Haymond MW, Cryer PE (1978) Norepinephrine: Hormone and neurotransmitter in man. *Am J Physiol* 234:E252–E256.
27. Astrup A, Bülow J, Madsen J, Christensen NJ (1985) Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am J Physiol* 248:E507–E515.
28. Malchow-Møller A, et al. (1981) Ephedrine as an anorectic: The story of the 'Elsinore pill'. *Int J Obes* 5:183–187.
29. Vansal SS, Feller DR (1999) Direct effects of ephedrine isomers on human beta-adrenergic receptor subtypes. *Biochem Pharmacol* 58:807–810.
30. Rothman RB, et al. (2003) In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *J Pharmacol Exp Ther* 307:138–145.
31. Astrup A, Lundsgaard C, Madsen J, Christensen NJ (1985) Enhanced thermogenic responsiveness during chronic ephedrine treatment in man. *Am J Clin Nutr* 42:83–94.
32. Parysow O, et al. (2007) Low-dose oral propranolol could reduce brown adipose tissue F-18 FDG uptake in patients undergoing PET scans. *Clin Nucl Med* 32:351–357.
33. Söderlund V, Larsson SA, Jacobsson H (2007) Reduction of FDG uptake in brown adipose tissue in clinical patients by a single dose of propranolol. *Eur J Nucl Med Mol Imaging* 34:1018–1022.
34. Barbaras L, Tal I, Palmer MR, Parker JA, Kolodny GM (2007) Shareware program for nuclear medicine and PET/CT PACS display and processing. *AJR Am J Roentgenol*, 188:W565-8. Available at http://www.bidmc.org/Research/Departments/Radiology/NMDownload.aspx.
35. Torriani M, et al. (2012) Deiodinase 2 expression is increased in dorsocervical fat of patients with HIV-associated lipohypertrophy syndrome. *J Clin Endocrinol Metab* 97: E602–E607.