Brown adipose tissue is involved in the seasonal variation of cold-induced thermogenesis in humans

Takeshi Yoneshiro,1 Mami Matsushita,2 Satoshi Nakae,3 Toshimitsu Kameya,4 Hiroki Sugie,4
Shigeho Tanaka,3 and Masayuki Saito2

1Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan;
2Department of Nutrition, School of Nursing and Nutrition, Tenshi College, Sapporo, Japan; 3Department of Nutritional Science, National Institute of Health and Nutrition, Tokyo, Japan; and 4LSI Sapporo Clinic, Sapporo, Japan

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Yoneshiro T, Matsushita M, Nakae S, Kameya T, Sugie H, Tanaka S, Saito M. Brown adipose tissue is involved in the seasonal variation of cold-induced thermogenesis in humans. Am J Physiol Regul Integr Comp Physiol 310: R999–R1009, 2016. First published March 30, 2016; doi:10.1152/ajpregu.00057.2015.—Brown adipose tissue (BAT) contributes to whole body energy expenditure (EE), especially cold-induced thermogenesis (CIT), in humans. Although it is known that EE and CIT vary seasonally, their relationship with BAT has not been investigated. In the present study, we examined the impact of BAT on seasonal variations of EE/CIT and thermal responses to cold exposure in a randomized crossover design. Forty-five healthy male volunteers participated, and their BAT was assessed by positron emission tomography and computed tomography. CIT, the difference of EE at 27°C and after 2-h cold exposure at 19°C, significantly increased in winter compared with summer, being greater in subjects with metabolically active BAT (High BAT, 185.6 kcal/day vs. 18.3 kcal/day, P < 0.001) than those without (Low BAT, 90.6 kcal/day vs. −46.5 kcal/day, P < 0.05). Multivariate regression analysis revealed a significant interaction effect between season and BAT on CIT (P < 0.001). The cold-induced drop of tympanic temperature (Tty) and skin temperature (Tskin) in the forehead region and in the supravacular region close to BAT deposits were smaller in the High BAT Group than in the Low BAT Group in winter but not in summer. In contrast, the drop of Tskin in peripheral regions distant from BAT was similar in the two groups in both seasons. In conclusion, CIT increased from summer to winter in a BAT-dependent manner, paralleling cold-induced changes in Tty/Tskin, indicating a role of BAT in seasonal changes in the thermogenic and thermal responses to cold exposure in humans.

energy expenditure; thermogenesis; brown adipose tissue; seasonal variation; body temperature

IN SMALL RODENTS, BROWN ADIPOSE TISSUE (BAT) has been recognized as the major site of sympathetically activated nonshivering thermogenesis, a physiological process during which heat production increases in response to environmental cues (7, 17). One of the typical examples is cold-induced thermogenesis (CIT), an increase in whole body energy expenditure (EE) seen when mammals are exposed to lower temperatures. When mice are exposed to cold, temperature-sensitive transient receptor potential (TRP) channels are activated at the peripheral tissues, such as the skin, and then sympathetic nerve activity in BAT is increased to activate a thermogenic molecule uncoupling protein 1 (UCP1) (28), which short-circuits the electrochemical gradient that drives ATP synthesis, thereby dissipating energy as heat. Heat generated in BAT is distributed to the rest of the body through the circulation to prevent body temperature drop (28). The indispensable role of BAT for thermoregulation has been clearly confirmed by findings that mice lacking UCP1 are intolerant to cold exposure (13) and have a much shorter life span in the cold (14).

Because BAT was once thought to be present in human infants but absent in human adults, its physiological role had been assumed to be significant only in infants whose shivering thermogenesis ability is still immature. However, the recent rediscovery of considerable amounts of BAT in adults rekindled research into its physiological significance (11, 39, 46, 48). Mild cold exposure (31, 39, 46, 51) or β3-adrenergic stimulation (12) gives rise to a parallel increase in the metabolic activity of BAT and whole body EE without significant shivering in adult humans. Moreover, the recruitment of BAT by chronic cold exposure increases the capacity of CIT without changes in fat-free mass (FFM), muscle shivering, and muscle mitochondrial uncoupling capacity (44, 52). Thus, it is obvious that BAT plays a significant role in CIT in adult humans, as in small rodents.

It has repeatedly been reported that daily EE in small rodents fluctuates according to ambient temperature or season in association with increased BAT thermogenic capacity (37, 49). Although the topic of seasonality in total EE is still controversial in humans, Plasqui and Westerterp (35) reported no seasonal change in total EE with decreased physical activity (PA) levels in winter, indicating an increase in PA-independent EE in response to lower ambient temperature. In fact, in humans, CIT increases in winter compared with summer (24, 47). As the metabolic activity of human BAT is maximal in winter (1, 33, 39), we hypothesized that any seasonal variation of CIT could be attributable to the difference in BAT activity. To test this idea, in the present study, we measured whole body EE and CIT of healthy men in both summer and winter, and then analyzed the relationship between their seasonal changes and BAT activity assessed by fluorodeoxyglucose (FDG)-positron emission tomography (PET) combined with computed tomography (CT). In addition to CIT, we also examined the response of body temperature to cold exposure, focusing on the possible involvement of BAT in the regulation of body temperature.

MATERIALS AND METHODS

Participants. Forty-five healthy male volunteers aged 20–31 years, who had been living in Sapporo, Japan, for ≥3 years, participated in this trial. Twenty subjects who participated in our previous study (26) were included in the present study, while the other 25 subjects were
newly recruited. They were carefully instructed regarding the study and gave their informed consent to participate. The protocol was approved by the institutional review boards of Tenshi College. Body weight and body fat mass were estimated by employing the mult frecuency bioelectric impedance method (HBF-361; Omron Health Care, Kyoto, Japan). Body mass index (BMI) was calculated as body weight in kilograms divided by the square height in meters. The FFM was calculated as the difference between the body weight and fat mass. Outdoor temperature, day length, duration of bright sunshine, and relative humidity in Sapporo were obtained from the Japan Meteorological Agency (http://www.jma.go.jp/jma/menu/ menureport.html) and National Astronomical Observatory of Japan (http://eco.mtk.nao.ac.jp/koyomi/).

FDG-PET/CT. All subjects underwent a standardized health examination and FDG-PET/CT after a 2-h cold exposure at 19°C in winter (December to March of 2011 to 2013) to evaluate the BAT activity of each subject (26, 39, 51–54). After overnight fasting for ~12 h, they visited the laboratory and were exposed to cold by being kept in an air-conditioned room at 19°C with standardized light clothing (a patient’s gown) and intermittently placed their feet on an ice block wrapped in cloth for ~4 min every 5 min to avoid cooling-associated pain. After 1 h under these cold conditions, they were given an intravenous injection of 18F-FDG (1.66–5.18 MBq/kg body wt) and kept under the same cold conditions. One hour after the 18F-FDG injection, FDG-PET/CT scans were performed by using a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Japan). BAT activity was quantified by calculating a standardized uptake value (SUV) of FDG in the supravacular fat deposits. According to the detection of the metabolically active BAT with a SUV of ≥2.0, subjects were divided into two groups: High BAT and Low BAT Groups (11, 27, 39, 51–54).

Measurement of energy expenditure and muscle shivering. Indirect calorimetry was performed in summer (July to September) and winter (December to March) of 2011 to 2013 in a randomized crossover design. Whole body EE was measured by using a respiratory gas analyzer connected to a ventilated hood (AR-1, Arco System, Kashiwa, Japan) (51, 52). After overnight fasting for ~12 h, subjects entered in an air-conditioned room and relaxed while wearing the standardized light clothing for more than 30 min under a thermoneutral condition at 27°C. Then oxygen consumption and carbon dioxide production were continuously recorded for 20–30 min at 27°C and 19°C experiment, the room temperature was automatically regulated at 27°C and 19°C, subjects reported perception to cold (feeling of cold) using a visual analog scale (VAS) (22, 44), and their cold sensation was scored as continuous values from 0 (never cold) to 10 (very cold).

**RESULTS**

Effects of season on thermogenesis and body temperature. The monthly mean of outdoor temperature and day length in 2011–2013 in Sapporo varied seasonally, being lowest in January and December, respectively (Fig. 1). Values for sun-

![Fig. 1. Monthly means of outdoor temperature, day length, sunshine duration, and relative humidity in Sapporo, Japan.](http://ajpregu.physiology.org/)

**Data analysis.** Data are expressed as means ± SE and are analyzed by either paired or unpaired t-tests, using a statistical software package (IBM SPSS Statistics 18.0, IBM Japan, Tokyo, Japan). To compare EE/CIT between the seasons or between the subject groups, whole body EE was adjusted for FFM, as previously reported (36). The adjusted EE was calculated as the mean EE plus measured EE minus the predicted EE, where the mean EE is the mean resting EE at 27°C in summer and winter, the measured EE is the EE measured in each subject, and the predicted EE is the calculated EE obtained by using the individual FFM in the linear regression equation. Correlations were assessed by using the Pearson’s or Kendall’s correlation coefficient, as appropriate. Independent associations of age, fat mass, FFM, outdoor temperature, day length, and BAT with EE/CIT were estimated by using stepwise multiple regressions. To assess the significance of interactions of BAT with season, subjects were coded as 0 (the Low BAT group) and 1 (the High BAT group), and the seasons were coded as 1 (summer) and 2 (winter). A cross-product term of the categorized variables was added to the multivariate regression model. A P value of less than 0.05 was considered to be statistically significant.
shine duration and humidity marginally fluctuated according to the season. Outdoor temperature and day length for the day of the experiments was lower (-4.2 ± 0.5 vs. 22.9 ± 0.3°C, \( P < 0.001 \)) and shorter (9.3 ± 0.1 vs. 14.2 ± 0.1 h/day, \( P < 0.001 \)), respectively, in winter than in summer. Body weight and BMI were almost the same in summer and winter (Table 1), while body fat content and FFM were higher \( (P < 0.001) \) and lower \( (P < 0.01) \), respectively, in winter.

In parallel with the decreased FFM, whole body EE at a thermoneutral condition (27°C) tended to be lower in winter than in summer \( (P = 0.09, \) Table 1\). In contrast, whole body EE after 2-h cold exposure at 19°C was higher in winter than in summer \( (P < 0.001) \). Significant positive correlations of whole body EE at 27°C and at 19°C to FFM were found in both seasons \( (P < 0.001, \) Fig. 2, A and B). RER decreased after cold exposure in both seasons \( (P < 0.001, \) Table 1). Compared with summer, the mean RER was lower in winter both at 27°C and at 19°C \( (P < 0.05) \). As there was a small but significant seasonal change in FFM, EE was adjusted for FFM by means of linear regression. The adjusted EE at 27°C was almost the same in summer and winter (Fig. 2C); it significantly increased at 19°C in winter \( (P < 0.001) \) but only slightly in summer. Fat oxidation increased at 19°C in both seasons \( (P < 0.001, \) Fig. 2D). Cold-induced increments in EE (CIT) and fat oxidation were markedly higher in winter than in summer \( (P < 0.001, \) Fig. 2, G and H). While CHO oxidation decreased after 2 h of cold exposure in both seasons \( (P < 0.001, \) Fig. 2E), the decrements were similar in the two seasons (Fig. 2F). No measurable change was observed in the EMG power at the pectoralis during cold exposure in both seasons, thus showing no seasonal variation (Fig. 2, F and J) and validating our model in capturing nonshivering thermogenesis.

\( T_{\text{skin}} \) in the supraclavicular, subclavicular, calf, hand, foot, and forehead regions (Fig. 3O) were decreased after cold exposure in both seasons \( (P < 0.001, \) Fig. 3, A–F). However, the cold-induced drops of \( T_{\text{skin}} \) in these regions were notably larger in summer than those in winter \( (P < 0.01, \) Fig. 3, G–L), indicating a diminished heat loss from the skin in summer. \( T_{\text{ty}} \) was also decreased during cold exposure in both seasons \( (P < 0.001, \) Fig. 3M); the \( T_{\text{ty}} \) drop was larger in summer than in winter \( (P < 0.001, \) Fig. 3N), suggesting a decreased ability to maintain \( T_{\text{ty}} \) in summer.

**Effects of BAT on seasonal changes in the responses to cold exposure.** All 45 subjects underwent FDG-PET/CT after 2-h cold exposure at 19°C. Thirty-three (73%) showed high activities of FDG uptake into BAT in the suprACLAVICULAR/PARASPINAL regions (HIGH BAT GROUP), while others showed undetectably low activities (LOW BAT GROUP). The quantitative BAT activity (SUV\(_{\text{max}}\)) of the HIGH and LOW BAT GROUPS was 6.21 ± 3.7 (minimum: 2.02 and maximum: 16.37) and 1.30 ± 0.09 (minimum: 0.87 and maximum: 1.73), respectively. The HIGH BAT GROUP tended to be younger and leaner than the LOW BAT GROUP (Table 1). FFM was similar for the two groups. There was no significant difference in seasonal changes in body composition between the two groups.

At thermoneutral 27°C, EE did not differ between the HIGH and LOW BAT GROUPS, either in summer or winter (Table 1, Fig. 4, A and B). Cold exposure at 19°C increased EE of both groups in winter \( (P < 0.001) \), but not in summer. Compared with the LOW BAT GROUP, EE at 19°C of the HIGH BAT GROUP was notably higher in winter \( (P = 0.01) \). Moreover, CIT remarkably increased in winter compared with summer \( (P < 0.05, \) Fig. 4C), being greater in the HIGH BAT GROUP than in the LOW BAT GROUP \( (P < 0.001) \). A significant positive correlation between BAT activity (SUV) and CIT were also found \( (r = 0.49, P < 0.001) \). Multivariate regression analysis revealed a significant interaction effect between BAT activity (SUV) and CIT on EE at 19°C \( (P = 0.01) \) and on CIT \( (P < 0.001) \), but not on EE at 27°C, independently of age and body composition. Cold exposure reduced RER and elevated fat oxidation in both groups \( (P < 0.01, \) Table 1 and Fig. 4, D and E). Furthermore,

### Table 1. Subject profiles and whole body EE in summer and in winter

<table>
<thead>
<tr>
<th></th>
<th>All ((n = 45))</th>
<th>High BAT Group ((n = 35))</th>
<th>Low BAT Group ((n = 12))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.4 ± 0.4</td>
<td>22.9 ± 0.5</td>
<td>24.6 ± 0.8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173.9 ± 0.8</td>
<td>173.9 ± 0.9</td>
<td>173.9 ± 0.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.7 ± 1.2</td>
<td>66.2 ± 1.3</td>
<td>65.2 ± 1.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.7 ± 0.4</td>
<td>21.9 ± 0.4</td>
<td>21.6 ± 0.5</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>14.9 ± 0.8</td>
<td>17.0 ± 0.8***</td>
<td>14.5 ± 0.9</td>
</tr>
<tr>
<td>Body fat content, %</td>
<td>10.1 ± 0.7</td>
<td>11.6 ± 0.7***</td>
<td>9.8 ± 0.9</td>
</tr>
<tr>
<td>Seasonal (\Delta) kg/m²</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>Seasonal (\Delta) %</td>
<td>0.5 ± 0.4</td>
<td>0.4 ± 0.5</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Seasonal (\Delta) kg</td>
<td>0.9 ± 0.3</td>
<td>-0.9 ± 0.3</td>
<td>-1.2 ± 0.4</td>
</tr>
<tr>
<td>Whole body EE at 27°C</td>
<td>1494 ± 20</td>
<td>1471 ± 24(*)(\dagger)</td>
<td>1493 ± 24</td>
</tr>
<tr>
<td>kcal/day</td>
<td>-23 ± 15</td>
<td>-29 ± 15</td>
<td>-7 ± 31</td>
</tr>
<tr>
<td>Whole body EE at 19°C</td>
<td>1499 ± 26</td>
<td>1631 ± 25(<em><strong>)</strong></em></td>
<td>1511 ± 31</td>
</tr>
<tr>
<td>kcal/day</td>
<td>132 ± 24†††††</td>
<td>139 ± 23†††††</td>
<td>1466 ± 47</td>
</tr>
<tr>
<td>RER at 27°C</td>
<td>0.83 ± 0.01</td>
<td>0.81 ± 0.01*</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>Seasonal (\Delta) RER</td>
<td>-0.02 ± 0.01</td>
<td>-0.02 ± 0.01</td>
<td>-0.02 ± 0.01</td>
</tr>
<tr>
<td>RER at 19°C</td>
<td>0.80 ± 0.01†††</td>
<td>0.77 ± 0.01***</td>
<td>0.80 ± 0.01†††</td>
</tr>
<tr>
<td>Seasonal (\Delta) RER</td>
<td>-0.02 ± 0.01</td>
<td>-0.03 ± 0.01</td>
<td>-0.03 ± 0.01</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. \(* P < 0.1, \* P < 0.05, \* * P < 0.01, \* * * P < 0.001 vs. summer, \* P < 0.05, \* * * P < 0.001 vs. 27°C. BAT, brown adipose tissue. BMI, body mass index; EE, energy expenditure; FFM, fat-free mass; RER, respiratory exchange ratio.

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cold-induced fat oxidation increased in winter in the High BAT Group (P < 0.01, Fig. 4F), but not in the Low BAT Group, suggesting the involvement of BAT on seasonal variations of fat oxidation during cold exposure. There was no notable difference in CHO oxidation between the High and Low BAT Groups (data not shown).

T_{skin} in the supraclavicular region, which is close to the BAT deposits (Fig. 3O), tended to be higher in winter than in summer for the High BAT Group at 19°C (P = 0.08, Fig. 5A), while that of the Low BAT Group was similar in the two seasons (Fig. 5H). Moreover, the T_{skin} drop in this region of the High BAT Group, which was significantly smaller than that of the Low BAT Group in winter (P < 0.05, Fig. 6A), remarkably increased in summer (P < 0.05), and the group difference disappeared. Simple linear regression analysis revealed a positive correlation between the supraclavicular T_{skin} at 19°C (r = 0.33, P = 0.03), but not at 27°C (r = 0.18, P = 0.26), and BAT activity (SUV). In contrast to the supraclavicular BAT region, the T_{skin} decreases in the subclavicular region and the peripheral calf, hand, and foot regions, which are far from the BAT deposits, were almost the same in the High and Low BAT Groups not only in summer but also in winter (Figs. 5, B–E, I–L, and Fig. 6, B–E). The response of T_{skin} in the forehead region also varied seasonally in both groups (P < 0.01, Fig. 5, F and M). T_{ty} for the High BAT Group at 19°C was evidently higher in winter than in summer (Fig. 5G, P < 0.001), whereas no seasonal difference was observed in the Low BAT Group (Fig. 5N). The drop of the forehead T_{skin} and T_{ty} for the High BAT Group in winter was significantly smaller than those in summer (P < 0.001) and of the Low BAT Group (P < 0.05, Fig. 6, F and G). Similarly, in the High BAT Group, but not in the Low BAT Group, cold sensation was less in winter (P < 0.05, Fig. 7, A–F). A negative correlation between changes in cold sensation and T_{ty} was observed (r = −0.23, P = 0.028).

DISCUSSION

In the present study, 45 healthy young males of normal body weight were recruited to minimize any possible effects of age, sex, or body composition on thermoregulation. FDG-PET/CT after 2-h cold exposure at 19°C in winter revealed 33 subjects showed significant FDG uptake into BAT (High BAT Group) while 12 showed undetectable FDG uptake (Low BAT Group), the prevalence being comparable to those obtained after mild cold exposure at 15–20°C (10, 22, 26, 27, 31, 39, 46, 48, 51–53), although there may be some ethnic differences (2). We then examined the responses of EE and body temperature to cold exposure in summer and winter in a randomized crossover design in the two subject groups. The major findings were 1) CIT and cold-induced fat oxidation increased in winter compared with summer, being greater in the High BAT Group than in the Low BAT Group, 2) the cold-induced drop of T_{ty} and
T_{\text{skin}} in the forehead and supraclavicular BAT regions was less in winter compared with summer, being more in the High BAT Group than the Low BAT Group, and 3) cold-induced drops of T_{\text{skin}} in the subclavicular and peripheral regions also decreased more in winter than in summer almost similarly in the two groups.

We have previously examined the impact of BAT on thermogenic capacity during cold exposure in winter and found a close correlation between BAT activity and CIT (51, 52). Several other groups also confirmed the involvement of BAT in CIT or nonshivering thermogenesis (5, 10, 31, 32, 44), although one study argued for its negligible role (27). Most of these studies, however, were carried out as a cross-sectional study with a small number of subjects. In the present study, we repeatedly measured CIT for all 45 subjects both in winter and in summer in a crossover design, which facilitates the discovery of seasonal variations for thermal response without having to take into consideration the interindividual differences and possible effects of order. CIT of the High BAT Group was markedly higher than that of the Low BAT Group in winter, again confirming the contribution of BAT to CIT. In contrast, CIT of the two groups in summer was very low and almost the same. While FDG-PET/CT was performed only in winter to evaluate the maximal BAT activity of each subject and to minimize the radiation exposure, it is highly likely that their BAT activity substantially decreased in summer. Indeed, it has been well documented that BAT prevalence/activity assessed by FDG-PET/CT decreases in summer and maximally increases in winter (1, 33, 39). Moreover, Kern et al. (19) reported the elevated UCP1 expression in the subcutaneous adipose tissue of lean subjects in winter. Taken together, these findings indicate that seasonal variations of CIT may be due to a parallel change in BAT activity. This seems consistent with the fact that aging diminishes both BAT activity and CIT (20, 53). In addition to CIT, the response of fat oxidation to cold increased in winter in a BAT-dependent manner. These results, being compatible with a report that cold-activated BAT uptake large amount of fatty acids (32), implying that human BAT uses fatty acids as an energy substrate for heat production.

It should be noted that a slight but significant seasonal increase in CIT was found, even in the Low BAT Group. This may be attributable to the residual activities of BAT, which is too low to be detected by FDG-PET/CT. It may also be possible that some BAT-independent thermogenic mechanisms, such as shivering thermogenesis in the skeletal muscle, are involved in CIT (45). In fact, Blondin et al. (4, 5) and Ouellet et al. (32) reported that muscle shivering intensity significantly increased following acute cold exposure and contributes to CIT along with BAT. However, the cold exposure...
condition used was apparently more severe than that of the present study: that is, in their studies, subjects were exposed to cold by wearing a liquid suit perfused with cold water at 18°C for 3 h. Indeed, CIT observed in their studies was ~1,900 kcal/day, which is 12 times higher than that observed in the present study in winter (160 kcal/day). Moreover, FDG uptake is markedly increased after cold exposure not only in BAT but also in skeletal muscle in their studies. By contrast, neither apparent signs of shivering nor FDG uptake into the skeletal muscle was observed under our experimental condition of 2-h cold exposure at 19°C (30, 31, 54), being consistent with a report (47) that muscle shivering is negligible under mild cold

Table 2. Independent associations of age, fat mass, fat-free mass, outdoor temperature/season, and BAT with energy expenditure/cold-induced thermogenesis

<table>
<thead>
<tr>
<th></th>
<th>Univariate Regression</th>
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<th>Multivariate Regression</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>β</td>
</tr>
<tr>
<td>Whole body energy expenditure at 27°C, kcal/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>0.158</td>
<td>0.137</td>
<td>−12.275</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>0.708</td>
<td>&lt;0.001</td>
<td>24.577</td>
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<tr>
<td>Fat mass, kg</td>
<td>0.376</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>BAT*</td>
<td>0.021</td>
<td>0.807</td>
<td>—</td>
</tr>
<tr>
<td>Outdoor temperature, °C</td>
<td>0.015</td>
<td>0.837</td>
<td>—</td>
</tr>
<tr>
<td>Day length, h/day</td>
<td>0.000</td>
<td>0.997</td>
<td>—</td>
</tr>
<tr>
<td>Interaction between BAT and seasonb</td>
<td>−0.029</td>
<td>0.725</td>
<td>—</td>
</tr>
<tr>
<td>Whole body energy expenditure at 19°C, kcal/day</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Age, yr</td>
<td>−0.164</td>
<td>0.122</td>
<td>−11.781</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>0.498</td>
<td>&lt;0.001</td>
<td>23.101</td>
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<tr>
<td>Fat mass, kg</td>
<td>0.345</td>
<td>&lt;0.001</td>
<td>—</td>
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<tr>
<td>BAT*</td>
<td>0.187</td>
<td>0.032</td>
<td>—</td>
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<tr>
<td>Outdoor temperature, °C</td>
<td>−0.245</td>
<td>&lt;0.001</td>
<td>−4.067</td>
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<tr>
<td>Day length, h/day</td>
<td>−0.192</td>
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<tr>
<td>Interaction between BAT and seasonb</td>
<td>0.292</td>
<td>&lt;0.001</td>
<td>51.022</td>
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<tr>
<td>Cold-induced thermogenesis, kcal/day</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>−0.045</td>
<td>0.671</td>
<td>—</td>
</tr>
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<td>Fat-free mass, kg</td>
<td>−0.110</td>
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<td>—</td>
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<tr>
<td>Fat mass, kg</td>
<td>0.048</td>
<td>0.656</td>
<td>—</td>
</tr>
<tr>
<td>BAT*</td>
<td>0.233</td>
<td>0.007</td>
<td>—</td>
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<tr>
<td>Outdoor temperature, °C</td>
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<td>−3.903</td>
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<td>Day length, h/day</td>
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<td>Interaction between BAT and seasonb</td>
<td>0.427</td>
<td>&lt;0.001</td>
<td>56.398</td>
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</tbody>
</table>

*aSubjects were divided into two groups corresponding to the BAT activity assessed by fluorodeoxyglucose-positron emission tomography combined with computed tomography (FDG-PET/CT) and coded as 0 (the Low BAT Group) and 1 (the High BAT Group). bThe cross-product term of BAT and season (summer: 1, winter: 2) were added to the regression models. cUnivariate regression analysis was performed using the Pearson’s product-moment correlation coefficient (c) or the Kendall’s tau rank correlation coefficient (d).
conditions comparable to ours. Collectively, it is unlikely that shivering thermogenesis contributes significantly to CIT observed in the present study. However, this does not necessarily exclude the partial contribution of nonshivering thermogenesis in skeletal muscle (3, 38) to the seasonal variation of CIT.

As thermogenic activity varies seasonally, body temperature response to cold exposure would be affected by season. In general, T_{skin} in the peripheral regions is decreased during cold exposure to maintain body heat loss from the skin (45). In the present study, T_{skin} in various regions significantly decreased following cold exposure, being larger in summer. These results, together with the seasonal variation of CIT, suggest that the responsiveness of T_{skin} during cold exposure is increased to compensate for the blunted CIT in summer. Indeed, we found a close positive correlation between CIT and the T_{skin} change in the distal calf, hand, or foot region (data not shown). These results agree with an earlier study showing a decreased CIT and larger T_{skin} drop in summer compared with winter (24).

Further, we focused on the relation of BAT to the seasonal variations of the T_{skin} response. Previously, we (51) and Symonds et al. (42) suggested a link between BAT and T_{skin} in the supraclavicular region close to the BAT deposits. Very recently, Boon et al. (6) documented that BAT activity/volume was positively related to the supraclavicular T_{skin} during cold exposure. Given that the metabolic activity of BAT affects T_{skin}, the BAT effect is expected to be lowered or disappear in summer because BAT activities of individuals of the High BAT Group would decrease in summer, while those of the Low BAT Group would be undetectably low throughout the year (39). Actually, the supraclavicular T_{skin} drop was smaller in the High BAT Group than in the Low BAT group in winter, but not in summer. While T_{skin} in the supraclavicular region might be affected by BAT thermogenesis, those in the subclavicular and some other peripheral regions distant from the BAT deposits would reflect heat dissipation from the skin rather than heat production (45). Interestingly, the T_{skin} drop in the subclavicular, calf, hand, or foot region was similar in the two subject groups not only in summer but also in winter. Such a region- and season-specific effect of BAT on T_{skin} strongly supports our contention that the observed differences in the supraclavicular T_{skin} drop are more likely attributable to the difference in BAT thermogenesis than to the difference in the heat dissipation from the skin.

Even though a significant contribution of BAT to CIT has been repeatedly confirmed as mentioned above, there is still limited evidence for its importance in thermoregulation. Although core or rectal temperature (T_{rec}) is likely to be maintained during mild cold exposure (44), T_{ty}, a measure of brain temperature, is likely to decrease (25). Our results indicate that T_{ty} is reduced during cold exposure to a greater degree in summer than in winter. Considering that the impaired ability to maintain T_{ty} in summer was accompanied by a diminished heat loss as noted above, such a discrepancy implies that the increased responsiveness of peripheral T_{skin} in summer lowers body heat loss from the skin but is not sufficient to cover the diminished CIT. Furthermore, it is striking that the T_{ty} drop was smaller in the High BAT Group than in the Low BAT Group only in winter. Collectively, it is likely that BAT thermogenesis is involved in the maintenance of T_{ty} and probably brain temperature during cold exposure.

There have been a number of epidemiological studies showing seasonal changes in body composition, with reduced lean body mass, body fat accumulation, and insulin resistance, in association with decreased PA and increased food intake, in winter (18, 35, 41). Consistent with these findings, our results confirmed an increased body fat content and decreased FFM in winter (18, 35, 41). Consistent with these findings, our results confirmed an increased body fat content and decreased FFM in winter (18, 35, 41).

Seasonal changes in BAT and CIT may be associated with those in some environmental factors, including temperature (7,
In the present study, univariate analyses showed inverse relationships of CIT with both outdoor temperature and day length. However, multivariate analyses confirmed a significant independent impact of outdoor temperature, but not of day length, suggesting that ambient temperature is the major regulator of the seasonal fluctuations of BAT/CIT.

The present study had several notable limitations. The limited view of muscle shivering in only the pectoralis using surface EMG may prevent us from assessing whole body shivering; thus, it might underestimate the contribution of shivering to CIT. Blondin et al. (4) documented a great variation of shivering activity between muscles with the highest in the pectoralis, and a significant positive correlation between FDG uptake and shivering activity. Ouellet et al. (32) also reported a parallel cold-induced uptake of FDG not only in BAT but also in skeletal muscle (longus colli). Thus, we cannot exclude a possible activation of shivering in some muscles other than the pectoralis, even under our mild cold condition, but as discussed above, its contribution to the observed CIT may be negligible.

Another limitation of the present study was that BAT activity was assessed only by FDG uptake. As the major substrate for BAT thermogenesis is fatty acids, it seems more rational to measure fatty acid oxidation as a thermogenic activity of BAT. In fact, Ouellet et al. (32) confirmed an increased uptake of a long-chain fatty acid analog, fluoro-thiaheptadecanoic acid, into BAT after cold exposure. Although the uptake of 2-deoxy-

Figure 6: Region- and season-specific impacts of BAT on $T_{ty}$ and $T_{skin}$, advocating the protective role of BAT thermogenesis for the maintenance of $T_{ty}$ during cold exposure. Cold-induced changes in $T_{sup}$ in the supra-clavicular (A), sub-clavicular (B), calf (C), hand (D), foot (E), and forehead regions (F), and those in $T_{ty}$ (G) for the High- and Low-BAT Groups in summer and in winter. Data are expressed as means ± SE. (*)$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

17, 19, 33, 54) and photoperiod (1, 9, 21, 43). In the present study, univariate analyses showed inverse relationships of CIT with both outdoor temperature and day length. However, multivariate analyses confirmed a significant independent impact of outdoor temperature, but not of day length, suggesting that ambient temperature is the major regulator of the seasonal fluctuations of BAT/CIT.
glucose (2-DG) and FDG is a reliable index of metabolic activity of glucose in individual tissues, including BAT, it is increased by various cues such as insulin (31), and therefore, 2-DG/FDG uptake does not always reflect the thermogenic activity of BAT. However, it is also true that glucose utilization is increased when BAT thermogenesis is activated. This may be indispensable for rapid recovery of cellular ATP levels by activating anaerobic glycolysis, as BAT thermogenesis is totally dependent on the uncoupling of oxidative phosphorylation, thereby resulting in a transient decrease in ATP production. In fact, we (54) confirmed in mice that noradrenaline-induced 2-DG uptake into BAT is totally dependent on the activation of UCP1 and parallels with BAT thermogenesis. Cypess et al. (12) also demonstrated a marked increase in FDG uptake into BAT by stimulation with a thermogenic β3-adrenergic agonist in humans. Biopsy studies in humans confirmed similar correlations between FDG uptake and UCP1 expression (23). At present, we cannot assess the relative contribution of fatty acids and glucose to BAT thermogenesis in humans, and the total BAT oxidative capacities, but it is evident that FDG uptake is useful to assess the thermogenic capacities of BAT, thereby being used as an index of BAT activity.

In the present study, BAT activity was assessed only in winter, but not in summer, because of the acceptable limits of radiation exposure of the participants. Nevertheless, the seasonal changes in BAT prevalence/activity and UCP1 expression had well been established in humans (1, 19, 33, 39). It should be emphasized that, in our previous study, all subjects with high BAT activity in winter showed much decreased activity in summer (39), whereas those with low BAT activity even in winter kept their low activity in summer. Thus, there is little doubt that BAT activities of our participants considerably decrease in summer.

Finally, our results indicate the protective role of BAT in the cold-induced drop of \( T_{by} \) and brain temperature, but they do not give any idea about the control of core temperature, which could not be measured in the present study due to technical difficulties. Although \( T_{by} \) is liable to change compared with \( T_{rec} \) (25), which does not change significantly during mild cold exposure (44), some studies have indirectly suggested a possible involvement of BAT in the control of core temperature (16, 40). This is to be explored in the future studies with a well-designed protocol in healthy humans.

Perspectives and Significance

The present study demonstrates a significant involvement of BAT in the seasonal variations of the cold-induced adaptive thermogenesis in healthy human adults. Since cold acclimation studies have demonstrated anti-obesity and anti-diabetic effects of human BAT (22, 52), it is possible that the seasonal decrease in adaptive thermogenesis may be associated with obesity and diabetes. To test this hypothesis in future studies, the other adaptive thermogenesis, especially diet-induced thermogenesis (DIT), should also be taken into account. To our knowledge, no study has shown seasonal fluctuations of DIT and its relationship to BAT in both animals and humans. Interestingly, an impact of \( UCP1 \) gene polymorphism on visceral fat area is diminished in summer (29), suggesting the association of the reduced BAT thermogenic capacity in summer with energy imbalance.

Finally, we also found that the decreased BAT activity was associated with the impaired ability to maintain \( T_{by} \) during cold exposure, supporting the hypothesis that BAT contributes to whole body EE and thermoregulation in humans, as in small rodents (7, 13, 14). The comparative approach to the study of body temperature seems highly likely to yield further insights into the contribution of human BAT to thermoregulation.

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No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
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