

## **High Incidence of Metabolically Active Brown Adipose Tissue in Healthy Adult Humans: Effects of Cold Exposure and Adiposity**

Masayuki Saito<sup>1</sup> Yuko Okamatsu-Ogura<sup>2</sup> Mami Matsushita<sup>1</sup> Kumiko Watanabe<sup>1</sup>, Takeshi Yoneshiro<sup>1</sup>, Junko Nio-Kobayashi<sup>3</sup>, Toshihiko Iwanaga<sup>3</sup>, Masao Miyagawa<sup>4</sup>, Toshimitsu Kameya<sup>5</sup>, Kunihiro Nakada<sup>5</sup>, Yuko Kawai<sup>5</sup> and Masayuki Tsujisaki<sup>5</sup>,

<sup>1</sup>Department of Nutrition, School of Nursing and Nutrition, Tenshi College, Sapporo 065-0013, Japan

<sup>2</sup>Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

<sup>3</sup>Graduate School of Medicine, Hokkaido University, Sapporo 060-8638

<sup>4</sup>PET Center, Ehime Hospital, Matsuyama 790-0024, Japan

<sup>5</sup>LSI Sapporo Clinic, Sapporo 065-8611, Japan

### **Correspondences to:**

Masayuki Saito, Ph.D.

e-mail: [saito@tenshi.ac.jp](mailto:saito@tenshi.ac.jp)

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*Objective:* The significant roles of brown adipose tissue (BAT) in the regulation of energy expenditure and adiposity are established in small rodents, but have been controversial in man. The objective is to examine the prevalence of metabolically active BAT in healthy adult humans, and to clarify the effects of cold exposure and adiposity.

*Research Design and Methods:* In vivo 2-[<sup>18</sup>F]fluoro-2-deoxyglucose (FDG) uptake into adipose tissue was measured in 56 healthy volunteers (31 males and 25 females) aged 23 - 65 y by positron emission tomography (PET) combined with X-ray computed tomography (CT).

*Results:* When exposed to cold (19 °C) for 2 hr, 17 out of 32 younger subjects (23-35 y.o.) and 2 out of 24 elderly subjects (38-65 y.o.) showed a substantial FDG uptake into adipose tissue of the supraclavicular and paraspinal regions, whereas they showed no detectable uptake when kept in warm (27 °C). Histological examinations confirmed the presence of brown adipocytes in these regions. The cold-activated FDG uptake was increased in winter compared to summer ( $p < 0.001$ ), and inversely related to body mass index ( $p < 0.001$ ), and total ( $p < 0.01$ ) and visceral ( $p < 0.001$ ) fat areas estimated from CT image at the umbilical level.

*Conclusions:* Our findings, being against the conventional view, indicate the high incidence of metabolically active BAT in adult humans, and suggest a role in the control of body temperature and adiposity.

Mammals have two types of adipose tissue, white (WAT) and brown (BAT) adipose tissues. These two tissues have quite opposite roles in whole body energy metabolism: that is, WAT is for energy storage and BAT for cold- and diet-induced thermogenesis, which significantly contributes to the control of body temperature and energy expenditure (1). BAT thermogenesis is principally dependent on the  $\beta$ -adrenergically mediated activation of lipolysis and subsequent degradation of fatty acids via uncoupling protein 1 (UCP1), which uncouples mitochondrial oxidative phosphorylation to dissipate the electrochemical gradient as heat instead of ATP synthesis. Thus, the  $\beta$ -adrenoceptor-UCP1 system has been expected as an intriguing target for the control of whole body energy balance, adiposity and obesity (2-5).

Almost all views about BAT thermogenesis have come from the studies using small rodents such as the mouse, rat and hamster. In humans, significant amounts of BAT are present in newborns, and may contribute to body temperature regulation during the neonatal period, probably in the same way as in small rodents. However, BAT seems to disappear rapidly during postnatal periods, and in adults is rather difficult to be identified by conventional anatomical examinations. Thus, it has been a general contention that BAT is absent or of minute amounts, and, if any, plays negligible roles in

adult humans (6-8).

The existence of metabolically active BAT in adult humans has been suggested by the current clinical studies using fluoro-deoxyglucose (FDG) - positron emission tomography (PET), one of the powerful diagnostic tools for malignant tumors: that is, PET sometimes detects symmetrical FDG uptake in the shoulder and thoracic spine regions, where no tumor is present. By simultaneous examinations with PET and X-ray computed tomography (CT), the site of the FDG uptake was identified as adipose tissue (9). Such FDG uptake is increased at lower environmental temperatures (10-12), and reduced by pretreatment with  $\beta$ -adrenergic blockers (13,14). These findings collectively suggest that the FDG uptake in adipose tissue at the specific regions reflects the metabolic activity of BAT (15).

However, almost all human studies so far reported seemed for more accurate diagnosis of cancer, not for the detection and evaluation of BAT itself. In this study, we performed FDG-PET/CT examinations in adult healthy volunteers under a warm and cold condition to clarify the effects of cold exposure, age, season, and some other body parameters including body fat. Our data indicate an unexpected high incidence of cold-activated BAT in adult healthy humans, and suggest a role in the regulation of metabolic thermogenesis and body fat content.

## **RESEARCH DESIGN AND METHODS**

Subjects recruited for this study were 56

healthy volunteers (31 males and 25 females) aged 23 - 65 y (Table 1). All subjects were carefully instructed about the study and gave their informed consent to participate. After a standardized health examination, they underwent FDG-PET /CT and other examinations once or twice from August of 2006 to next March. The protocol was approved by the Institutional Review Boards of Tenshi College.

**FDG-PET/CT:** After fasting for 6 – 12 hr, the subjects were kept in an air-conditioned room at 19 °C with light clothing (usually a T-shirt with underwear), and put their legs on an ice block intermittently (usually for 4 min at every 5 min) . After one hour under this “cold” condition, they were given intravenous injection of <sup>18</sup>F-FDG (259 MBq), and kept under the same cold condition. In some cases, the subjects were kept at 26-28 °C with standard clothing and without leg-icing (“warm” condition). One hour after the <sup>18</sup>F-FDG injection, whole-body PET/CT scans were performed on a PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Tochigi, Japan) in a room at 24 °C. With the CT parameters of 120 kv and real exposure control, unenhanced low-dose spiral axial 2-mm collimated images were obtained. This was used for PET attenuation correction as well as anatomic localization. Subsequently, full-ring PET was performed in 6 incremental table positions, each approximately 15 cm in thickness. The total time of these scans was about 30min.

PET and CT images were co-registered

and analyzed by VOX-BASE workstation (J-MAC system, Sapporo, Japan). Two experienced blinded observers assessed the FDG uptake, particularly in both sides of the neck and paravertebral regions, by visually judging the radioactivity greater than background. In parallel, the FDG uptake in the neck region was quantified, and expressed as relative to that in the whole brain.

**Anthropometric and body fat measurement, and blood analysis:** Body mass index was calculated as body weight (kg)/height (m)<sup>2</sup>, and percent of body fat was estimated by the multi-frequency bioelectric impedance method (InBody 320 Body Composition Analyzer, Biospace, Seoul, Korea). The abdominal and subcutaneous fat areas at the level of L4-L5 were estimated from the CT images.

Serum levels of leptin and adiponectin were measured using respective ELISA kits: Human leptin ELISA kit, B-Bridge, Mountain View, CA, USA; Human adiponectin ELISA kit, Otsuka Pharmaceutical, Tokyo, Japan. Other blood parameters were analyzed in a company of laboratory testing services (SRL Inc., Tokyo, Japan).

**Histological examinations:** An autopsy tissue specimen was obtained from fat depots of the supraclavicular region of a 21-year old man, and stained with hematoxylin-eosin or an anti-serum against rat UCP1 (16).

**Data analysis:** Data are expressed as means ± SD, and analyzed by paired or student’s t-test. Chi-squared test was used for the data of the prevalence of BAT. SPSS

soft-wear package (Version 10.0, Chicago, USA) was used for correlation analysis between BAT and adiposity-related parameters.

## **RESULTS**

Since one of the typical methods to activate BAT in rodents is cold exposure, first, we examined the acute effects of cold exposure on FDG uptake. For this, healthy subjects were overnight fasted, and kept in a room at 28 °C (warm condition), or at 19 °C with light clothes and intermittent putting on an ice-cooled footrest (cold condition). Two hours later, they underwent FDG-PET/CT examination. Under the warm condition, a clear FDG uptake was detected in the brain, heart, and the oropharyngeal region, while no FDG signal in adipose tissue (Fig. 1B). When the same subjects were kept under the 2-hr cold condition, a clear and intense FDG uptake was found in adipose tissue at the supraclavicular and paraspinal regions (Fig. 1A).

During a 7-month period from August of 2006 to next March, we performed a total of 71 FDG-PET/CT examinations under the 2-hr cold condition for 56 healthy volunteers aged 23 - 65 years. As summarized in Table 2, cold-activated FDG uptake in the supraclavicular region was detected in 18 out of 55 subjects (33 %) in winter from January to March. Although the incidence of such FDG uptake seemed not to show apparent gender difference, it changed with age ( $p < 0.01$  by Chi-square test), being 52% (16/31) and 8 % (2/24) in younger (23-35 y.o.) and elderly

(38-65 y.o.) subjects, respectively. Table 2 confirms again the stimulatory effect of acute cold exposure on FDG uptake as typically shown in Fig. 1: that is, 8 subjects who showed clear FDG uptake under the cold condition were tested again under the warm condition at 28 °C in 2 weeks, but none of them showed detectable FDG uptake in the adipose tissue including supraclavicular and paraspinal regions, regardless of the test seasons.

Table 2 also suggests some seasonal variations of the incidence, being low in summer from August to September. This was clearly demonstrated from the results of some individual subjects: that is, in summer, the FDG uptake was detected in 2 out of 8 subjects. When the same subjects were examined again in winter, it was found in 6 subjects, 4 of which showed no FDG uptake in summer and other 2 showed lower uptake in summer (Fig. 2B). Moreover, FDG uptake was sometimes detected both at the supraclavicular and paraspinal regions in winter, but only in the supraclavicular region in summer (Fig. 2A). Thus the incidence and intensity of cold-activated FDG uptake showed seasonal variations, being higher in winter.

In small rodents, BAT thermogenesis is recognized as a significant component of whole body energy expenditure, and thereby to contribute to the regulation of body fat content (1, 2). To examine whether this is also the case in human, first, we analyzed the relationship between the cold-activated FDG uptake into BAT and adiposity in a total of 19 subjects bearing detectable BAT. As shown in Fig. 3,

FDG uptake into the supraclavicular adipose tissue showed significant inverse relations to BMI ( $r = -0.674$ ,  $p < 0.001$ ), total fat ( $r = -0.564$ ,  $p < 0.01$ ) and visceral fat ( $r = -0.681$ ,  $p < 0.001$ ). Weaker but significant inverse relations were also found to subcutaneous fat ( $r = -0.492$ ,  $p < 0.05$ ) and plasma insulin level ( $r = -0.473$ ,  $p < 0.05$ ), but not to plasma levels of leptin, adiponectin and T3 (data not shown).

We also compared the similar parameters in subjects bearing detectable and undetectable amounts of cold-activated BAT (Table 3). As expected from the results in Table 2, the mean age was significantly lower in the group bearing detectable BAT than that without BAT. Body weight, BMI, visceral and subcutaneous fat areas estimated from CT tended to be lower in the group bearing detectable BAT, but the difference was statistically not significant. Since these parameters of adiposity are much influenced by ageing, being higher in elderly subjects, next, we compared the two groups of 32 younger subjects (23-35 y.o. in Table 2). The mean age was comparable in the two groups (27.3 vs. 29.1 y.o. in males, 30.3 vs. 29.5 y.o. in females), the effects of ageing may be canceled. There was no difference in height, body weight, and blood parameters examined between the two groups in both males and females. The group bearing detectable BAT tended to show decreased BMI, body fat content and visceral fat area compared to that without BAT, but again the difference was statistically not significant (data not shown).

In a separate study, we examined histologically an autopsy sample of fat depots

obtained from the supraclavicular region, and found numerous multi-locular adipocytes expressing UCP1 protein (Fig. 4).

## **DISCUSSION**

In this study, we performed FDG-PET/CT examination in adult healthy volunteers, particularly focusing on the effects of cold exposure and adiposity-related parameters on FDG uptake into adipose tissue in some specific regions, where UCP1-positive brown adipocytes were detected. The major findings were: 1) FDG uptake in adipose tissue in the supraclavicular and paraspinal regions was negligible in warm condition, but markedly increased after 2-hour cold exposure, 2) Cold-activated FDG uptake was increased in winter compared to summer, 3) It was detected in about half of younger subjects, but much less in elderly subjects, and 4) It decreased with increasing adiposity assessed by BMI and body fat content.

It has been reported in rodents that cold exposure markedly increased 2-DG uptake into BAT, but only slightly into white adipose tissue (17-20). The stimulatory effects of cold exposure were mimicked by electrical stimulation of sympathetic nerves into BAT and  $\alpha$ -adrenergic agonist administration, but abolished by surgical severing of the sympathetic nerves or  $\alpha$ -adrenergic blockade.

We (21) demonstrated that the  $\alpha$ -adrenergically stimulated 2-DG uptake into BAT is totally dependent on the activation of UCP1, and thereby a metabolic marker of BAT. Our present findings of the increased FDG

uptake into adipose tissues of the specific regions after 2-hour cold exposure are quite similar to those in rodents, and thus support the idea that it reflects the activation of BAT, and that adult humans have, more or less, metabolically active BAT. These seem consistent with previous case reports about patients with malignant lymphoma and some other types of tumor, where the incidence of FDG-uptake in the supraclavicular region is higher when the examination was performed at lower room temperatures (10-12).

In our study, cold-activated FDG uptake was markedly increased when examined in winter compared to summer. Cohade et al. (10) reported that the incidence of FDG-uptake into in the supraclavicular region showed seasonal variations, being higher in January to March. It is known in mice and rats that chronic cold exposure (cold acclimation) results in hyperplasia of BAT and increased 2DG uptake (1, 18-20). Moreover, cold acclimation has been shown in rodents to induce an apparent “trans-differentiation” of WAT to BAT (22-24). Considering that the average outdoor temperature in our study was -1.6 °C in winter and 21.3 °C in summer, it is quite likely that the increased FDG uptake in winter is attributable to hyperplasia of BAT and/or “trans-differentiation” of WAT to BAT.

Collectively, it can be concluded that the cold-activated FDG uptake in the supraclavicular and paraspinal regions is an index of the amount and activity of BAT. It is to be noted that about half of younger (23-35 y.o.) subjects have metabolically active

BAT, being quite against the widely accepted view that the amount and activity of BAT are negligible in adult humans. Although the prevalence of BAT was much decreased in elderly subjects (38-65 y.o.), the prevalence in our study is much higher than those previously reported in clinical studies (10), where FDG uptake in the shoulder region is detected in some patients (<10%) at relatively low room temperatures (~22 °C), but not found at higher temperature (>25 °C). The reason for the high prevalence in our study must be that our subjects were kept at a lower temperature (19 °C) and stimulated by intermittent ice-cooling of legs. In other word, our condition of cold exposure may be more appropriate to detect BAT.

More interesting in our results is the relationship between BAT and adiposity of the subjects: that is, cold-activated FDG uptake into BAT showed significant inverse relations to BMI, body fat and visceral fat. Weaker but significant inverse relations were also found to subcutaneous fat and plasma insulin level.

This seems quite compatible with those in rodents that the amount and activity of BAT is decreased in obesity. There is an ample of evidence that BAT thermogenesis is a significant component of whole body energy expenditure, and thereby contributes to the regulation of energy balance and body fat content (1). Our present results thus imply a significant role of BAT in the control of adiposity via the regulation of energy expenditure in humans in the same way as in rodents. Being consistent with this idea, the

subjects bearing detectable BAT tended to be low in BMI and body fat content compared to those without BAT, though the difference did not reach statistically significant levels.

Another point to be noted is that the incidence of cold-activated BAT decreased in elderly subjects. In a total of 24 elderly subjects, BAT was detected in only two males (Table 2). Interesting is that these two males are rather lean (BMI = 22.2 and 20.6) compared with other 10 male subjects bearing no BAT with BMI of 24.4. Although these results are preliminary and to be confirmed in larger numbers of subject, they may imply that the disappearance of BAT accelerates the development of obesity with aging. This idea seems consistent with our previous observation that UCP1 deficiency in mice increases susceptibility to diet-induced obesity with age (25).

The present results thus collectively suggest that cold-activated BAT participates, more or less, in the control of energy expenditure and adiposity in humans, as does it in small rodents. This may ask for re-evaluation of the physiological relevance of BAT in humans. For example, it is not rare that experimentally and/or clinically estimated basal metabolic rate, as well as post-prandial thermogenesis (diet-induced thermogenesis), considerably varies among individuals even after correcting possible contributing factors such as gender, age and body dimensions. It may be possible that such variations are due to the individual differences in BAT activity. Moreover, our results may highlight again a

pharmacological approach to obesity treatment, which has repeatedly been confirmed in experimental animals (3, 4, 26, 27) but not yet approved in human: that is, agonists specific to  $\alpha$ 3-adrenergic receptor effectively reduce adiposity in small rodents and dogs, but to much less extents or little in humans. Based on our results, it may be likely that  $\alpha$ 3-adrenergic agonists are effective only for individuals who keep active BAT, as do they in wild-type, but not in UCP1-deficient, mice (4). Our present findings may give a clue to revisit such intriguing subjects.

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**Table 1** Subject profiles

	Male	Female
Number	31	25
Age (y.o.)	35.8 ± 9.0 (23 - 65)	38.8 ± 8.8 (25 - 65)
Height (cm)	170.7 ± 5.2 (154 - 181)	158.0 ± 4.4 (148 - 166)
Body weight (kg)	68.8 ± 7.6 (53.2 - 91.0)	52.5 ± 5.4 (40.6 - 63.4)
BMI	23.8 ± 2.6 (17.7 - 31.0)	21.1 ± 2.3 (16.2 - 26.9)
Body fat (kg)	16.4 ± 4.8 (5.7 - 36.8)	14.9 ± 4.4 (7.4 - 26.2)
Blood parameters		
Glucose (mg/dl)	88.7 ± 5.6 (71 - 102)	83.4 ± 5.1 (71 - 96)
HA1c (%)	4.8 ± 0.2 (4.5 - 5.5)	4.8 ± 0.2 (4.0 - 5.1)
Total cholesterol	195 ± 21 (115 - 251)	203 ± 22 (159 - 272)
HDL-cholesterol (ng/dl)	61.6 ± 11.8 (35 - 90)	73.0 ± 12.0 (51 - 98)
Triglyceride (mg/dl)	113 ± 57 (19 - 395)	67 ± 25 (29 - 183)
Insulin (ng/ml)	4.5 ± 2.5 (1.1 - 10.4)	3.5 ± 1.3 (1.3 - 5.3)
Leptin (ng/ml)	5.0 ± 4.1 (0.3 - 26.1)	11.1 ± 7.1 (1.4 - 28.6)
Adiponectin (ug/dl)	10.3 ± 3.2 (3.9 - 18.0)	15.1 ± 4.6 (3.3 - 24.7)
T3 (pg/ml)	2.0 ± 0.6 (1.0 - 3.7)	1.9 ± 0.8 (0.5 - 4.4)

Values are means ± SD with minimum and maximum values in parentheses.

**Table 2.** Prevalence of cold-activated BAT in adult humans.

During a 7-month period from August of 2006 to next March, a total of 71 FDG-PET/CT examinations were conducted under the 2-hr cold condition for 56 healthy volunteer subjects as in Fig. 1A. Some of them (the number in parentheses) underwent the examination again under the warm condition as in Fig. 1B in 2 weeks after that under the cold condition. The number of subjects who showed apparent FDG uptake into BAT (adipose tissue in the supraclavicular region) is presented in numerator with the total number in denominator.

The mean outdoor temperature was also shown with the highest and lowest in parentheses. Summer: August 29 – September 28, 2006. Autumn: October 5 – October 19, 2006. Winter: January 29 – March 6, 2007.

Season	Summer	Autumn	Winter	
Outdoor temperature (C)	21.3 (19.6-28.3)	11.7 (7.6-16.1)	-1.6	(-5.2-1.8)
Number of subject	8	8	31	24
Age (y.o.)	23-31	23-32	23-35	38-65
Prevalence				
Total	2/8 (0/1)	3/8 (0/3)	16/31 (0/4)	2/24
Male	1/5	2/6 (0/2)	8/19 (0/3)	2/12
Female	1/3 (0/1)	1/2 (0/1)	8/12 (0/1)	0/12



**Table 3** Comparison between subjects bearing detectable (+) and undetectable (-) amounts of BAT.

BAT	Male		Female	
	-	+	-	+
Number	21	10	16	9
Age (y.o.)	38.4 ± 10.0	31.3 ± 6.7*	43.6 ± 8.6	30.3 ± 1.7*
Height (cm)	170 ± 6	170 ± 4	158 ± 5	159 ± 3
Body weight (kg)	71.3 ± 6.8	65.3 ± 6.6	54.5 ± 4.6	49.0 ± 5.7
BMI	24.4 ± 2.3	22.7 ± 2.8	22.0 ± 2.2	19.4 ± 2.1
Body fat (%)	24.5 ± 5.5	20.6 ± 5.0	29.4 ± 5.1	24.5 ± 5.4
Body fat (kg)	17.7 ± 4.8	13.8 ± 4.6	16.2 ± 3.6	12.5 ± 4.0
Fat area (cm <sup>2</sup> )				
Total	236 ± 82	152 ± 87	222 ± 85	159 ± 76
Visceral	71 ± 27	47 ± 19	51 ± 24	30 ± 7
Subcutaneous	165 ± 64	104 ± 68	171 ± 67	129 ± 71

Values are means ± SD, \*p<0.05 vs BAT (-) by t-test.

### Figure 1. Whole body FDG-PET images under cold or warm condition

**A:** A 25-year old male subject was fasted for 12 hr, and kept in an air-conditioned room at 19 °C with light clothing, and put his legs on an ice block intermittently (approximately for 4 min at every 5 min). After 1 hr under this “cold” condition, he was given an intravenous injection of <sup>18</sup>F-FDG, and kept under the same cold condition. One hour after the <sup>18</sup>F-FDG injection, whole-body PET/CT scans were performed in a room at 24 °C.

**B:** Two weeks after the first examination in the cold condition (A), the same subject underwent FDG-PET/CT examination as previously, but he was kept at 27 °C with standard clothing and without leg-icing (warm condition) for 2 hr before the examination.

### Figure 2. FDG uptake into BAT in summer and winter

**A:** A 30-year old female subject underwent FDG-PET/CT examination under the cold condition as in Figure 1 in August 29, 2006 (summer) and again in February 22, 2007 (winter).

**B:** Eight subjects (5 males and 3 females) underwent FDG-PET/CT examination under the cold condition in summer (August 29 – September 28, 2006) and again in winter (January 29 - March 6, 2007). FDG uptake into the supraclavicular region was densitometrically quantified, normalized and expressed as relative to that into the brain. Thick bars are means with SD.

### Figure 3. Cold-activated BAT and adiposity

Cold-activated FDG uptake into the supraclavicular region of 19 subjects was quantified as in Fig. 2, and plotted against BMI, total, and visceral fat areas estimated from CT images at the umbilical (L4, L5) level.

**Figure 4. Histological identification of UCP1-positive brown adipocytes in fat depots obtained from the supraclavicular region**

Tissue sections were stained with hematoxylin-eosin (A) or anti-serum against rat UCP1 (B)

.

Figure 1

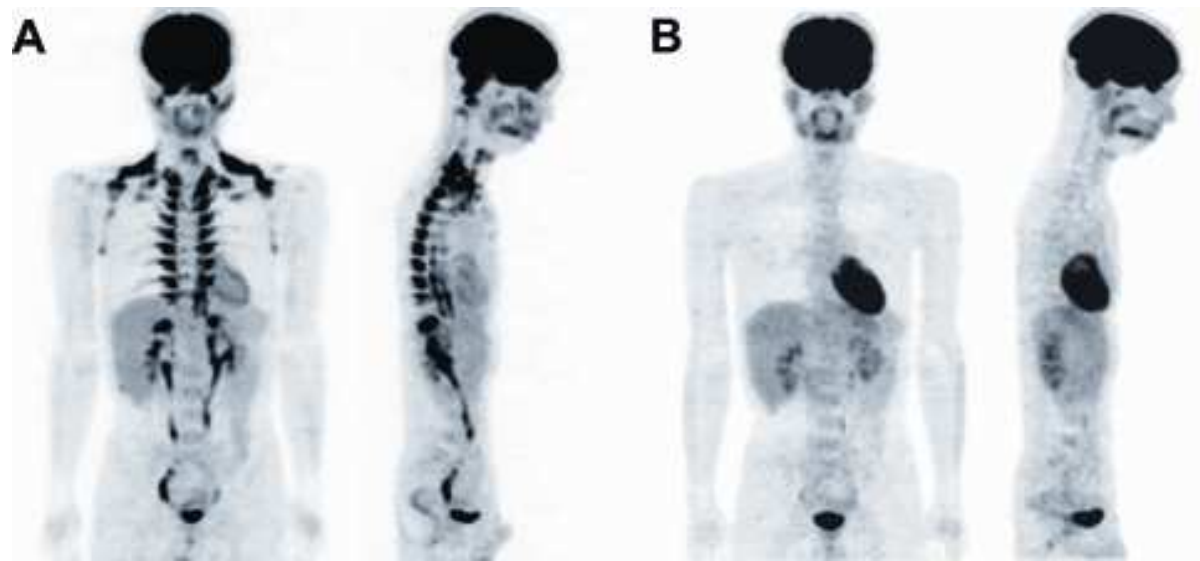


Figure 2

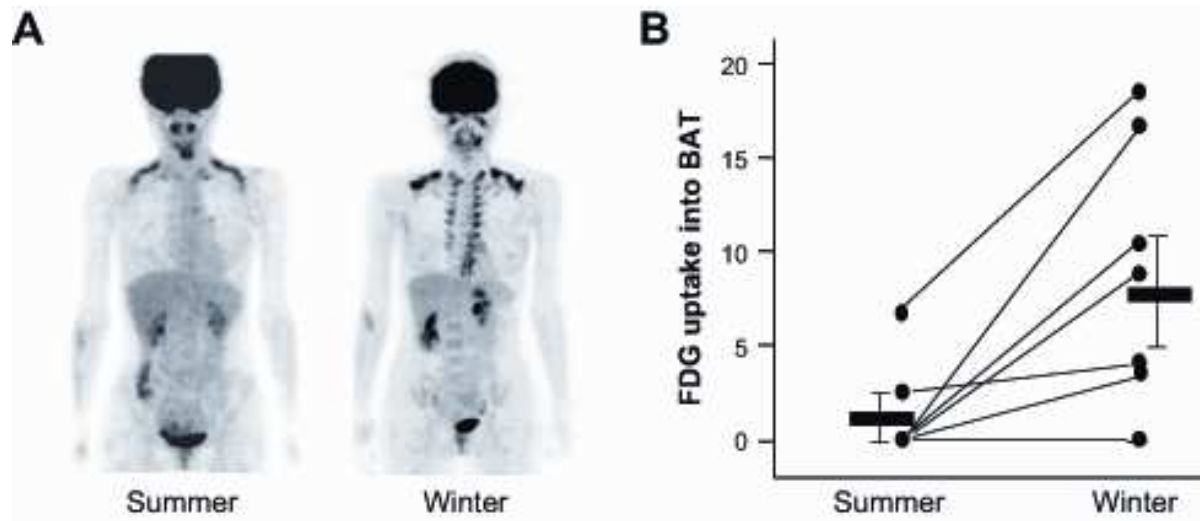


Figure 3

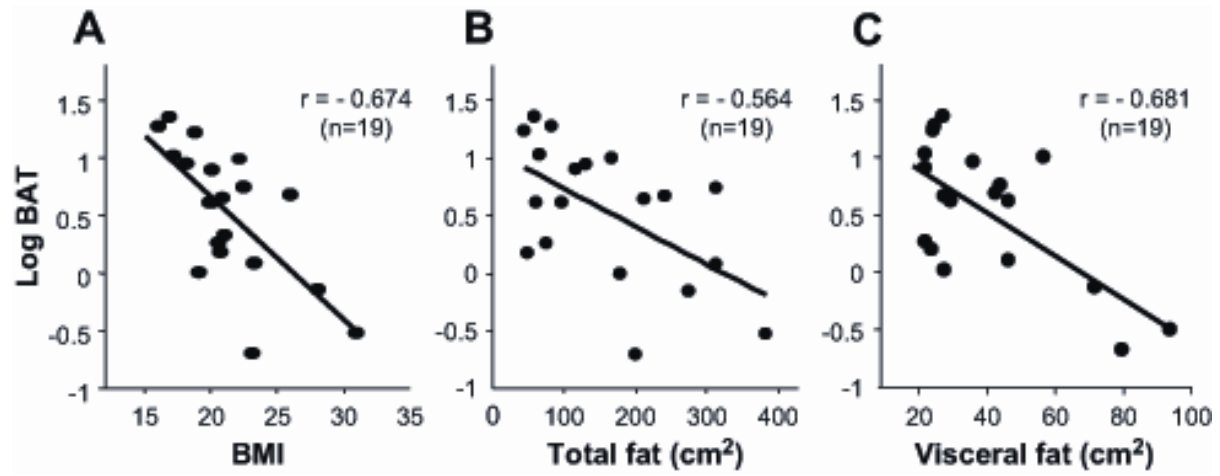


Figure 4

