Cell Metabolism
Short Article

Different Metabolic Responses of Human Brown Adipose Tissue to Activation by Cold and Insulin

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SUMMARY

We investigated the metabolism of human brown adipose tissue (BAT) in healthy subjects by determining its cold-induced and insulin-stimulated glucose uptake and blood flow (perfusion) using positron emission tomography (PET) combined with computed tomography (CT). Second, we assessed gene expression in human BAT and white adipose tissue (WAT). Glucose uptake was induced 12-fold in BAT by cold, accompanied by doubling of perfusion. We found a positive association between whole-body energy expenditure and BAT perfusion. Insulin enhanced glucose uptake 5-fold in BAT independently of its perfusion, while the effect on WAT was weaker. The gene expression level of insulin-sensitive glucose transporter *GLUT4* was also higher in BAT as compared to WAT. In conclusion, BAT appears to be differently activated by insulin and cold; in response to insulin, BAT displays high glucose uptake without increased perfusion, but when activated by cold, it dissipates energy in a perfusion-dependent manner.

INTRODUCTION

Brown adipose tissue (BAT) is capable of adaptive thermogenesis because of the presence of uncoupling protein 1 (UCP1) in its numerous mitochondria. BAT dissipates energy as heat in response to stimulation by the sympathetic nervous system (Cinti, 2006). The existence of active BAT in healthy adult humans has been debated and was not confirmed until recently (reviewed by Enerbäck, 2010). When using positron emission tomography (PET) with 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) tracer, combined with computed tomography (CT) for diagnostic imaging of metabolically active tumors and metastases, a confounding symmetrical pattern of increased metabolism is often observed in the supraclavicular region (Nedergaard et al., 2007). On the basis of PET/CT-guided biopsies from healthy subjects, it was recently established that this tissue corresponds to metabolically active BAT since it both presents histological features of BAT and expresses mRNA and proteins that are expected for BAT (Virtanen et al., 2009). Whether BAT plays a significant role in human metabolism is still unclear. However, recent studies indicate that BAT may be more abundant in leaner individuals (e.g., van Marken Lichtenbelt et al., 2009).

To elucidate the characteristics of BAT activation, we studied a group of 27 healthy adults by using PET/CT imaging with [^{15}O]H_2O and [^{18}F]FDG as tracers during cold exposure and, in a warm environment, either with or without insulin stimulation. We measured perfusion and glucose uptake rates in BAT and compared them to those in skeletal muscle and subcutaneous white adipose tissue (WAT). Perfusion rate is generally matched to the oxygen consumption of a tissue (Segal, 2005), and in the case of BAT, determination of perfusion rate can be used to estimate thermogenesis. We found that activation of energy-consuming BAT by cold leads to an increased perfusion rate that is associated with whole-body energy expenditure. Classic brown adipocytes have been shown to share a common origin with skeletal muscle cells (Seale et al., 2008). While skeletal muscle is known to be a highly insulin-sensitive tissue, little is known about the insulin sensitivity of human BAT. This study was set up to address this issue. Our data suggest that BAT is a highly insulin-sensitive tissue type, since the insulin-stimulated glucose uptake rate in BAT is not significantly different from that in skeletal muscle.

RESULTS

Cold Exposure and Glucose Uptake

In this study, the adipose tissue in which the glucose uptake rate was higher than 3.0 μmol/100 g/min during cold exposure was

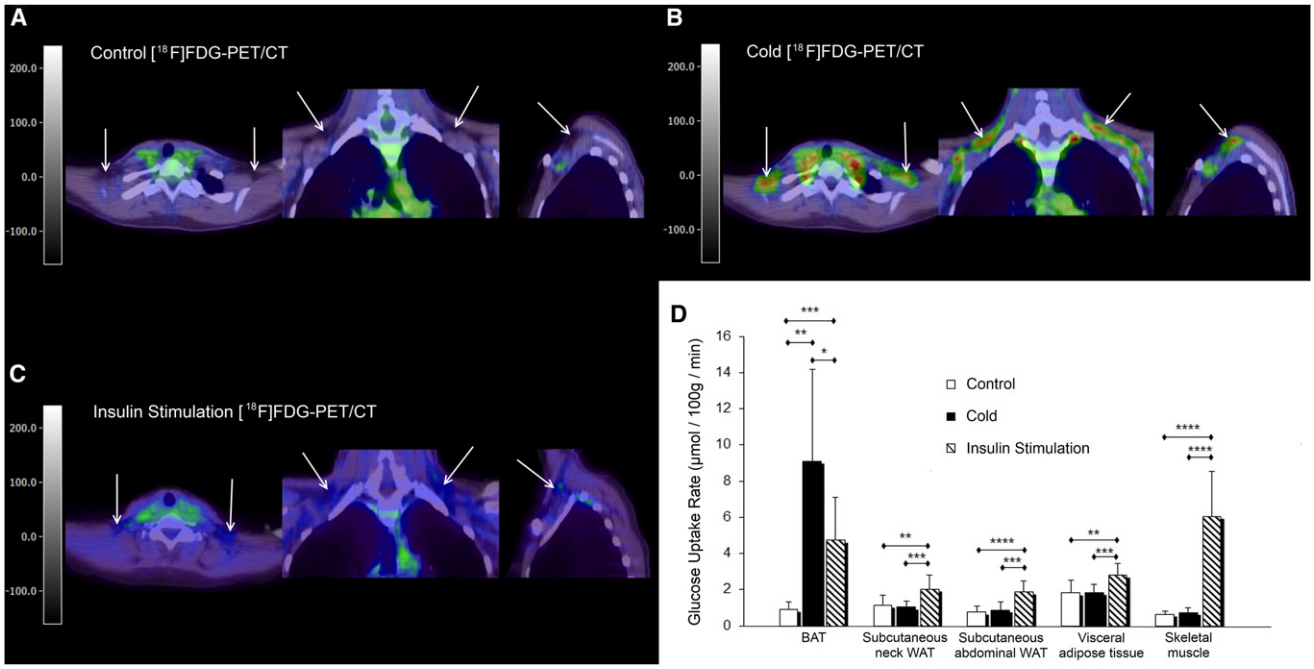

Figure 1. Glucose Uptake

(A) Control [^{18}F]FDG-PET/CT image showing minimal BAT activity.

(B) Cold exposure [^{18}F]FDG-PET/CT image showing cold-activated BAT.

(C) Insulin stimulation [^{18}F]FDG-PET/CT image showing insulin-mediated BAT activity.

(D) Glucose uptake rates in BAT, subcutaneous neck WAT, subcutaneous abdominal WAT, VAT, and skeletal muscle determined under control conditions, during cold exposure, and during insulin stimulation. Results are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. (A)–(C) from left to right: transaxial, coronal and sagittal views. The grayscale refers to Hounsfield units of the CT image. Arrows indicate supraclavicular adipose depot.

considered to represent active BAT. This threshold was chosen after qualitative interpretation of PET/CT images and determination of BAT glucose uptake rate under control conditions, where the rate was always lower than 1.7 μmol/100 g/min. Metabolic activation of BAT was detected in 19 out of 27 subjects (70%) in cold exposure [^{18}F]FDG-PET/CT scans. Active BAT was mostly found in the supraclavicular area but also in paraspinal and para-aortic regions, the axillary depot, the mediastinum, and close to the adrenal glands in conjunction with supraclavicular uptake (data not shown). The estimated mass of detected active BAT ranged from 9 to 90 g, the mean mass being 34 ± 22 g. No significant differences in BAT activation could be found between males and females. Only the subjects with cold-activated supraclavicular BAT were included in the further analysis of BAT metabolism.

Cold-induced BAT glucose uptake rate was determined and compared to WAT, visceral adipose tissue (VAT), and skeletal muscle glucose uptake rates. This comparison was considered important because different tissues may contribute unequally to cold-induced changes in glucose metabolism.

The BAT glucose uptake rate was markedly increased (0.9 ± 0.4 versus 9.1 ± 5.1 μmol/100 g/min, $p = 0.002$) by cold exposure (Figures 1A and 1B). Glucose uptake rate in BAT was increased on average by a factor of 12 in response to cold. However, the subcutaneous abdominal WAT glucose uptake rate did not change with cold exposure (0.7 ± 0.3 versus 0.9 ± 0.5 μmol/100 g/min, $p = 0.47$). Glucose uptake rates in VAT (1.8 ± 0.7 versus 1.8 ± 0.5 μmol/100 g/min, $p = 0.91$) and skeletal muscle (0.7 ± 0.2 versus 0.7 ± 0.3 μmol/100 g/min, $p = 0.26$) also remained unchanged. Glucose uptake results are summarized in Figure 1D. These results indicate that the glucose uptake rates in subcutaneous WAT, VAT, and skeletal muscle are quite similar and unaffected by cold, while BAT glucose uptake can be stimulated significantly by cold exposure.

Cold exposure significantly increased concentrations of norepinephrine in plasma (1.9 ± 0.5 versus 4.9 ± 1.5 nmol/l, $p < 0.0001$) (Figure S1A), but no significant associations were found between activation of BAT by cold and the concentrations of norepinephrine, its metabolite dihydroxyphenylglycol (DHPG), or cyclic adenosine monophosphate (cAMP) in plasma. On the other hand, cold exposure decreased plasma insulin concentration (4.1 ± 1.8 versus 2.2 ± 1.0 mU/l, $p < 0.001$) (Figure S1E).

Effect of Insulin on Glucose Uptake

Insulin is a major regulator of glucose uptake in most tissues. As little is known about the insulin sensitivity of human BAT, we assessed the influence of insulin on BAT, WAT, and skeletal muscle glucose uptake (Figure 1D). The insulin-stimulated glucose uptake rate in the supraclavicular adipose tissue (Figure 1C), identified to contain cold-activated BAT, was 5-fold higher than when studied without insulin stimulation (0.9 ± 0.4 versus 4.7 ± 2.4 μmol/100 g/min, $p < 0.001$, control versus insulin stimulation, respectively) and not significantly different from that in skeletal muscle (6.0 ± 2.5 μmol/100 g/min, $p = 0.19$). Insulin also enhanced glucose uptake in subcutaneous abdominal WAT (0.7 ± 0.3 versus 1.9 ± 0.6 μmol/100 g/min, $p < 0.0001$) and VAT (1.8 ± 0.7

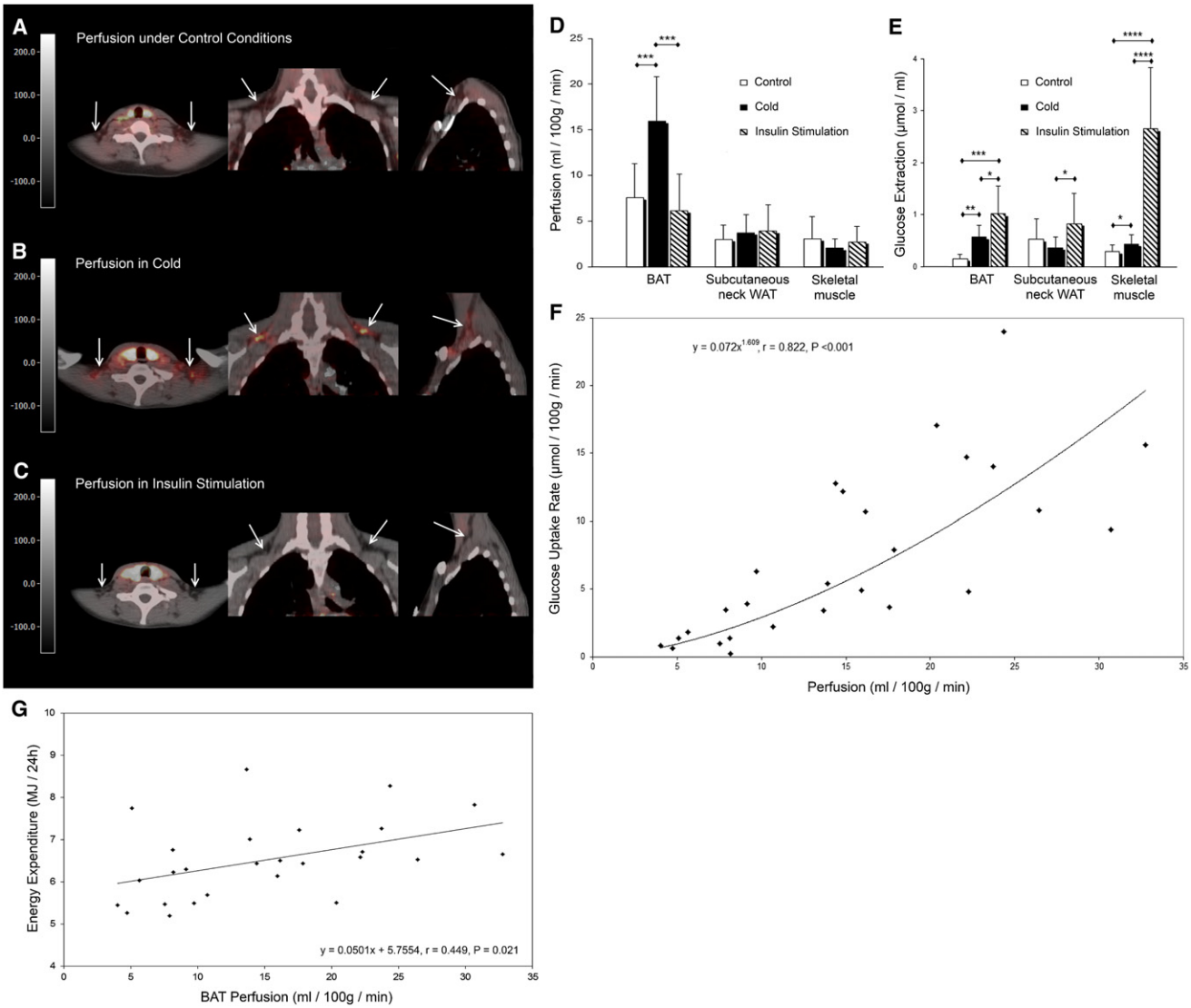

Figure 2. Perfusion and Glucose Extraction

(A) PET/CT image showing unaffected perfusion in the supraclavicular BAT under control conditions.

(B) PET/CT image after cold exposure showing increased perfusion in the supraclavicular BAT.

(C) PET/CT image showing unaffected perfusion in the supraclavicular BAT during insulin stimulation.

(D) Perfusion of BAT, subcutaneous neck WAT, and skeletal muscle determined under control conditions, during cold exposure, and during insulin stimulation. Results are expressed as means ± SD. ***$p < 0.001$.

(E) Glucose extraction of BAT, subcutaneous neck WAT, and skeletal muscle determined under control conditions, during cold exposure, and during insulin stimulation. Results are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

(F) Relationship between cold-activated perfusion and glucose uptake rate in the supraclavicular adipose tissue.

(G) Association of supraclavicular adipose tissue perfusion and whole-body energy expenditure.

versus 2.8 ± 0.7 µmol/100 g/min, p = 0.002), but these effects remained lower than in BAT (p = 0.003 and p = 0.01, subcutaneous WAT and VAT, respectively). The subjects who lacked cold-activated BAT showed insulin-mediated glucose uptake in the supraclavicular adipose tissue depot (1.9 ± 0.1 µmol/100 g/min) similar to that in subcutaneous abdominal WAT.

Relationship between Perfusion and Metabolism

BAT is highly vascularized. The blood flow provides oxygen for BAT mitochondria, and also transfers heat from BAT to other parts of the body. We quantified perfusion in supraclavicular BAT, subcutaneous neck WAT, and skeletal muscle (Figures 2A–2C).

The BAT perfusion more than doubled in response to cold (7.5 ± 3.7 versus 15.9 ± 4.9 ml/100 g/min, p = 0.0002), while no changes were observed in subcutaneous WAT (3.0 ± 1.6 versus 3.7 ± 2.0 ml/100 g/min, p = 0.18) or in skeletal muscle (3.8 ± 3.6 versus 2.0 ± 1.1 ml/100 g/min, p = 0.08) (Figure 2D). Subsequently, glucose extraction from blood was determined (Figure 2E). Cold exposure increased glucose extraction from blood

in BAT (0.1 ± 0.1 versus 0.6 ± 0.2 μmol/ml, p = 0.004), but not in subcutaneous WAT. The glucose extraction in skeletal muscle was also slightly increased by cold exposure (0.3 ± 0.1 versus 0.4 ± 0.2 μmol/ml, p = 0.03). The glucose uptake rate and perfusion in the supraclavicular adipose tissue were associated in a curvilinear fashion during cold exposure (r = 0.822, p < 0.001) (Figure 2F). Interestingly, a positive association was found between BAT perfusion and whole-body energy expenditure (r = 0.449, p = 0.021) during cold exposure (Figure 2G).

The effect of insulin on BAT perfusion and glucose extraction was also tested. Hyperinsulinemic euglycemia did not enhance perfusion in BAT (7.5 ± 3.7 versus 6.1 ± 4.0 ml/100 g/min, p = 0.38), WAT (3.0 ± 1.6 versus 3.9 ± 2.9 ml/100 g/min, p = 0.35), or skeletal muscle (3.8 ± 3.6 versus 2.7 ± 1.6 ml/100 g/min, p = 0.34) (Figure 2D). Glucose extraction was significantly increased by insulin both in BAT (0.1 ± 0.1 versus 1.0 ± 0.5 μmol/ml, p = 0.002) and in skeletal muscle (0.3 ± 0.1 versus 2.6 ± 1.2 μmol/ml, p < 0.0001) (Figure 2E).

Differences between Subjects with and without Active BAT

Concentrations of thyroid-stimulating hormone (TSH) and the thyroid hormones free triiodothyronine (T3) and free thyroxine (T4) in plasma were measured in conjunction with screening and cold exposure. TSH was slightly higher among the subjects with cold-activated BAT (2.9 ± 1.8 versus 1.7 ± 0.8 mU/l, p = 0.039). Plasma TSH concentrations usually rise with age, but in our material, subjects with active BAT tended to be younger than those without active BAT. No differences were detected in the screening values of free T4 (14.9 ± 2.2 versus 14.8 ± 2.6 pmol/l, p = 0.885) or free T3 (4.8 ± 0.6 versus 4.5 ± 0.5 pmol/l, p = 0.300). Plasma TSH was decreased in response to cold, while free plasma T3 was slightly decreased only among subjects with active BAT (Figures S1C and S1D).

During cold exposure, normalized whole-body energy expenditure tended to be higher in subjects with active BAT (6.7 ± 0.9 versus 6.1 ± 0.8 MJ/24 hr, p = 0.109).

We did not observe significant differences in whole-body insulin-stimulated glucose uptake (M value), fasting plasma glucose, oral glucose tolerance, or body mass index (BMI). Measurements are summarized in Table 1.

Gene Expression in BAT and WAT

Little is known about the expression levels of genes related to insulin signaling and glucose uptake in human BAT. Hence, we analyzed the levels of some important players in these events by quantitative real-time PCR and compared the expression levels in BAT to those in WAT (Figure 3). The only gene that was found to be differently expressed in BAT versus WAT was *GLUT4*, which was significantly more highly expressed in BAT. While a trend toward higher expression of *GLUT1* and lower expression of *IRS1* in BAT versus WAT was seen, neither *IRS2* nor *INSR* showed any signs of being differently expressed in the two tissues. The higher expression of *GLUT4* in BAT as compared to WAT might partly explain the higher insulin-stimulated increase in glucose uptake rate in BAT than in subcutaneous WAT. The *UCP1* expression levels were determined as a control for BAT versus WAT identity, and they were confirmed to be several-fold higher in BAT as compared to WAT samples (Figure 3).

Table 1. Division of Subjects by Cold Activation of BAT

	Cold-Activated BAT	No Cold Activation	P value
Age (years)	38.7 ± 10.0	41.5 ± 9.7	0.514
BMI (kg/m^2)	22.7 ± 2.2	22.7 ± 2.6	0.981
Waist (cm)	75.2 ± 8.6	77.8 ± 7.3	0.466
M value (μmol/kg/min)	47.6 ± 22.5	45. 8 ± 18.1	0.858
Plasma thyroid-stimulating hormone (mU/l)	2.9 ± 1.8	1.7 ± 0.8	0.039
Free plasma thyroxine (pmol/l)	14.9 ± 2.2	14.8 ± 2.6	0.885
Free plasma triiodothyronine (pmol/l)	4.8 ± 0.6	4.5 ± 0.5	0.300
Measurements in Cold Exposure	n = 19	n = 8	n/a
Plasma thyroid-stimulating hormone (mU/l)	2.1 ± 1.3	1.4 ± 0.5	0.220
Free plasma thyroxine (pmol/l)	14.4 ± 2.0	14.9 ± 3.3	0.730
Free plasma triiodothyronine (pmol/l)	4.5 ± 0.7	4.5 ± 0.8	0.984
Plasma norepinephrine (nmol/l)	5.5 ± 2.6	5.0 ± 1.5	0.572
Plasma DHPG (nmol/l)	14.8 ± 7.1	13.2 ± 3.9	0.543
Plasma cAMP (nmol/l)	17.7 ± 5.6	17.9 ± 4.1	0.930
Plasma insulin (mU/l)	2.2 ± 1.1	2.2 ± 0.8	0.936
Plasma lactate (mmol/l)	1.0 ± 0.4	0.9 ± 0.2	0.224
Energy expenditure (MJ/24 hr)	6.7 ± 0.9	6.1 ± 0.8	0.109
Respiratory quotient	0.83 ± 0.17	0.80 ± 0.03	0.641
Measurements under Control Conditions	n = 8	n = 4	n/a
Plasma norepinephrine (nmol/l)	2.0 ± 0.6	1.8 ± 0.3	0.645
Plasma DHPG (nmol/l)	8.0 ± 1.3	7.3 ± 1.7	0.403
Plasma cAMP (nmol/l)	13.4 ± 2.3	13.8 ± 2.5	0.790
Plasma insulin (mU/l)	5.3 ± 3.3	3.8 ± 1.3	0.403
Energy expenditure (MJ/24 hr)	5.5 ± 0.4	5.4 ± 0.5	0.655
Respiratory quotient	0.79 ± 0.03	0.82 ± 0.01	0.212
Measurements in Insulin Stimulation	n = 11	n = 3	n/a
Plasma norepinephrine (nmol/l)	2.2 ± 0.9	3.0 ± 1.5	0.299
Plasma DHPG (nmol/l)	10.1 ± 2.9	13.4 ± 7.8	0.279
Plasma cAMP (nmol/l)	13.3 ± 4.1	16.4 ± 4.1	0.263
Plasma insulin (mU/l)	68.6 ± 11.3	68.7 ± 7.8	0.997
Energy expenditure (MJ/24 hr)	6.3 ± 0.6	5.8 ± 0.3	0.200
Respiratory quotient	0.92 ± 0.03	0.93 ± 0.05	0.634

The subjects have been divided into two groups, "Cold-Activated BAT" and "No Cold Activation," based on cold exposure PET/CT findings. Values are expressed as means ± SD.

DISCUSSION

Only a few studies on the existence of BAT in healthy adults have been published. The main objective of this study was to quantify

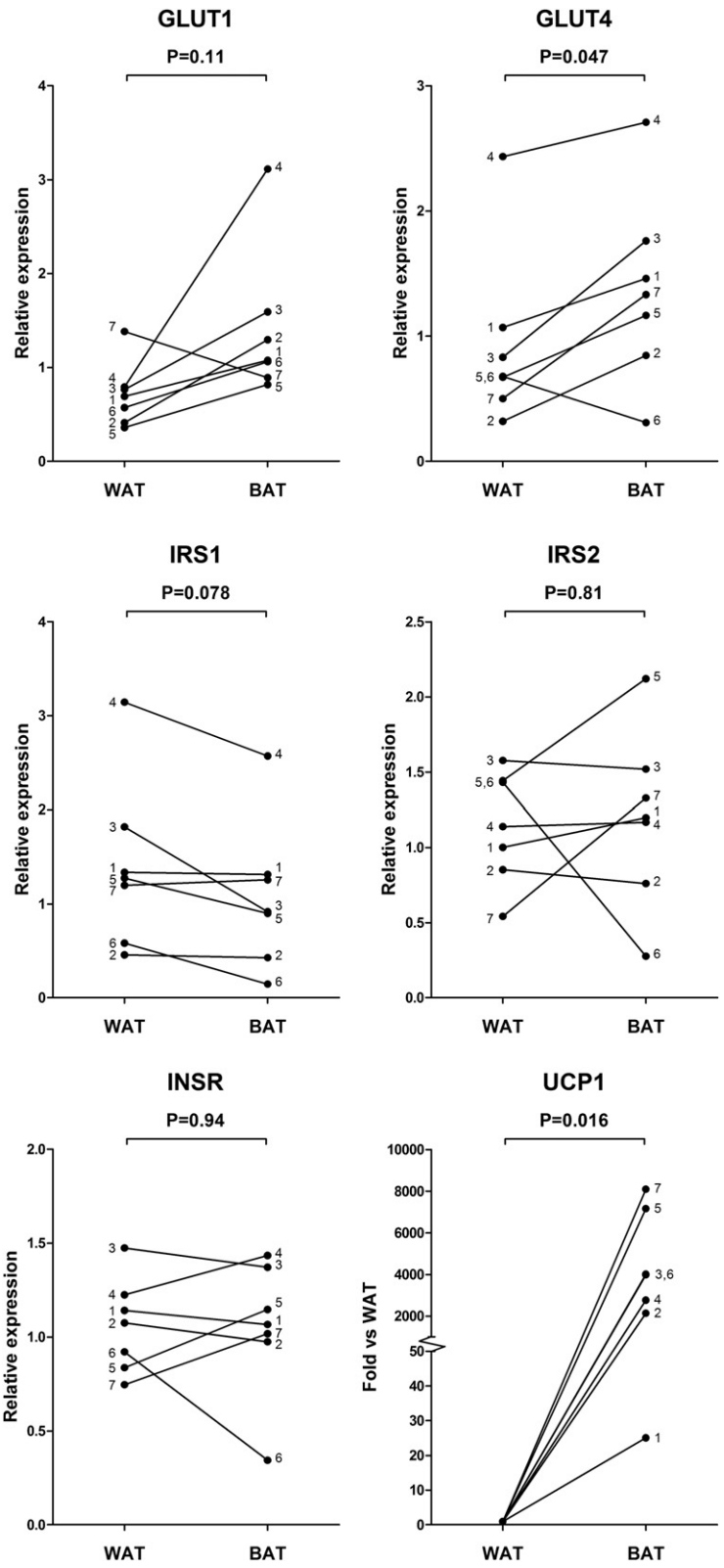

Figure 3. Expression of Glucose Uptake-Related Genes in WAT versus BAT

The relative expression levels of five genes involved in insulin signaling and glucose uptake (*GLUT1*, *GLUT2*, *IRS1*, *IRS2*, and *INSR*) were measured in WAT and BAT samples from seven subjects. To validate the BAT versus WAT identity of the samples, their *UCP1* expression levels were measured and confirmed to be several-fold higher in BAT as compared to WAT. Each of the seven subjects is labeled with a number (1–7).

BAT glucose uptake and perfusion in healthy adults and to test how these parameters respond to cold exposure and increased insulin levels.

Blood flow in the human supraclavicular BAT has not been studied before. Our observation of increased perfusion in BAT in response to cold reflects the dense vasculature of the tissue. The association we could show between BAT perfusion and glucose uptake rate is actually very similar to the response seen in exercising skeletal muscle (Pearson's $r = 0.636$, $p < 0.01$, data not shown).

We found that the concentrations of norepinephrine and its main metabolite, DHPG, were substantially increased in plasma during cold exposure. However, similar increases in these biomarkers, thought to reflect sympathetic nervous system activation, were seen both in subjects with and without cold-activated BAT. This suggests that the subjects who fail to activate BAT in response to this treatment either require more pronounced stimulation or lack BAT altogether. The latter view is supported by the finding that the subjects who lacked cold-activated BAT showed insulin-mediated glucose uptake in the supraclavicular adipose depot similar to that in subcutaneous WAT, while the BAT of subjects with cold-activated BAT was highly insulin sensitive since its insulin-stimulated glucose uptake rate was not significantly different from that of skeletal muscle.

Plasma insulin was decreased by cold exposure. This may suggest that insulin is not a significant player in the norepinephrine-driven acute cold-induced thermogenesis. It has been demonstrated using hibernoma-derived tumor cells that norepinephrine-induced expression of *UCP1* mRNA is dependent on insulin only during prolonged culture periods (Kozak et al., 1992). Studies on streptozotocin-treated mice suggest that insulin deficiency progressively decreases the thermogenic capacity of BAT (Shibata et al., 1987). Shibata et al. also showed that insulin administration does not acutely restore the thermogenic response of BAT.

Although insulin infusion stimulates glucose uptake in WAT, the glucose uptake rate remains much lower than in BAT. Our gene expression analysis of human adipose tissue showed that *GLUT4* is more abundantly expressed in BAT as compared to WAT, a feature that might partly explain why BAT seems to be a more insulin-sensitive tissue than WAT. During hyperinsulinemic euglycemia, we did not detect increased perfusion in BAT, showing that the insulin-mediated activation of BAT metabolism is not dependent on increased perfusion. This

may be because increased perfusion is not required to meet oxygen demand during insulin-stimulated thermogenesis, or because the glucose is only transported into the brown adipocytes and no thermogenesis takes place. During hyperinsulinemic euglycemia, we did not observe increased plasma norepinephrine levels, although plasma concentration of DHPG was slightly elevated. Insulin has been shown to increase plasma norepinephrine when infused at 15-fold concentration compared to our study (Kern et al., 2005), and an insulin infusion comparable to our study has been shown to increase skeletal muscle sympathetic nerve activity (Bisquolo et al., 2005). Hence, we conclude that although we did not observe increased plasma norepinephrine levels, it cannot be excluded that the sympathetic nerve activity is increased in BAT during hyperinsulinemic euglycemic clamp. It seems that the mechanism behind BAT activation by insulin infusion is distinct from cold activation, since cold exposure decreases plasma insulin concentration.

Insulin stimulates glucose uptake in brown adipocyte cell models (Klein et al., 2002) and in rodent BAT (Inokuma et al., 2005). That the presence of insulin-sensitive BAT would be beneficial for whole-body metabolism is supported by results from studies on genetically modified mice having increased amounts of BAT (Cederberg et al., 2001; Seale et al., 2011). However, skeletal muscle would clearly remain the organ determining whole-body insulin-stimulated glucose disposal rate in adult humans, even if BAT was expanded considerably. Our aim was to explore the normal physiology of BAT. Therefore, a rather homogeneous group of healthy subjects with normal glucose tolerance and a limited range of BMI were included in the study. The probability of detecting significant differences between subjects with and without active BAT was therefore rather small. Nevertheless, subjects with active BAT did tend to have higher whole-body energy expenditure during cold exposure. Furthermore, we found that perfusion in BAT is positively associated with whole-body energy expenditure during cold exposure, unlike, e.g., plasma concentrations of thyroid hormones or catecholamines (Table S1). It has been estimated that BAT thermogenesis accounts for 40% of total oxygen consumption in cold-acclimated rats during cold exposure (Foster and Frydman, 1979). Our results suggest that active BAT induces an increase in energy expenditure in a perfusion-dependent manner in humans. A recent study has shown an association between the cold-induced rise in energy expenditure and BAT activity (Yoneshiro et al., 2011). These findings indicate a role for BAT in human energy homeostasis.

Plasma TSH was found to be significantly elevated among subjects with cold-activated BAT. TSH receptors are present in adipose tissue (Sorisky et al., 2008), and interestingly, TSH receptor activation has been linked to an increase in *UCP1* expression in preadipocytes (Zhang et al., 2006). Therefore, TSH may promote formation of BAT. On the other hand, TSH concentration was decreased by cold exposure (Figure S1C), and free plasma T3 in BAT-positive subjects was also slightly decreased (Figure S1D). In contrast, active BAT in rodents appears to generate T3 for the plasma pool (Silva and Larsen, 1985). However, it should be pointed out that complex interactions between BAT and thyroid hormones are unlikely to be proven by measuring plasma concentrations of these hormones.

In conclusion, both glucose uptake and perfusion are increased in human BAT in response to cold, indicating active thermogenesis. We also show that human BAT is a highly insulin-sensitive tissue type and that its insulin-stimulated uptake of glucose is not dependent on increased perfusion. Our findings on cold-activated perfusion and insulin-mediated glucose uptake in human BAT reinforce the view that BAT plays a role in human metabolism.

EXPERIMENTAL PROCEDURES

Subjects

All subjects (age 40.2 ± 9.4 years, BMI 22.8 ± 2.2 kg/m^2, 20 females and 7 males) provided written informed consent. The study protocol was reviewed and approved by the Ethics Committee of the Hospital District of Southwest Finland, and the study was conducted according to the principles of the Declaration of Helsinki. The subjects were screened for medical history and status, and those who were healthy and had normal glucose tolerance and cardiovascular status, as assessed on the basis of routine laboratory tests, 2 hr oral glucose tolerance test, electrocardiograms, and measured blood pressure, were enrolled in the study.

Study Design

The subjects underwent two PET/CT sessions on separate days after overnight fasting. One session was performed during cold exposure and the other either with or without insulin stimulation in a warm environment. The first 12 subjects were assigned to a group without insulin stimulation and the next 15 subjects to a group receiving insulin stimulation. One subject in the latter group attended only the cold exposure session and withdrew from the study before the insulin stimulation.

On the cold exposure day, the subjects spent 2 hr wearing light clothing in a room with an ambient temperature of 17°C ± 1°C before moving into the PET/CT room, which had an air temperature of 23°C. During the PET/CT session, one foot of the subject was placed intermittently (5 min in/5 min out) in cold water at a temperature of 8°C ± 1°C. The small temperature fluctuations in the prescan room and cold water bath did not affect the glucose uptake in BAT during the PET/CT sessions (Figure S2). On the insulin stimulation day, plasma insulin was increased using a primed-continuous (1 mU · kg^{-1} · min^{-1}) infusion of insulin (Actrapid, Novo Nordisk A/S), and normoglycemia was maintained with an infusion of 20% glucose adjusted according to plasma glucose measurements (DeFronzo et al., 1979). In the control group, isotonic saline was infused instead. Whole-body energy expenditure was measured with an indirect calorimeter (Deltatrac II, Datex-Ohmeda) during the PET/CT sessions. Energy expenditure was normalized to fat-free mass, measured with a bioimpedance-based method (Omron BF400, Omron Healthcare).

Biopsy Procedure

Seven subjects provided an additional written informed consent for a biopsy of supraclavicular adipose tissue to be performed. The site of the biopsy was decided by the cold exposure [^{18}F]FDG-PET/CT image that showed activated BAT. A subcutaneous WAT sample was collected from the same incision. The biopsies were obtained under local lidocaine-epinephrine anesthesia by a plastic surgeon at normal room temperature (20°C). Immediately after removal, the tissue samples were snap-frozen in liquid nitrogen.

PET Scanning Protocol

PET/CT (GE Discovery VCT) scanning began from the clavicular region, the level of clavicles being in the middle of the axial field of view (AFOV). First, a 900 MBq bolus of [^{15}O]H_2O was administered intravenously, and a dynamic emission scan with variable frame lengths (6 × 5 s, 6 × 15 s, 8 × 30 s) was performed to determine tissue perfusion. After sufficient decay of [^{15}O]H_2O, a 185 MBq bolus of the glucose analog [^{18}F]FDG was administered, and a dynamic emission scan with variable frame lengths (1 × 1 min, 6 × 0.5 min, 1 × 1 min, 3 × 5 min, 2 × 10 min) was started simultaneously in the clavicular region. Next, the AFOV was placed more caudally on the lower thoracic region,

and a dynamic emission scan with constant frame lengths (5 × 3 min) was performed. Third, the AFOV was placed even more caudally to the abdominal region, and a dynamic emission scan was performed again with constant frame lengths (5 × 3 min).

PET Analysis

The regions of interest (ROIs) were manually outlined in the fusion image, composed of summed dynamic [^{18}F]FDG PET image and the CT image. The glucose uptake rate value was based on ROIs drawn on 2–3 adjacent transaxial planes on both lateral aspects for defined structures of supraclavicular BAT and deltoid muscle and, in the case of abdominal subcutaneous WAT, on planes superior to the umbilicus. Posterior subcutaneous neck WAT was outlined in the midline and VAT at the level of the umbilicus. Subsequently, regional time activity curves (TACs) were generated.

Regional TAC data were analyzed against current radioactivity in arterial plasma using the model of Patlak (Patlak and Blasberg, 1985). A lumped constant value of 1.14 was used for adipose tissue (Virtanen et al., 2001) and of 1.20 for skeletal muscle (Peltoniemi et al., 2000). The glucose extraction was determined by dividing the glucose uptake rate with the corresponding perfusion rate.

The method of measuring tissue perfusion with [^{15}O]H_2O is based on the principle of exchange of inert gas between blood and tissues (Kety and Schmidt, 1945). A two-compartment model including fractional arterial blood volume (de Langen et al., 2008) was linearized and fitted with the subject's whole-blood input function to PET data voxel-by-voxel using the Lawson-Hanson nonnegative least-squares technique to produce parametric perfusion images. The kinetic modeling allows exact quantification even in the presence of flow heterogeneity and nonuniform partition coefficient of water. ROIs of regional glucose uptake analysis were employed in the determination of perfusion.

RNA Preparation, cDNA Synthesis, and Quantitative Real-Time PCR

Total RNA was isolated from the attained biopsies and used for cDNA synthesis as previously published (Virtanen et al., 2009). Quantitative real-time PCR reactions were performed using the Power SYBR Green PCR Master Mix (Applied Biosystems) and an ABI PRISM 7900HT machine (Applied Biosystems). Relative gene expression levels were determined by the standard curve method using *β-actin* as the normalizing gene. The primers used in this study are found in Table S2.

Statistical Analyses

Results are expressed as means ± SD. Two-tailed paired and unpaired Student's t tests were applied to test the statistical significance of differences between the results recorded under control conditions, during cold exposure, and during insulin stimulation. Differences between subjects with and without cold-activated BAT were evaluated with unpaired t test. Gene expression levels in WAT versus BAT were analyzed using Wilcoxon matched pairs signed ranks test.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, two tables, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at doi:10.1016/j.cmet.2011.06.012.

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