High Prevalence of Brown Adipose Tissue in Adult Humans

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Context: Positron emission tomography (PET)-computed tomography (CT) has identified metabolically active supraclavicular fat in adult humans based on uptake of labeled glucose and confirmed to be brown adipose tissue (BAT) histologically. However, PET-CT has estimated a prevalence of BAT as low as 5% in adult humans, casting doubt on its significance. The true prevalence of BAT is unknown because of the suboptimal sensitivity of standard PET-CT.

Objective: The objective of the study was to determine whether BAT is present in PET-negative supraclavicular fat.

Design: This was a prospective cohort study.

Setting: The study was conducted at a tertiary referral hospital.

Patients: Seventeen patients who underwent preoperative PET-CT for staging of head and neck malignancy participated in the study.

Main Outcome: The main outcome was signature BAT gene transcripts and protein in biopsies of supraclavicular fat with sc fat as negative control.

Results: PET-CT was positive in three and negative in 14 patients. PET-positive fat harbored multilobulated lipid droplets and stained strongly for uncoupling protein 1 (UCP1). These features are absent in sc fat. By contrast, PET-negative fat contained a predominance of cells with unilobulated lipid droplets, with scattered cells containing multilobulated lipid droplets and variable UCP1 staining. Molecular analyses of fat biopsies showed lower but clear expression of UCP1, NDUFS3 (NADH dehydrogenase (ubiquinone) iron-sulfur protein 3), β_3-adrenoceptor, and PRDM16 (PR domain containing 16) transcripts.

Conclusions: BAT is present in supraclavicular fat, regardless of PET status. BAT is highly prevalent in adult humans, and its abundance determines PET status. **(*J Clin Endocrinol Metab* 96: 2450–2455, 2011)**

Brown adipose tissue (BAT) plays an important role in thermogenesis and energy homeostasis in rodents (1). It contains a unique protein, uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation, releasing energy stored in the mitochondrial proton electrochemical gradient as heat. However, the role of BAT has traditionally been considered unimportant in adult humans. It was believed to be present only in newborns and rapidly lost within the first few years of life (2).

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Abbreviations: BAT, Brown adipose tissue; BMI, body mass index; CI, confidence interval; CT, computed tomography; FDG, ^{18}F-deoxyglucose; NDUFS3, NADH dehydrogenase (ubiquinone) iron-sulfur protein 3; PET, positron emission tomography; PET+ve, PET positive; PET−ve, PET-negative; PRDM16, PR domain containing 16; SUV, standard uptake value; UCP1, uncoupling protein 1.

	PET positive	PET negative
N	3	14
Female	3	1
Age (years)	33 ± 8	67 ± 4
BMI (kg/m^2)	22.2 ± 1.0	25.3 ± 1.1
SUV	2.5 ± 0.3	0.1 ± 0.0

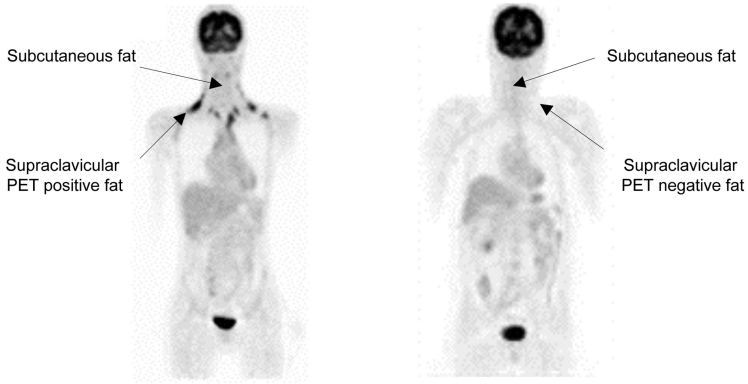

FIG. 1. PET-guided fat biopsy. Patient characteristics and diagram indicates locations of intraoperative adipose tissue biopsies from patients with positive and negative PET-CT, respectively.

Recently this view has been challenged by functional imaging using ^{18}F-deoxyglucose (FDG)-positron emission tomography (PET)-computed tomography (CT), which has revealed adipose tissue of high metabolic activity located mainly in the cervical and supraclavicular areas in adult humans (3). Histological examination has confirmed FDG avid depots to be BAT, based on the presence of UCP1-positive adipocytes with multilobulated lipid droplets (4–7). The observation that body mass index (BMI) is lower among those with positive PET scans has suggested a physiological role of BAT in regulating energy homeostasis in humans (4, 8).

Most studies to date have reported a prevalence of BAT of 5–10% in adult humans (9–15). However, sensitivity and reproducibility of PET-CT performed at ambient temperature are low (4). We hypothesized that BAT persists in adulthood and that its true prevalence is underestimated by metabolic imaging. The true prevalence of BAT in adult humans can be determined only from direct histological analysis of neck adipose tissue. Our aim was to determine whether BAT is present in PET-negative but in lesser abundance than in PET-positive individuals.

Subjects and Methods

Subjects

We recruited 17 consecutive patients with known head and neck malignancies from St. Vincent's Hospital (Sydney, Australia) who required PET-CT for staging before surgery. All patients were euthyroid biochemically and none was taking medications known to affect BAT activity (*e.g.* β-blockers and tranquilizers). We compared the histological and molecular characteristics of adipose tissue obtained from defined sites (see below). Patients were stratified as PET-positive (PET+ve) (n = 3) and PET-negative (PET−ve) (n = 14) patients, based on FDG-PET-CT imaging. St. Vincent's Hospital Humans Research Ethics Committee approved the study. All participants provided consent to the approved study.

Biopsy procedures

Standard preparation for a PET-CT study involved a 6-h fast before iv administration of 380–400 MBq FDG. The patients rested quietly in a room for 60 min at a temperature of approximately 20–22C before moving to the scanning room. Guided by PET-CT (Fig. 1), open biopsies were obtained intraoperatively from the supraclavicular fossae within fat delineated by CT under direct PET-CT guidance, within a region bounded by the sternocleidomastoid muscle anteriorly, sternoclavicular joint caudally, anterior edge of posterior scalenus muscle posteriorly, and lateral edge of posterior scalenus laterally. A positive scan was defined by a maximal standard uptake value (SUV) of FDG of at least 2.0 g/ml within fat tissue of at least 4 mm in diameter, delineated by CT. PET+ve and PET−ve adipose fat were therefore obtained from the same anatomical location (*i.e.* supraclavicular fossae) and differed only by SUV. UCP1 protein and gene transcripts were not detected in sc fat in previous studies (4, 6, 7). A separate biopsy of sc fat in the midline region was therefore obtained from each patient as negative control. Each fat sample was divided into two pieces. One was fixed in 10% formalin for histological examination. The other piece was snap frozen in liquid nitrogen in preparation for mRNA extraction to prepare cDNA for use in quantitative RT-PCR analysis.

Definition of BAT

BAT was defined histologically by the presence of multilobulated lipid droplets or UCP1 staining. BAT is characterized by high level of expression *UCP1*, β_3-adrenoceptor (*ADRB3*), NADH dehydrogenase (ubiquinone) iron-sulfur protein 3 (*NDUFS3*), and PR domain containing 16 (*PRDM16*) (6, 7). We compared the expression of these transcripts as well as β_1-adrenoceptor *(ADRB1)* and β_2-adrenoceptor *(ADRB2)* in supraclavicular and sc fat obtained from PET+ve and PET−ve patients.

Analysis of mRNA levels

RNA collection

RNA was extracted from whole adipose tissue or cultured adipocytes using Trizol/RNeasy mini spin columns (QIAGEN, Doncaster, Australia) and further purified with ethanol precipitation. Average yield was 5–7 μg per 100 mg whole tissue. RNA

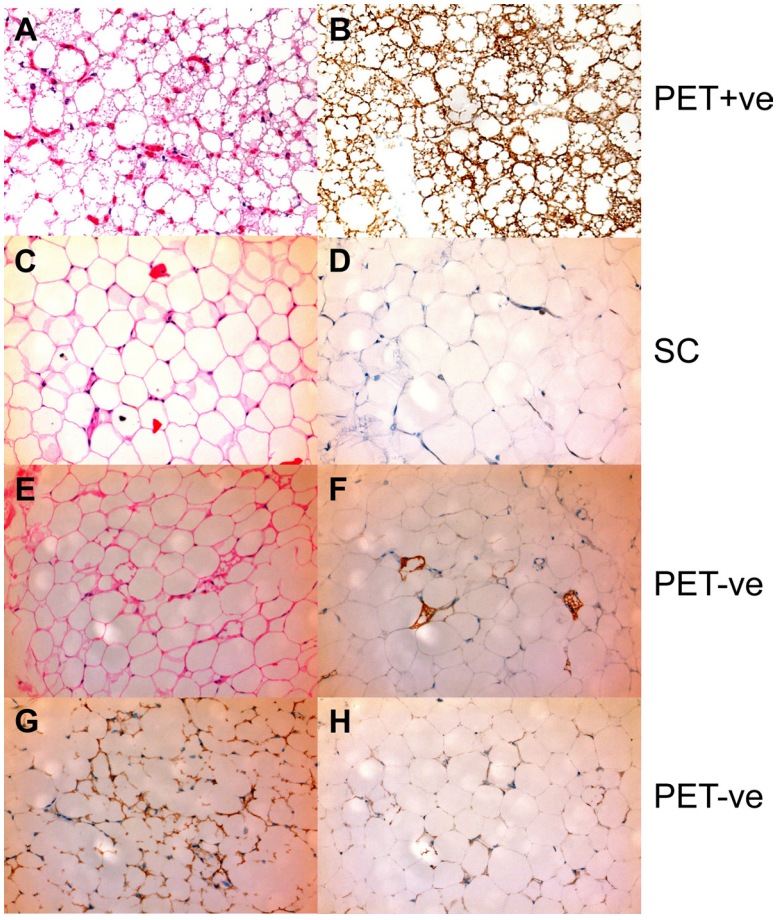

FIG. 2. Histologic analyses (× 20) of PET+ve, PET−ve supraclavicular and sc adipose tissue. Sections of PET+ve fat [A: hematoxylin and eosin (H&E); B: UCP1 immunohistochemistry] showed adipocytes with multilobulated lipid droplets stained strongly for UCP1. Adjacent sc fat showed adipocytes with unilobulated lipid droplets negative for UCP1 (C: H & E; D: UCP1). PET−ve fat showed predominantly cells with unilobulated lipid droplets interspersed with islands of cells with mutilobulated fat droplets (E: H & E) with UCP1 staining (F–H: UCP1).

concentration and quality was assessed by NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

Quantitative RT-PCR

For quantification of target gene mRNA levels, 2 μg of RNA was reverse transcribed with a high-capacity cDNA kit (Applied Biosystems, Foster City, CA) in a total volume of 20 μl. Due to sample limitation, original cDNA was preamplified using Taqman PreAmp master mix (Applied Biosystems) following the manufacturer's instructions. Standard TaqMan cycling conditions were used with TaqMan gene expression assays using the Applied Biosystems 7900HT (Applied Biosystems). All reactions were performed in triplicate.

To control for variability in amplification due to differences in starting mRNA concentrations, β-actin was used as an internal standard. The relative expression of target mRNA was computed from the target cycle threshold values and the β-actin cycle threshold value using the standard curve method (sequence detection systems chemistry guide; Applied Biosystems).

To check for preamplification uniformity, relative quantification was repeated with the *UCP1* TaqMan Gene Expression Assay to compare amplification of cDNA to amplification of preamplified cDNA using the $\Delta\Delta C_T$ method, as described by the manufacturer.

Immunohistochemistry

Adipose tissue biopsies were fixed in 10% formalin and molded in paraffin. Tissue sections were stained with hematoxylin-eosin for morphology. Sections for immunohistochemistry were subjected to heat-induced antigen retrieval. After washing in PBS, sections were blocked for 1 h in 1% BSA and 0.1% Tween 20 in PBS, and incubated with primary antibody [rabbit polyclonal to UCP1 (1:500, Abcam, Cambridge, UK)]. This was followed by 3 × 10-min washes in PBS and incubation with secondary antibody. Sections were sealed with coverslips and dried for 10 min. Slides were imaged using an Eclipse E800 microscope (Leica, Heerbrugg, Switzerland). The specificity of the UCP1 antibody for BAT was validated by antibody omission and by the finding of absent staining in skeletal muscle biopsies obtained from the anterior neck using the same protocol (data not show). Abundance of UCP1-positive cells was determined in each subject by counting across five ×20 fields; 525 ± 12 cells were counted per subject. Results are expressed as percentage UCP1-immunoreactive adipocytes of the total number of counted adipocytes.

Statistical analysis

The data were analyzed using SPSS Statistics version 17 (SPSS Inc., Chicago, IL). Results are presented as mean ± SEM. Differences in continuous variables were analyzed by the unpaired *t* test. Data not normally distributed were log transformed before statistical analysis. $P < 0.05$ was considered statistically significant.

Results

We studied 17 patients who underwent PET-CT. Three had a positive PET-CT. SUV of PET+ve fat were on average 2- to 3-fold higher than those of PET−ve fat. The SUV of PET−ve and sc fat were similar (0.1 ± 0.0 *vs.* 0.1 ± 0.0, P = NS).

On histological analysis, all PET+ve fat showed cells containing multilobulated lipid droplets (Fig. 2A), characteristic of BAT. These cells displayed strong immunoreactivity for UCP1 (Fig. 2B). Gene expression analysis revealed high levels of *UCP1* and *NDUFS3* mRNA, more than 4 orders higher compared with sc fat ($P < 0.001$). *PRDM16* expression was also significantly higher ($P < 0.001$), as were those of *ADRB3* (Fig. 3A-C). The abundance of *ADRB1* and *ADRB2* mRNA transcripts was not different between PET+ve and sc fat or between PET+ve and PET−ve fat (Fig. 3B).

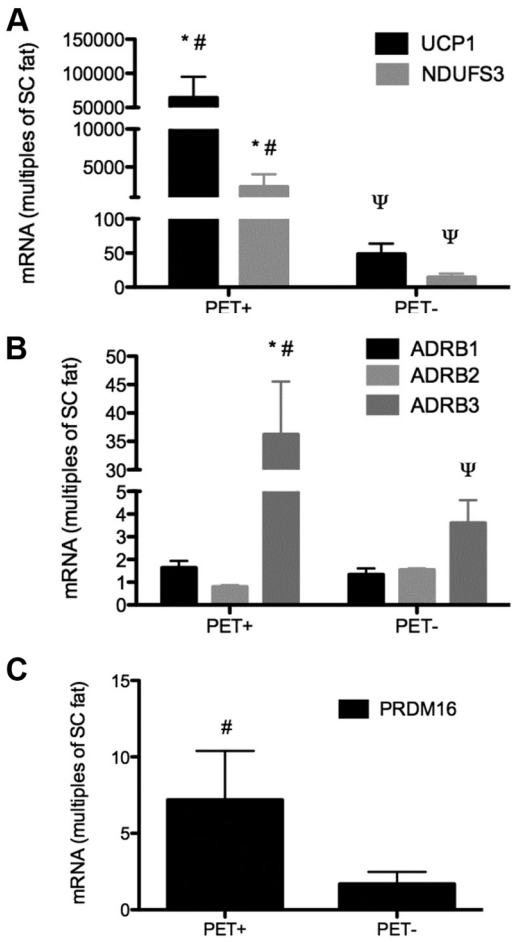

FIG. 3. Gene expression analyses of PET+ve and PET−ve supraclavicular fat. The mean levels of expression of *UCP1* and *NDUSF3* (A); *ADRB1*, *ADRB2*, and *ADRB3* (B); and *PRDM16* in PET+ve and PET−ve fat (C) are expressed as multiples of the corresponding transcripts in sc fat, obtained by quantitative real-time PCR analysis. *, $P < 0.01$ compared with PET−ve fat; #, $P < 0.001$ compared with SC fat; ψ, $P < 0.01$ compared with sc fat.

Histological analysis of sc fat revealed cells with unilobulated lipid droplets (Fig. 2C), with no UCP1 staining (Fig. 2D). The expression levels of *UCP1*, *NDUFS3*, and *ADRB3* were barely detectable in sc fat. Subcutaneous fat displays characteristic of white adipose tissue.

Histological analysis of PET−ve fat revealed a mixed appearance characterized by a predominance of cells with unilobulated lipid droplets, characteristic of white adipose tissue, indistinguishable from sc fat, interspersed with distinct islands of cells with multilobulated lipid droplets (Fig. 2E). Immunohistochemistry demonstrated these islands of cells to be positive for UCP1 (Fig. 2, F–H). In three samples, staining was evident among single cells with multilobulated lipid droplets (Fig. 2F). From 14 PET−ve fat biopsies, UCP1-positive cells were identified in 13 samples. The proportion of UCP1-positive cells from supraclavicular fat ranged from 3 to 28% in PET−ve, compared with 95–100% in PET+ve fat.

All 14 PET−ve samples contained higher levels of expression of *UCP1*, *NDUFS3*, and *ADRB3* mRNA, compared with sc fat. In PET−ve fat, the transcript abundance of *UCP1* was on average 49.8 times greater [95% confidence interval (CI) 20.5, 80.2; $P < 0.01$], *NDUFS3* 4.4 times higher (95% CI, 3.9, 24.6; $P < 0.01$), and *ADRB3* was 3.6 fold higher (95% CI, 2.4, 6.9; $P < 0.01$) than in sc fat (Fig. 3, A–C). However, *PRDM16* expression was not increased compared with sc fat.

In the 17 patients studied, BMI correlated negatively with *UCP1* mRNA abundance in supraclavicular fat ($R^2 = 0.44$, $P = 0.004$).

Discussion

This study evaluated the prevalence of BAT in supraclavicular fat of adult humans by systematic histological examination and molecular analysis in 17 patients, 14 of whom were PET−ve after FDG imaging. Signature transcripts of BAT were present in all patients, whereas UCP1 immunoreactivity was detected in all but one PET−ve patient. The abundance of brown adipocytes was 50–70% lower in PET−ve fat compared with PET+ve fat. The results show BAT to be present in all patients with abundance strongly associated with PET status.

The high prevalence stands in marked contrast to the low frequency of 5–10% reported from PET-CT studies (9–15). We recently reported that PET-CT imaging undertaken in ambient conditions is poorly reproducible and insensitive (4). Among patients with BAT, the probability of a PET scan being positive was only 13%. The extrapolated prevalence of 64% from that study markedly underestimates the universal presence of BAT uncovered in the present study.

BAT is regulated by the sympathetic nervous system. Animal studies have demonstrated that the proliferation and differentiation of brown adipocytes are stimulated by noradrenaline (1). The β-adrenergic system also stimulates the biogenesis of mitochondria; expression of NDUFS3, a component of the respiratory chain (16); and UCP1 in BAT. Recent studies on the cellular origin of BAT have identified novel regulators. PRDM16, a zinc finger protein is a pivotal transcriptional regulator of the developmental fate of precursor adipomyocyte (17). The results showed that the genes that define BAT function in rodents, such as *UCP1* and *NDUFS3*, those governing differentiation, development, and proliferation, such as *PRDM16* and *ADRB3*, are similarly found in human BAT.

The detection of significant abundance of *ADRB* transcripts in supraclavicular adipose tissue suggests that in humans the sympathetic nervous system plays an impor-

tant regulatory role in brown fat biology, as it does in rodents (1). The critical role of ADRB3 in modulating BAT function is well established. The identification of both *ADRB1* and *ADRB2* in both PET+ve and PET−ve fat suggests that these two ADRB subgroups may regulate BAT activity in humans, in addition to the dominant role of ADRB3 in BAT. Evidence supporting a role for ADRBs other than ADRB3 comes from the observation that propranolol, a β-blocker with poor ADRB3 affinity at therapeutic doses, completely abolishes FDG uptake in BAT on PET-CT (18). Recent evidence also suggests an interplay between skeletal muscle and BAT in mediating cold-induced thermogenesis and propranolol does not inhibit cold-induced thermogenesis completely in humans (19). Collectively these findings suggest ADRB1 and ADRB2 play an underappreciated regulatory role in thermogenesis and in human BAT and may explain the lack of success of β_3-agonists in the activation of human BAT (20, 21).

There are limitations in our study. First, the investigations were restricted to patients with head and neck malignancies in whom indications for PET-CT imaging offered the opportunity to correlate metabolic function to histology. This resulted in inevitable heterogeneity in patient characteristics, such as age, gender, BMI, and dietary factors. Rigorous control of these factors may have changed the detection frequency of BAT by metabolic imaging but unlikely to have yielded positive scans in all patients. However, the presence of BAT transcripts in all 17 patients with a heterogeneous clinical history provides strong evidence that BAT is present in vast majority of adult humans.

Second, we cannot exclude the possibility that malignancy may trigger the proliferation and activation of BAT in otherwise BAT-negative fat. However, this is unlikely to be a confounding factor because we did not observe an association between active malignancy and the presence BAT in our previous investigation in more than 3000 patients with a history of malignant disease (4). Confirmation that BAT is highly prevalent in the general population requires the undertaking of similar studies in healthy individuals. However, radiation exposure from PET imaging precludes such studies being undertaken in normal healthy subjects. The radiation exposure from a single conventional PET-CT study exceeds the limits set by the Australian Radiation Protection and Nuclear Safety Agency. The paucity of human data in the field reflects the ethical and logistic challenges posed by undertaking BAT research in otherwise normal human subjects. Despite these limitations, our finding of a high prevalence provides evidence supporting a potentially important metabolic role of BAT in adult life.

Our findings suggesting the almost universal presence of BAT by histologic analysis, regardless of FDG uptake, is supported by cold stimulation studies. In a study of 24 healthy men, 23 individuals (96%) returned a positive PET-CT after cold exposure (7). Based on the discrepancies in prevalence estimates of BAT between ambient and cold condition, it can be estimated that cold stimulation converts more than 80% of negative PET-CT to positive scans. What determines conversion of a negative to a positive PET-CT after acute cold exposure? Our histological-functional evaluation indicates that there is 15- to 20-fold more BAT in PET+ve than in PET−ve fat. Thus, it is likely that cold exposure stimulates activity by at least the same magnitude since proliferation is unlikely to occur after such a brief duration of cold exposure. The large capacity for BAT adaptation would suggest the possibility of pharmacological stimulation to enhance BAT activity as an approach for obesity treatment. The finding of a significant negative correlation between BMI and *UCP1* mRNA level in our study supports a causative link between BAT thermogenic potential and whole-body adiposity.

In summary, BAT is present in supraclavicular fat and absent in sc fat in all patients, regardless of imaging status. Previous evidence suggesting a high prevalence of BAT in adult humans has been indirect and circumstantial. Our study is the first to provide definitive histological and molecular evidence supporting the universal presence of BAT in adult humans. BAT detection by PET-CT under standard ambient conditions is determined by the abundance of BAT. Fat in the supraclavicular fossa displays a spectrum of BAT abundance ranging from scattered to uniform composition of brown adipocytes. We conclude that BAT is present in the majority of, if not all, adult humans. The conversion of PET−ve to PET+ve supraclavicular fat indicates enormous BAT activity capacity, which may be harnessed pharmacologically for the treatment of obesity.

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