Effect of Nicotine and Ephedrine on the Accumulation of ^{18}F-FDG in Brown Adipose Tissue

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This study evaluated the effect of various β-adrenergic agonists on ^{18}F-FDG uptake in brown adipose tissue (BAT) in rats using ex vivo biodistribution studies. **Methods:** Caffeine (10 mg/kg of body weight, $n = 4$), ephedrine (5 mg/kg of body weight, $n = 4$), nicotine (0.8 mg/kg of body weight, $n = 9$), or a mixture of nicotine and ephedrine (0.8 mg/kg of body weight and 5 mg/kg of body weight, respectively, $n = 9$) was injected into the peritoneal cavity of female Lewis rats 30 min before intravenous ^{18}F-FDG injection. One hour after injection of ^{18}F-FDG, the animals were sacrificed, and BAT, other major organs, and blood were extracted. The biodistribution results were compared with body temperature data. **Results:** In the rats injected with nicotine or ephedrine, the mean uptake of ^{18}F-FDG, in percentage injected dose (%ID)/(g of interscapular BAT) × (kg of body weight), was significantly increased (7.9-fold for nicotine and 3.7-fold for ephedrine), compared to the control rats. Nicotine had the strongest effect on ^{18}F-FDG uptake in BAT. Caffeine increased BAT uptake slightly, but this increase did not reach statistical significance. The combination of nicotine and ephedrine increased the uptake 12.0-fold, compared with control rats; more than either nicotine or ephedrine alone. Uptake of ^{18}F-FDG in most other major organs did not change significantly. The effect of nicotine was blocked by prior injection of β-adrenergic antagonists. A transient decrease in body temperature was observed in the nicotine-injected group, and this effect was canceled by prior injection of β-adrenergic antagonists. No significant change in baseline temperature was seen before or after β-adrenergic agonist injection. **Conclusion:** Nicotine caused a greater increase in ^{18}F-FDG uptake in BAT than did other interventions, and the effect was increased when nicotine was combined with ephedrine. The effect of nicotine was completely blocked by prior injection of β-adrenergic antagonists, indicating that β-adrenergic agonists increase the metabolism of BAT. These preclinical data suggest that patients should avoid nicotine and ephedrine before undergoing ^{18}F-FDG PET to minimize ^{18}F-FDG uptake in BAT.

Brown adipose tissue (BAT) is located mainly in the supraclavicular region and has thermogenic activity different from that of white adipose tissue. ^{18}F-FDG uptake in the posterior neck was initially reported in 1996 (1). However, it was only after the introduction of PET/CT fusion technology that this focal uptake was recognized to be not in muscle but in fat—likely BAT (2,3).

^{18}F-FDG uptake in BAT may sometimes be indistinguishable from uptake in tumors or lymph nodes. Tumor or metastatic lymph node ^{18}F-FDG uptake may be masked by high ^{18}F-FDG uptake in BAT, leading to false-negative interpretations and possibly incorrect patient management. Castelluci et al. reported that ^{18}F-FDG uptake in BAT is the most common nontumoral site of focal intense uptake seen in follow-up studies of patients treated for lymphoma (4). Increased uptake in the supraclavicular region has been reported to occur in 2.5%–4.0% of patients undergoing ^{18}F-FDG PET/CT studies (3). It is more common in women and is likely more common in winter (2,3). Understanding the function of BAT and the factors that influence BAT activity are important for the interpretation of ^{18}F-FDG PET/CT studies. Low ^{18}F-FDG uptake in BAT is desirable for optimal PET interpretation.

BAT differs from white adipose tissue morphologically. BAT is so named because of its brownish color on direct visual inspection, a color that results from its rich vascularization and increased mitochondrial content. BAT is generally located in deep cervical regions, including the supraclavicular area, the interscapular and paravertebral regions, the upper abdomen, and areas near large vessels. Functionally, BAT is characterized by a unique metabolic pathway that results in the generation of heat. In BAT, oxygen consumption by mitochondria is not coupled with

adenosine triphosphate synthesis, but the energy that is released from the oxidation of reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate is completely converted to heat. BAT is important as a thermogenic organ by producing heat to maintain body temperature for many hibernating mammals, especially the young.

Heat production by BAT is triggered by the activation of the sympathetic nervous system. Norepinephrine released from sympathetic nerve terminals binds to β3-adrenergic receptors on the surface of BAT and leads to β-oxidation of free fatty acids. Norepinephrine, independent of insulin, also activates BAT glucose transport by glucose transporter-1 and potentially by glucose transporter-4 (5). Uncoupling protein-1 is critical for the upregulation of glucose use and heat production by BAT. The study of ^{18}F-FDG uptake into BAT has the potential to be a good tool for evaluating BAT activity.

We previously demonstrated that BAT activity is reduced by brief β-blocker interventions (6). In this study, we evaluated ^{18}F-FDG uptake in the BAT of rats using ex vivo biodistribution and applied core temperature monitoring to determine the effect of various β-adrenergic agonists on BAT activity. All the pharmacologic agents used in this study were previously reported to be activators of the sympathetic nervous system. Caffeine is an alkaloid that increases the activity of the sympathetic nervous system. Ephedrine is a sympathetic nerve stimulant that evokes thermogenesis and is used as a drug for weight loss, which also requires the presence of sympathetic nervous activity (7). Yoshida et al. reported that nicotine increases norepinephrine turnover and thermogenesis in BAT in mice (8). The original impetus to perform this trial stemmed from the clinical observations of one of the authors, David L. Lilien, of an intense pattern of ^{18}F-FDG uptake in the supraclavicular area fat of a clinical patient who had evidence of severe and ongoing nicotine use immediately before ^{18}F-FDG PET.

MATERIALS AND METHODS

Animals

Eight- to 9-wk-old female Lewis rats (mean body weight, 197 g) were used in this study. Rats have a relatively large amount of BAT in the interscapular region (9). All animals were kept in animal housing facilities for at least 1 wk before the start of the experiments. Food and water were given ad libitum. This study was performed as part of a project approved by the animal research committee at the Johns Hopkins Medical Institutions.

Monitoring of Body Temperature

The body temperature of the rats was monitored using a telemetric system (Vital View; Mini Mitter Co., Inc.). A signal transmitter (PDT-400 E-Mitter; Mini Mitter) was transplanted into the peritoneal cavity of rats while they were under anesthesia, and the rats were used for BAT studies 3–4 wk later. Body temperature was measured every 30 s during the study. The E-Mitter charges a battery from the radiofrequency field produced by a receiver and

transmits the data automatically to a computer. Using this system, body temperature data from rats can be collected noninvasively before and during the experiment.

We also checked the variance among the E-Mitter signal transmitters. For this purpose, we put the devices into water baths of various temperatures and compared the results with the readings of a mercury thermometer. All values from the 8 E-Mitter devices were within ±0.5°C of the measurements obtained with the thermometer.

Pharmacologic Intervention

Either caffeine (10 mg/kg of body weight), ephedrine (5 mg/kg of body weight), nicotine (0.8 mg/kg of body weight), a mixture of nicotine and ephedrine, or a saline control was injected by intraperitoneal injection in the same volume (100 μL). The drug or saline control was administered 30 min before intravenous injection of ^{18}F-FDG. For studies with β-adrenergic antagonists, we used reserpine (4 mg/kg of body weight) intraperitoneally 4 h before ^{18}F-FDG injection or propranolol (5 mg/kg of body weight) intraperitoneally 50 min before ^{18}F-FDG injection. All pharmacologic agents were warmed to 37°C in a water bath before administration to prevent them from cooling the body. The dose and time of injection for each drug were determined according to published articles reporting the expected time of onset and maximal pharmacologic effects on target tissues (6,10–15).

Biodistribution Study

After fasting overnight, all rats received a 7.4-MBq (200-μCi) injection of ^{18}F-FDG via the tail vein without anesthesia. All rats were sacrificed 60 min after ^{18}F-FDG injection. Blood, major organs (including back and leg muscles), interscapular BAT, and white adipose tissue surrounding BAT were removed and weighed. The radioactivity in each organ was counted using a γ-counter. The percentage injected dose (%ID) per gram of tissue, standardized by kilogram of body weight (%ID/[g of tissue] × [kg of body weight]), was calculated and compared among tissues. All procedures were performed at room temperature in a climate-controlled room.

Statistical Analysis

The data, calculated as %ID/(g of tissue) × (kg body weight), for the groups receiving the drugs were compared with the control group using the Mann–Whitney U test. A P value of less than 0.05 was considered statistically significant.

RESULTS

The rats were divided into 5 experimental groups that included the control group (saline injection, 7 rats), caffeine group (4 rats), ephedrine group (4 rats), nicotine group (9 rats), and combination group (nicotine and ephedrine, 9 rats). In the control group, BAT uptake was 0.073 %ID·kg/g—lower than uptake in other major organs.

Ephedrine and nicotine increased the ^{18}F-FDG uptake 3.7- and 7.9-fold, respectively, over uptake in the control group. In the groups injected with ephedrine and nicotine, the increase in BAT uptake achieved statistical significance (both: $P < 0.05$). The combination of ephedrine and nicotine increased uptake of ^{18}F-FDG in BAT to a level greater than that reached by ephedrine or nicotine alone ($P < 0.05$). The combination was 12-fold greater than in the control group ($P < 0.05$), and uptake of ^{18}F-FDG was higher in BAT than in any other measured organ (Fig. 1). The mean %ID/g × (kg
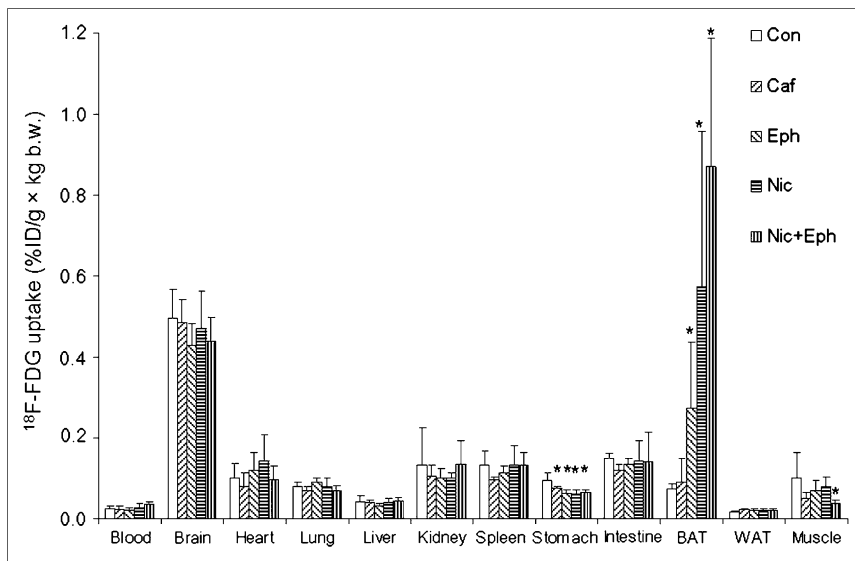
FIGURE 1. Groups of rats ($n = 4$ or $n = 9$) received a different pharmacologic agent 30 min before administration of ^{18}F-FDG. No major changes in ^{18}F-FDG uptake (mean ± SD) were seen in major organs except for BAT. Caffeine-injected group showed slight increase in uptake. Ephedrine, nicotine, and their combination displayed a significant increase in ^{18}F-FDG uptake. b.w. = body weight; Con = control; Caf = caffeine; Eph = ephedrine; Nit = nicotine; WAT = white adipose tissue. *$P < 0.05$.

of body weight) in BAT was 1.24-fold greater in the caffeine-injected group than in the control group, but this value did not achieve statistical significance ($P = 0.60$). No significant change in ^{18}F-FDG uptake was seen in other major organs except for stomach, which showed lower uptake in the stimulated groups than in the controls ($P < 0.05$).

Next, we added 2 other experimental groups. In one group ($n = 4$), reserpine was administered 4 h before the ^{18}F-FDG injection in an attempt to counteract the effects of nicotine on BAT. In the second group ($n = 4$), propranolol was injected 50 min before the ^{18}F-FDG injection to evaluate its inhibitory effect on nicotine-induced BAT stimulation. The %ID/g × (kg of body weight) data in the above-described 7 experimental groups are shown in Figure 2. In the reserpine and propranolol groups, ^{18}F-FDG uptake in BAT was substan-
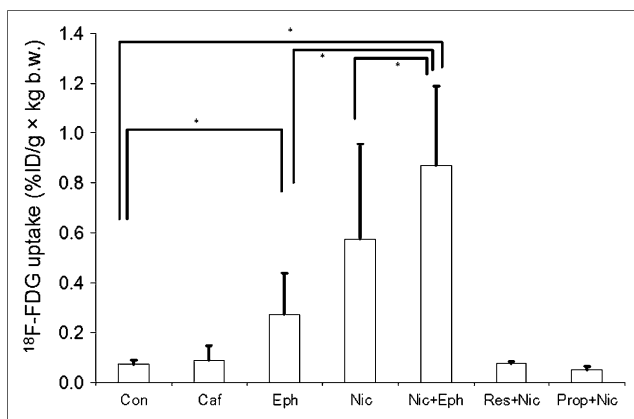
tially reduced to the same level as in the control group, in comparison to the nicotine-alone group ($P < 0.05$). Changes in ^{18}F-FDG uptake were observed in various tissue samples other than BAT in the drug administration groups. Uptake in heart and white adipose tissue was lower in the propranolol group than in the control groups ($P < 0.05$). Low uptake in the stomach was observed in the caffeine, ephedrine, nicotine, and nicotine plus ephedrine groups (each: $P < 0.05$). Low uptake in muscle was observed in the nicotine plus ephedrine group ($P < 0.05$) (Table 1).

Body temperature changes associated with pharmacologic interventions were observed in the Mini Mitter data. In the nicotine-injected group, a temporary decrease in core body temperature was observed in all rats immediately after injection. Rat body temperature decreased to a nadir at about 18–24 min after injection and gradually increased during the hour after the nadir. The temperature decrease was 0.47°C–2.25°C (average, 1.22°C), compared with baseline temperatures (average body temperature during 1 h before nicotine injection). No temperature change was observed in the control group. In some rats, core body temperature continued to increase and rise above baseline levels. In the group of rats treated with propranolol before nicotine administration, a temporary decrease in body temperature was not observed.

The relationship between tissue uptake as measured by %ID/g × (kg of body weight) and change in body temperature is shown in Figure 3. A change in body temperature was defined as follows: baseline (average body temperature during 1 h before pharmacologic administration) minus the lowest measured body temperature during the 30 min after pharmacologic intervention. Each experimental group exhibited a characteristic distribution of ^{18}F-FDG in BAT. Positive correlations between a drop in body temperature and uptake in BAT were seen only in the nicotine plus ephedrine groups (correlation coefficient, 0.721).

FIGURE 2. ^{18}F-FDG uptake (mean ± SD) in control group was low. Ephedrine and nicotine administration increased BAT uptake significantly (*$P < 0.05$). Pretreatment with propranolol and reserpine completely blocked BAT activation by nicotine (*$P < 0.05$). b.w. = body weight; Con = control; Caf = caffeine; Eph = ephedrine; Nic = nicotine; Res = reserpine; Prop = propranolol.

TABLE 1
Result of Biodistribution Studies

Site	Con (n = 7)	Caf (n = 4)	Eph (n = 4)	Nic (n = 9)	Nic + Eph (n = 9)	Res + Nic (n = 4)	Pro + Nic (n = 4)
Blood	0.026 ± 0.004	0.024 ± 0.006	0.022 ± 0.007	0.029 ± 0.008	0.035 ± 0.007	0.018 ± 0.002	0.034 ± 0.007
Brain	0.496 ± 0.070	0.484 ± 0.056	0.428 ± 0.054	0.470 ± 0.092	0.441 ± 0.059	0.541 ± 0.026	0.429 ± 0.034
Heart	0.101 ± 0.034	0.081 ± 0.032	0.120 ± 0.046	0.143 ± 0.064	0.096 ± 0.032	0.133 ± 0.026	0.034 ± 0.007*
Lung	0.079 ± 0.010	0.068 ± 0.011	0.090 ± 0.012	0.080 ± 0.020	0.069 ± 0.013	0.093 ± 0.008	0.068 ± 0.008
Liver	0.043 ± 0.012	0.039 ± 0.008	0.031 ± 0.005	0.041 ± 0.009	0.044 ± 0.008	0.042 ± 0.006	0.052 ± 0.007
Kidney	0.131 ± 0.092	0.105 ± 0.026	0.101 ± 0.024	0.101 ± 0.011	0.134 ± 0.058	0.087 ± 0.012	0.092 ± 0.008
Spleen	0.132 ± 0.036	0.095 ± 0.008	0.113 ± 0.016	0.132 ± 0.049	0.132 ± 0.032	0.146 ± 0.015	0.096 ± 0.014
Stomach	0.095 ± 0.018	0.074 ± 0.006*	0.063 ± 0.009*	0.061 ± 0.010*	0.065 ± 0.006*	0.084 ± 0.006	0.088 ± 0.009
Intestine	0.149 ± 0.013	0.119 ± 0.014	0.135 ± 0.013	0.145 ± 0.047	0.141 ± 0.073	0.155 ± 0.008	0.175 ± 0.011
Muscle	0.100 ± 0.063	0.050 ± 0.017	0.067 ± 0.026	0.080 ± 0.024	0.038 ± 0.009*	0.076 ± 0.009	0.051 ± 0.015
WAT	0.017 ± 0.002	0.023 ± 0.003	0.022 ± 0.004	0.020 ± 0.005	0.020 ± 0.005	0.023 ± 0.004	0.015 ± 0.001*
BAT	0.073 ± 0.015	0.090 ± 0.058	0.272 ± 0.164*	0.574 ± 0.382*	0.870 ± 0.319*	0.128 ± 0.016	0.096 ± 0.017

*$P < 0.05$ vs. control.

Con = control; Caf = caffeine; Eph = ephedrine; Nic = nicotine; Res = reserpine; Pro = propranolol; WAT = white adipose tissue.
Data are mean (±SD) %ID/(g of tissue) × (kg of body weight).

DISCUSSION

Both ephedrine and nicotine markedly increased uptake in BAT. The administration of nicotine and ephedrine in combination showed the strongest effects. Increased uptake in BAT with the combination of nicotine and ephedrine was

FIGURE 3. Extent of ^{18}F-FDG uptake and temperature decrease from baseline after injection of nicotine alone or with other interventions. Group-specific distribution is present. Groups combining nicotine and ephedrine show both large decrease in body temperature and large increase in ^{18}F-FDG uptake. In nicotine group, drop in body temperature was prominent but increase in ^{18}F-FDG uptake was not as high as in nicotine plus ephedrine group. In control, reserpine plus nicotine, and propranolol plus nicotine groups, decreases in body temperature and increases in ^{18}F-FDG uptake were minimal. Significant correlation between decreased body temperature and increased ^{18}F-FDG uptake was seen only in nicotine plus ephedrine group. b.w. = body weight; Ephe = ephedrine; Nic = nicotine; Pro = propranolol; Res = reserpine.

also significantly higher (both $P < 0.05$) than the increase with exposure to nicotine or ephedrine alone. Our study was not designed to determine whether the effects of the 2 drugs are mediated by the same pathways. Although we were able to show that caffeine slightly increased ^{18}F-FDG uptake in BAT, this effect did not achieve statistical significance.

No significant change in ^{18}F-FDG uptake was seen in other major organs except for stomach and muscle. ^{18}F-FDG uptake in stomach was decreased in all pharmacologic intervention groups. Low ^{18}F-FDG uptake in muscle was observed in the nicotine plus ephedrine group. Decreases of stomach uptake were abrogated by prior injection of a β-blocker, suggesting that this effect may be mediated by the sympathetic nervous system. Activation of the sympathetic nervous system has been reported to decrease blood flow to the stomach. Smoking is a known risk factor for gastroduodenal ulcer. Some reports suggest nicotine induces norepinephrine release in the stomach and reduces blood flow (16). A decrease in blood flow to the stomach may contribute to decreased gastric ^{18}F-FDG uptake, indicating a potential use for these pharmacologic agents in reducing physiologic uptake in the stomach on ^{18}F-FDG PET scans. A significant increase in ^{18}F-FDG uptake was seen in BAT only with ephedrine and nicotine.

We injected a β-blocker before nicotine administration to evaluate a potential antagonistic effect on nicotine stimulation of BAT. The dose of β-blocker used in this study was same as that described in prior studies and was potent enough to block the effects of the sympathetic nervous system on BAT activation (6). Both reserpine and propranolol inhibit the function of the sympathetic nervous system, but there are differences between the 2 drugs. Reserpine inhibits the release of noradrenaline from nerve terminals.
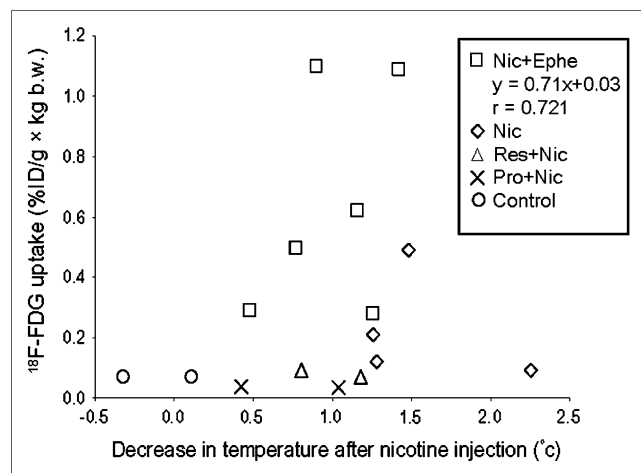
Several doses are required to deplete noradrenaline completely from the synaptic cleft. On the other hand, because propranolol is a nonselective blocker of the β-adrenergic receptor, propranolol likely reacts faster and more robustly if used in high concentrations. The groups pretreated with propranolol showed a difference in uptake in other organs. Specifically, uptake in heart and white adipose tissue were substantially reduced. The decreases in ^{18}F-FDG uptake in the heart were considerable—71.6% lower than in the control group. This finding is similar to previously described results (6) and may be due in part to effects of the sympathetic nervous system on cardiac glycolysis.

BAT is well known to be a key organ in maintaining body temperature. We anticipated finding some relationship between an increase in body temperature and the extent of ^{18}F-FDG uptake in BAT. Ephedrine is known to be an agent that leads to thermogenesis. Astrup et al. suggested that thermogenesis after ephedrine administration does not result from increased temperature in BAT itself but from increased blood flow around the intrascapular tissue (17).

We monitored real-time core body temperature using an implanted transmitter to evaluate the relationship between ^{18}F-FDG uptake and body temperature. In our study, the increase in body temperature after nicotine injection was not significant. Instead, we observed a temporary drop in body temperature after intraperitoneal injection of nicotine. In most cases, body temperature recovered within 30 min. The fact that the control and propranolol pretreatment groups did not show this temperature drop supports the concept that it resulted from the nicotine injection and was mediated by the sympathetic nervous system. The fact that a drop in body temperature was also observed in the reserpine pretreatment group suggests that pretreatment with reserpine before nicotine stimulation was not enough to deplete noradrenaline vesicles from the sympathetic nerve terminal.

As shown in Figure 3, we compared the degree of ^{18}F-FDG uptake (%ID/g × [kg of body weight]) with the degree of temperature decrease. A characteristic temperature distribution was seen in each group. The group pretreated with both nicotine and ephedrine showed both a large drop in temperature and a large increase in ^{18}F-FDG uptake in BAT. The group pretreated with only nicotine also showed a large drop in temperature drop and increase in ^{18}F-FDG uptake, although not to as great an extent as in the group pretreated with both nicotine and ephedrine. The control and propranolol pretreatment groups showed a lesser drop in temperature and increase in ^{18}F-FDG uptake in BAT.

A temperature drop was observed in the reserpine group, but uptake in BAT was low. We could not readily explain this drop in body temperature. We warmed the intraperitoneal injection before administration to ensure that the injection would not have a direct cooling effect on the body. It is well known that nicotine contracts peripheral arteries and induces a temporary cooling of the distal extremities. In this study, we measured the core body temperature in the peritoneal cavity.

Another possible explanation is that blood vessels inside the abdomen had contracted, causing a temporary drop in intraperitoneal temperature. A drop in temperature is mediated mainly by an α-adrenergic effect, but uptake in BAT is mainly a β-effect. The nonselective adrenergic antagonist propranolol inhibits both effects.

Some reports suggest that BAT may act as a buffer for energy consumption and storage. BAT is activated after a high-calorie meal to consume the excess energy in a so-called diet-induced thermogenesis system. BAT is the main organ that produces diet-induced thermogenesis, and a lack of its function is considered to be one cause of obesity (18). Other reports have said that uncoupling protein 1 double-knock-out mice displayed increased susceptibility to diet-induced obesity with age (19). Recently, BAT has also been found to be important in understanding the mechanism of glucose tolerance (20,21). These reports indicate that BAT has a critical role in energy consumption and in the pathogenicity of obesity and diabetes.

Many reports suggest that cigarette smokers weigh less than nonsmokers and that they gain weight with smoking cessation. A study of chronic smoke exposure to hamsters showed an increased mass of BAT in the studied animals (22). The key cause of this phenomenon was thought to be nicotine. Another study showed that chronic nicotine treatment results in loss of body weight without a change in food intake (15). In our study, because the pharmacologic intervention was not chronic, the effect was evident but short. Chronic exposure to nicotine is reported to result in chronic activation of BAT and may lead to a reduction in body weight.

The activity of BAT can be measured by various methods, including the weight of BAT, oxygen consumption, blood flow, and other parameters. ^{18}F-FDG is a tracer that assesses the activity of glucose transport in tissue—one aspect of activation of energy consumption. This study was of ex vivo biodistribution, but in vivo biodistribution can also be studied, as visualized by PET (6). PET has this advantage over existing methods and is easy to apply to humans. ^{18}F-FDG will be a good tool for analyzing the function of BAT and for evaluating the possible use, as antiobesity agents, of new drugs that activate BAT. These preclinical data suggest that a patient should avoid both ephedrine and nicotine (smoking) before undergoing ^{18}F-FDG PET. One should keep in mind that patients smoking cigarettes may present with high uptake of ^{18}F-FDG in BAT. The data also suggest that β-blockers or reserpine may be useful tools for minimizing ^{18}F-FDG uptake in BAT.

CONCLUSION

Uptake of ^{18}F-FDG into BAT was activated by intraperitoneal injections of nicotine and ephedrine. The effect was additive when nicotine and ephedrine were used in combination. The effect of nicotine was abrogated by pretreatment

with a β-blocker. The body temperature of rats temporarily decreased soon after nicotine injection. The extent of the drop in body temperature has some correlation with the extent of ^{18}F-FDG uptake in BAT.

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