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Research Article

Brown Adipose Tissue Can Be Activated or Inhibited within an Hour before ^{18}F-FDG Injection: A Preliminary Study with MicroPET

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Brown adipose tissue (BAT) is emerging as a potential target for treating human obesity. It has been indicated that BAT is rich in innervations of sympathetic nerve control. Using ^{18}F-FDG microPET imaging, this study aims at evaluating how factors related to sympathetic activation/inhibition changed BAT metabolism of mice. BAT ^{18}F-FDG uptake were semiquantitatively evaluated in different groups of mice under temperature (cold or warm stimulus) or pharmacological interventions (norepinephrine, epinephrine, isoprenaline, or propranolol) and were compared with the corresponding controls. It was found that BAT activation can be stimulated by cold exposure ($P = 1.96 \times 10^{-4}$), norepinephrine ($P = .002$), or both ($P = 2.19 \times 10^{-6}$) within an hour before ^{18}F-FDG injection and can also be alleviated by warming up ($P = .001$) or propranolol lavage ($P = .027$). This preliminary study indicated that BAT function could be evaluated by ^{18}F-FDG PET imaging through short-term interventions, which paved the way for further investigation of the relationship between human obesity and BAT dysfunction.

1. Introduction

Brown adipose tissue (BAT) is a kind of fat tissue different from white adipose tissue (WAT). In mammals, BAT has its function mainly in nonshivering thermogenesis, responsible for control of body temperature and regulation of energy balance [1]. BAT is commonly found in human newborns and small mammals, and it is previously thought that there is only a vestigial amount of BAT in adulthood [2]. Recently, ^{18}F-FDG PET/CT demonstrated a relatively broad distribution of functional BAT in adult human, mainly along the way of sympathetic nerve control, such as paravertebral and periadrenal regions [3, 4]. These functional BATs have drawn our attention as a potential therapeutic target for inducing weight loss through its energy expenditure pathway [2, 5], because excision or denervation of interscapular BAT can produce abnormal increase in the amounts of WAT in those animals, meaning that they are becoming fatter [6]. Some other studies, though showing limited success, have already tried to activate BAT by stimulating SNS to control body weight, using, for instance, β_3-adrenoceptor agonists CL-316243 or L-796568 [7, 8]. But none of them monitored BAT metabolic function during their researches.

Cold stimulus has been proved to increase ^{18}F-FDG uptake, an indicator of BAT metabolism, in either animal or human studies [9, 10]. Cold can activate sympathetic nerve system (SNS) to excrete norepinephrine, and then the BAT, which is rich in sympathetic nerve terminals, will produce more heat [11]. Genomic studies have indicated that the heat production is related to uncoupling protein 1 (UCP1), which is highly expressed in the mitochondria of BAT cells and can convert glucose and free fatty acid into heat [12]. For further evaluation of how the influence factors related to SNS changed the BAT metabolism, this study investigated several interventions to stimulate or inhibit SNS and BAT metabolism of mice, and under the application of microPET, BAT metabolic statuses were clearly seen in vivo, showing different degree of FDG uptake under different interventions which could be semiquantitatively analyzed in real time. MicroPET served as a useful tool in monitoring

BAT function under different interventions and showed the potential to guide further study of the relationship between BAT metabolism and obesity and diabetes.

2. Materials and Methods

2.1. Animals and Grouping. Five-to-ten-wk female Kunming mice (provided by Beijing Medical College Animal Breeding Center) were enrolled in this study (mean body weight = 25 g). All animals were kept in the Animal Breading Center of PUMCH with constant room temperature of 21°C. Food and water were given ad libitum. Control mice ($n = 12$) were examined under room temperature of 20-21°C. Three intervention groups were involved in this study (Table 1). In physical intervention group, mice were exposed to cold ($n = 6$, 6°C-7°C, $t = 1$ h), cold and then warm ($n = 6$, 6°C-7°C, $t = 1$ h, and then 35°C-36°C, $t = 1.5$ h), or only warm ($n = 6$ and $t = 1.5$ h). For pharmacological stimulation group, under 21°C mice received intraperitoneal injection of norepinephrine (NE, 0.4 mg/kg, $n = 5$), epinephrine (0.02 mg/kg, $n = 5$), isoprenaline (0.016 mg/Kg, $n = 6$), respectively, or both cold and NE (NE: 0.04 mg/kg, $n = 7$). For pharmacological inhibition group, cold pre-exposure mice received intragastric administration of propranolol (13.2 mg/kg, $n = 3$) or saline ($n = 3$).

2.2. Temperature Interventions. Control mice were kept in the examination room with constant temperature of 20°C-21°C without physical intervention or pharmacological stimulation. For cold intervention, we put the mice in a plastic box and kept them in a temperature-regulative refrigerator (BC-185FA, Aucma, China) with a constant temperature of 6°C-7°C (measured by a thermometer in the refrigerator) for 1 h. For warm stimuli, we placed the mice in a plastic box laid above a power-regulative heating pad (ML-1.5-4, Tianjin Tester Company, China) with the temperature of 35°C-36°C (measured by a thermometer in the box) for 1 h and kept warming the mouse for 0.5 h after FDG injection. All these animals were provided with food and water supply during the interventions.

2.3. Pharmacological Interventions. Norepinephrine (Tianjin Jinhui Amino Acids Co.), epinephrine (Beijing Yongkang Phar Co.), and isoprenaline (Shanghai Harvest Phar Co.) were diluted with saline and injected into peritoneal cavity of mouse in the volume of 100 μL (0.4 mg/kg), 100 μL (0.02 mg/kg), and 30 μL (0.016 mg/Kg), respectively, 1 h before FDG injection. Propranolol tablets (Tianjin Lisheng Phar Co.) were dissolved in saline to 1 mg/mL and administered intragastrically through a small animal lavage needle in the volume of 300 μL (13.2 mg/kg) per mouse 1 h before FDG injection. To monitor the combination effect of cold stimuli and NE, we performed the cold stimuli protocol as stated and gave NE injection with 0.04 mg/kg 1 h before FDG injection. All of these pharmacological interventions were performed under room temperature of 21°C, and after interventions, water and food were provided.

Table 1: Groups according to interventions and the number of mice in each group.

Interventions	Mouse number
Temperature intervention	**18**
Cold exposure	6
Cold exposure + warm stimuli	6
Warm stimuli	6
Pharmacological stimulation	**23**
Norepinephrine (NE)	5
epinephrine	5
isoprenaline	6
NE + cold exposure	7
Pharmacological inhibition	**6**
Cold + propranolol	3
Cold + saline	3
Controls	**12**

2.4. MicroPET Protocol. All mice were examined under nonfasted status. After physical or pharmacological interventions, 3.7 MBq ^{18}F-FDG was injected into peritoneal cavity for each mouse. Water and food was supplied after FDG injection. Anesthesia using Summit AS-1-000-7 animal anesthesia system (USA) was performed with 1.5% isoflurane (with O_2 combination of 2 liter per minute) 40 min later. PET data acquisition procedure was performed under Siemens Inveon system 10 min after anesthesia when mice were totally unconscious. Acquisition procedure lasted for 300 sec for each mouse. Anesthesia was continued during the scanning process with the same flow rate through a facemask designed for small animal.

2.5. Data Analysis. All microPET images were studied by two researchers (Wu and Cheng) on a high-resolution computer screen applying ASIPro VM software. Both visual and semiquantitative analyses were performed for each mouse. For visual analysis, the two researchers compared the interscapular BAT ^{18}F-FDG uptake color intensity of mouse from different intervention groups. For semiquantitative analysis, 3D round regions of interests (ROI) were placed carefully over the interscapular BAT and brain on microPET images for each mouse, respectively. ^{18}F-FDG uptake value was acquired in the form of nanocurie per cubic centimeter (nCi/cc) automatically after placing ROIs. Uptake ratio (R) of maximum interscapular BAT uptake and mean brain uptake was calculated for each mouse and compared between different interventions.

2.6. Statistical Analysis. Data were reported as means ± SD. Statistical analysis was performed using SPSS software, Version 17.0. One way ANOVA was used, and $P < .05$ was considered significant.

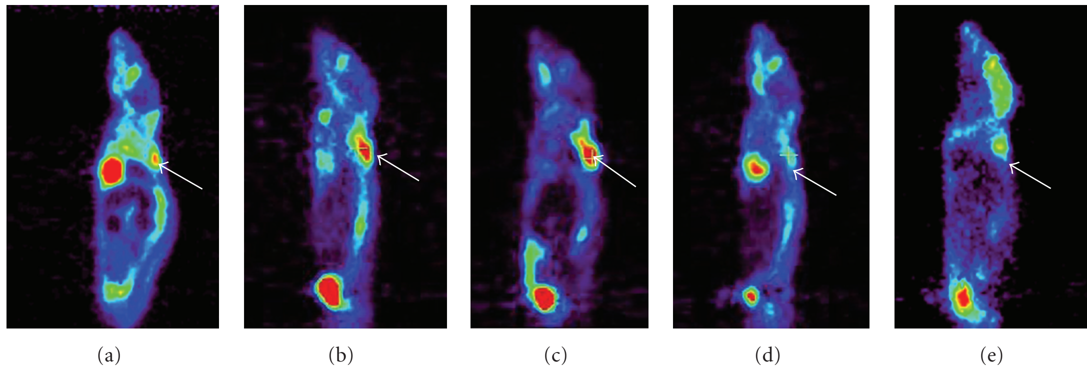

FIGURE 1: Comparison of interscapular BAT uptake in mice receiving different interventions from microPET sagittal images. For visual analysis, microPET images showed that mouse interscapular BAT ^{18}F-FDG uptake (arrow) varied under different interventions. (c) Cold plus NE interventions showed highest BAT uptake ($R = 15.64 \pm 5.58$, $P = 2.19 \times 10^{-6}$) compared to (b) cold exposure ($R = 10.22 \pm 4.13$, $P = 1.96 \times 10^{-4}$) and (a) control ($R = 4.08 \pm 1.32$), while (d) warming ($R = 2.13 \pm 0.43$, $P = .001$) and (e) propranolol ($R = 1.30 \pm 0.16$, $P = .027$) showed decreased BAT uptake in cold pre-exposure mice.

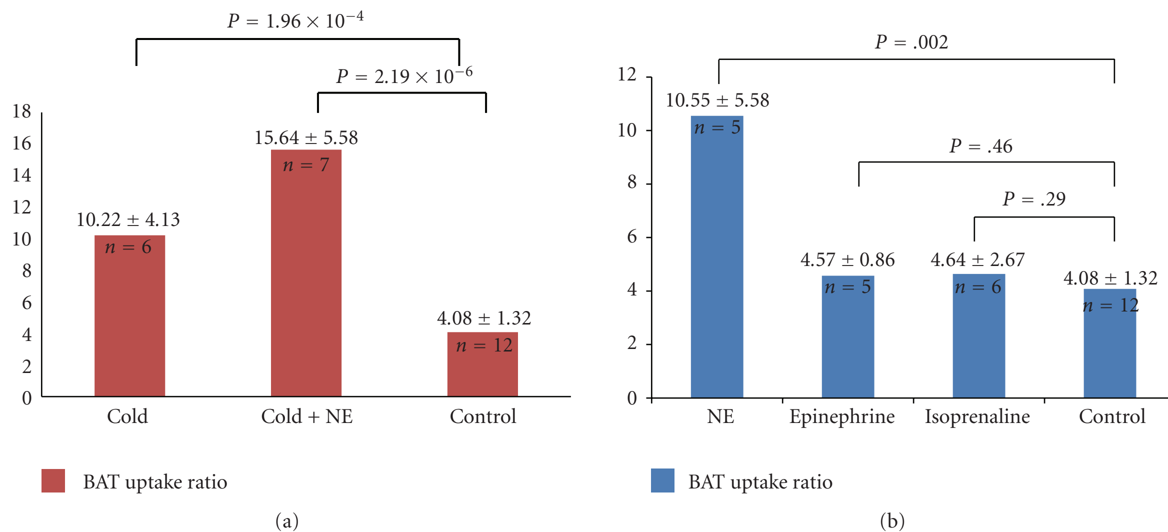

FIGURE 2: Comparison of the effects of different interventions to stimulate interscapular BAT in mice. (a) BAT FDG uptake ratio in cold, cold + NE and control group. Cold together with NE intervention showed the highest uptake in BAT. (b) BAT FDG uptake ratio in NE, epinephrine, isoprenaline, and control group. All these pharmaceutics increase BAT activity, but only NE showed significant difference ($P = .002$).

3. Results

In visual analysis, different interscapular BAT ^{18}F-FDG uptake intensity was found under different interventions as demonstrated by the microPET images (Figure 1). Cold and NE exposure caused the highest BAT uptake, followed by cold exposure and the control, while propranolol lavage and warming up showed decreased BAT uptake in cold pre-exposed mice.

In semiquantitative analysis, BAT ^{18}F-FDG uptake was significantly higher under cold exposure compared to control mice under room temperature (R: 10.22 ± 4.13 versus 4.08 ± 1.32, $P = 1.96 \times 10^{-4}$) and highest with both cold exposure and NE stimulations (R: 15.64 ± 5.58 versus 4.08 ± 1.32, $P = 2.19 \times 10^{-6}$). A stimulation with only NE (R: 10.55 ± 5.85), epinephrine (R: 4.57 ± 0.86), or isoprenaline (R: 4.64 ± 2.67) at room temperature all increased BAT uptake compared with the controls (R: 4.08 ± 1.32), but only NE showed significant difference compared with the controls ($P = .002$) (Figure 2).

For BAT inhibition, warming could significantly reduce BAT uptake in mice with cold pre-exposure (R: 2.13 ± 0.43 versus 10.22 ± 4.13, $P = .001$) and without cold pre-exposure (R: 2.48 ± 0.88 versus 4.08 ± 1.32, $P = .017$). In addition, propranolol could significantly reduce BAT uptake in cold pre-exposed mice (R: 1.30 ± 0.16 versus 3.09 ± 0.90, $P = .027$) (Figure 3).

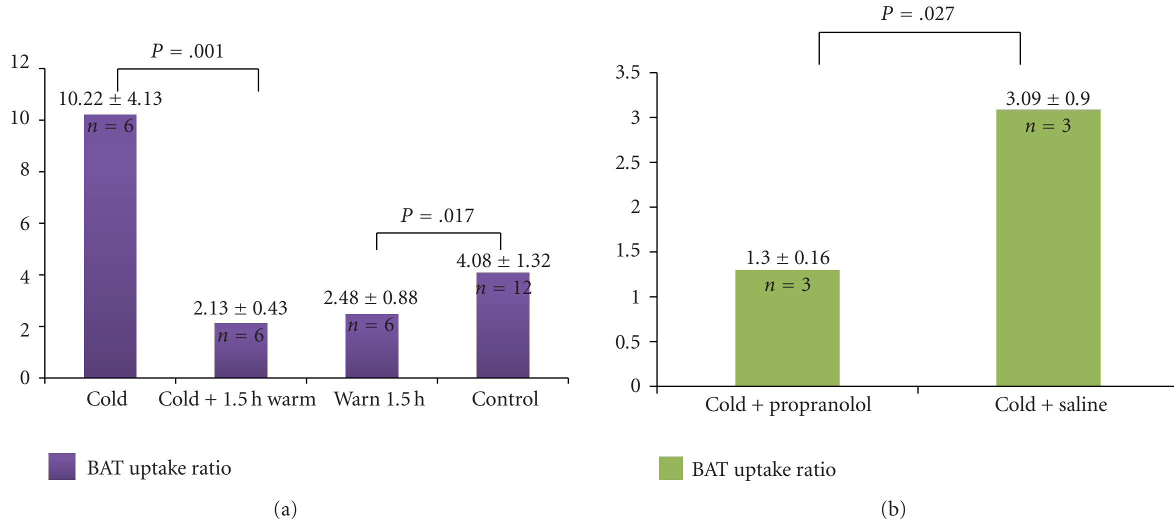

Figure 3: Comparison of the effects of warming and propranolol to inhibit interscapular BAT in mice. (a) BAT FDG uptake ratio under warm intervention. Warming could significantly decrease BAT metabolism in mice with or without cold pre-exposure ($P = .001$ and .017, resp.). (b) BAT FDG uptake ratio under propranolol intervention. Propranolol could significantly decrease BAT activity in cold pre-exposure mice ($P = .027$).

4. Discussion

Brown adipose tissue (BAT) has been shedding new light on weight control of human recently, but few researches focused on monitoring BAT metabolic function using molecular imaging. It is well known that BAT has more sympathetic innervations than WAT [13], which means that BAT may be activated by stimulating SNS. For future investigation of new methods to control obesity through activation of BAT function, the present study aimed mainly at establishing a technology platform using microPET to evaluate the BAT function through interventions with factors related to SNS activation or inhibition. Considering that the SNS might probably be modulated by short-term interventions, the temperature control or drug administration was generally set as 1 h before FDG injection in this study. Moreover, peritoneal injection or lavage was used to obtain a more stable effect. The results proved that these choices were feasible.

BAT can bring in false positive results in PET images of human by showing increased radioactive uptake especially in the supraclavicular area [14, 15]; many studies, therefore, try to find methods to inhibit BAT uptake in humans, for instance, by keeping the patients warm or giving orally propranolol administration [16–18]. Our study provided new data concerning these two methods in inhibiting BAT uptake that only 1.5 hour of warm intervention (1 h before and 0.5 hour after FDG injection) could quickly and markedly inhibit BAT metabolism (R: 2.48 ± 0.88 versus 4.08 ± 1.32, $P = .017$), and compared with propranolol intervention, warming seemed to be more effective in reducing BAT activity ($P = .001$). Based on this result, in clinics, to prevent BAT uptake in PET images, providing a warm environment for patients right before PET scan would be a more effective, convenient, and safer way than propranolol intake.

Cold stimulus was proved useful to increase BAT metabolism in either animal or human studies [9, 10] because it can activated sympathetic nerve terminals surrounding the BAT to excrete NE for more heat production [11]. In our study, cold exposure also significantly increased BAT activity (R: 10.22 ± 4.13 versus 4.08 ± 1.32, $P = 1.96 \times 10^{-4}$), and this effect was more obvious when combined with NE injection ($P = 2.19 \times 10^{-6}$), well confirming that BAT metabolism was correlated with catecholamine system. Moreover, cold effect could be quickly suppressed by warming (R: 2.13 ± 0.43 versus 10.22 ± 4.13, $P = .001$) or propranolol administration afterwards (R: 1.30 ± 0.16 versus 3.09 ± 0.90, $P = .027$), both of which were antagonistic to sympathetic-adrenal system, thus prohibiting BAT activity.

In our present study the β-adrenoceptor agonists (NE, epinephrine and isoprenaline) all increased interscapular BAT activity in mouse, but only NE showed a significant difference to controls ($P = .002$, .459, and .293, resp.). We think the dosage insufficiency of epinephrine and isoprenaline we gave mice may answer this question. With limited researches and few examples to follow, we gave a tentative dosage of epinephrine (0.02 mg/kg) and isoprenaline (0.016 mg/Kg) to mice, which is about the clinical dosage for children (about half of adult dose), much smaller than the advised dose for mouse (about 9-fold clinical dosage for adults) based on surface area per kilogram [19]. However, the dosage of NE (0.4 mg/kg) we gave mice was 11 times the dosage for human adult, so only NE was effective in activating BAT metabolism. Baba et al. [20] used epinephrine in 5 mg/kg, and microPET scan showed significant BAT uptake compared to controls; therefore, we think increasing dosage in future study may overcome this problem.

There are some limitations in this study. For instance, only female mice were enrolled in this study, and the mouse number in each intervention group was not big enough

($n = 3 \sim 7$). Furthermore, some uncontrollable factors such as environmental noise and stimuli caused by experimental handling may potentially influence the nerve system of mouse and thus the activity of BAT. In future studies, we will make improvement in these aspects.

In general, this preliminary study gives new prospective in studying the stimulation and inhibition of BAT of mouse by a quantitative analyzing tool of microPET. By applying microPET, the BAT activate status can be evaluated in vivo semiquantitatively in real-time.

5. Conclusion

Environment temperature control can significantly stimulate or alleviate BAT uptake of ^{18}F-FDG within 1 h in mice. Stimulating sympathetic nerve system by norepinephrine can significantly increase the metabolism of BAT within 1 h, while inhibiting SNS by warming or propranolol can alleviate BAT function. This preliminary study with ^{18}F-FDG microPET warrants further investigations of the mechanism of BAT and various methods of intervention according to clinical purposes. However, better optimization of different intervention conditions as well as some other methods for activating BAT should be further explored and studied in the hope of future human application.

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