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Kinetics of Human Brown Adipose Tissue Activation and Deactivation

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Abstract

Brown adipose tissue (BAT) has been identified as a potential target in the treatment and prevention of obesity and metabolic disease. The precise kinetics of BAT activation and the duration of stimulus required to recruit metabolically active BAT, and its subsequent deactivation, are not well-understood. In this clinical trial, 19 healthy adults (BMI: 23.7±0.7 kg/m^2, Age: 31.2±2.8 y, 12 female) underwent three different cooling procedures to stimulate BAT glucose uptake, and active BAT volume was determined using ^{18}F-Fluorodeoxyglucose (FDG) PET/CT imaging. We found that 20 minutes of pre-injection cooling produces activation similar to the standard 60 minutes (39.9 mL vs. 44.2 mL, p= 0.52), indicating that BAT activity approaches its peak function soon after the initiation of cooling. Furthermore, upon removal of cold exposure, active BAT volume declines (13.6 mL vs. 44.2 mL, p=0.002), but the deactivation process persists even hours following cessation of cooling. Thus, the kinetics of human BAT thermogenesis are characterized by a rapid increase soon after cold stimulation but a more gradual decline after rewarming. These characteristics reinforce the feasibility of developing mild, short-duration cold exposure to activate BAT and treat obesity and metabolic disease.

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Conflict of Interest

No conflicts of interest are reported

Introduction

Obesity and the resulting metabolic diseases are worldwide health concerns[1] and are independently associated with all-cause mortality[2]. Obesity is primarily defined by an excess of white adipose tissue (WAT), whereas a greater presence of brown adipose tissue (BAT) is associated with lower body mass index (BMI)[3,4,5] and improved glucose metabolism[6]. Due to BAT's ability to consume glucose and free fatty acids in response to cold exposure[7,8,9], cooling has been a common method for studying BAT's potential for the prevention and treatment of obesity[10]. As cold exposure is widely explored and reproducibly activates BAT thermogenesis, there is a need to standardize cooling protocols to best compare results across the field[11,12]. Furthermore, determining ideal conditions with which to target BAT for its thermogenic capabilities can be challenging because BAT may need along-acting and persistent stimulus. On the other hand,a short-duration of activation may sufficiently activate BAT glucose uptake, similar to skeletal muscle[13].The precise kinetics describing BAT activation in response to cold, when better understood, could lead to novel treatments for hyperglycemia and hypertriglyceridemia.

Two hours of cold exposure is the standard duration used to study cold-induced BAT metabolism[11], yet it is unclear if the full exposure time is necessary to achieve the same amount of BAT thermogenesis. In rats, BAT thermogenesis increases substantially above baseline in less than 5 minutes[14]. Furthermore, it is unclear whether human BAT immediately suppresses its thermogenic processes following removal of a cold stimulus. When using mild cold to activate BAT to minimize subjects' discomfort and maximize adherence to the cooling protocol, it is particularly important to determine the minimal effective duration of exposure.

While human BAT might achieve peak thermogenesis soon after initiation of cooling, its metabolic activity may decline much more slowly after a cold stimulus is removed. Evidence for this delayed process is suggested by the large retrospective studies of BAT prevalence using ^{18}F-fluorodeoxyglucose positron emission tomographic/computed tomographic (^{18}F-FDG PET/CT).In these studies, patients were not deliberately exposed to cold during the 60 minutes prior to FDG injection, yet 5-10% had detectable BAT glucose uptake[15,16,3]. These observations suggest several possibilities related to the nature of the cold-exposure experienced by these subjects including exposure well before FDG injection; cold-acclimation; and cold temperatures in the rooms where the patients waited for the FDG injection to distribute to metabolically active tissues. In all such scenarios, BAT glucose uptake was maintained during the scan acquisition. Therefore, we hypothesized that BAT can be activated using a duration of cold exposure shorter than two hours and that BAT remains activated even hours following removal of a cold stimulus. We tested these hypotheses in 19 healthy female and male volunteers who underwent three different cooling conditions: a "standard" condition of 60 minutes of pre-FDG cooling; 20 minutes of pre-FDG cooling; and also 120 minutes of cooling followed by 180 minutes of warming prior to ^{18}F-FDG PET/CT imaging.

Materials and Methods

Study Population

Healthy volunteers (12 women, 9 men) were recruited through electronic advertisements (Table 1). Each subject participated in 2-3 separate in-patient study visits and wore standard hospital scrubs during each visit. This study followed institutional guidelines and was approved by the Institutional Review Boards of Beth Israel Deaconess Medical Center (BIDMC) and Joslin Diabetes Center. Written informed consent was obtained from all volunteers.

Cold Exposure Design

Cooling was performed with a cooling vest with circulating water at 12.8-16.1°C in a room at 20 °C[17]. Three cooling designs were used (Fig 1A): Short Cooling, Standard Cooling, and Rewarming. For the Short Cooling day, FDG was administered after 20 minutes of cold exposure, and subjects remained in the cooling vest for 60 more minutes. On the Standard Cooling day, FDG was injected after 60 minutes of cold exposure and the volunteer wore the cooling vest for another 60 minutes[17]. On the Rewarming day, volunteers wore the cooling vest for 120 minutes, the vest was removed for 120 minutes of resting at 23°C (re-warming), the FDG was injected, then the subjects rested at 23°C for 60 more minutes.

Imaging Protocol and Quantification of FDG Uptake

PET/CT images were obtained from the base of the skull to the kidneys with an i.v. bolus administration of 444 MBq (12 mCi) of ^{18}F-FDG and image acquisition using a Discovery LS multidetector helical PET/CT scanner (GE Medical Systems). Areas of ^{18}F-FDG uptake on PET co-localizing with regions of fat were identified on CT (-250 to -10 Hounsfield Units) were quantified by their Standard Uptake Value (SUV): average activity per unit volume within the region of interest divided by the injected dose per body mass in kg. The SUV threshold used was >1.0 g/mL. We then applied a post-hoc individualized adjustment for lean body mass (LBM) applied to BAT volume: ((1.5 g/mL) * (0.73))/ (individualized LBM); where 0.73 is the average LBM proportion of this cohort. Regions of interest were drawn axially on each image slice around metabolically active adipose tissue within the scanning window.[18]

Statistical Analysis

The goal of this pilot study was to detect the difference in BAT activation between Standard cooling and Rewarming. We calculated that we would need to study 12 subjects to reject the null hypothesis that this response difference is 0, with a power of 0.80 and an α of 0.05. Prism software (version 7; GraphPad, La Jolla, CA, USA) was used for statistical analysis. All the data are expressed as the mean ± standard deviation. Significant differences between means were identified using Wilcoxon Signed Rank tests. Differences were considered significant at $P < 0.05$.A Student's *t*-test was used to compare male vs. female demographics.

Results

BAT Kinetics

The standard two hours of cold exposure (60 minutes pre-injection) induced the highest detectable volume of activated BAT (44.2 ± 36.9 mL). Twenty minutes of pre-FDG cold exposure yielded a similar amount of BAT volume compared to the Standard Cooling (39.9 ± 24.2 mL, p=0.52) (Fig 1C). In fact, the majority of subjects had more detectable BAT with the shorter duration of pre-injection cold exposure (Fig 1D).

When subjects were re-warmed for three hours following two hours of cold exposure, a substantially lower volume of BAT remained metabolically active (13.6 ± 18.7 mL) when compared to Standard Cooling (p = 0.002) (Fig 1E). Under conditions of re-warming following cold exposure, nearly every subject had less activated BAT than without re-warming, yet several subjects still had detectable BAT (Fig 1F). The tissue SUVmean, another measure of BAT metabolic activity, was highest under standard cooling (1.68 ± 0.52 g/mL), followed by 20 minutes of pre-injection cooling (1.55 ± 0.43 g/mL, P=0.27), while the Rewarming condition had the lowest SUVmean of 1.21 ± 0.18 g/mL (P=0.02 compared with Standard cooling).

Discussion

The elucidation of the kinetics of BAT activation is another important step in understanding its regulation under standard physiological conditions. Our results demonstrate that 80 minutes of cold exposure elicits similar BAT activation as 120 minutes, indicating that brown adipocytes are recruited soon after cold exposure to maintain heat production. During the re-warming period, those subjects with the highest amount of BAT volume demonstrated the greatest reductions in detectable BAT, whereas among those with little to moderate amounts, the amounts of detectable BAT were not substantially different during different cold exposure conditions. This distinction suggests that subjects with large volumes of BAT have more flexibility in the activation and de-activation in response to temperature challenges.

The evidence here indicates the novel finding that the thermogenic effects of cooling are not immediately suppressed in the absence of a cold stimulus, suggesting that BAT continues to utilize glucose for hours after initial recruitment. These findings are corroborated by another study demonstrating that heat production remains elevated for at least one hour during re-warming following cold exposure[19]. In fact, longer cold challenges (up to 24-hours) have shown that human core and skin temperatures level out after 6 hours[20].

There are several methodological considerations that impact the interpretation of our data. First, we focused the BAT quantification on the principal cervical-supraclavicular-axillary BAT depots rather than including other, smaller depots; however, this approach leads to volumes that correlate well with total-body BAT metabolic activity[18]. We also could not distinguish beige/brite from brown adipocytes[21,22] with FDG PET/CT alone, so the different contributions from these cell types[23] to glucose uptake could not be determined. In addition, we did not collect skin or core temperature measures or calculate energy expenditure in

order to assess the intensity of the cold stimulus, so it is not clear if a stronger or weaker cold challenge would yield the same findings. The FDG dose administered in this study limited the number of testable conditions to three, so we could not also measure baseline BAT activity in the absence of cold. However, a parallel study of ours that employed this negative control showed that there was a median volume of 0 mL detectable BAT[17]. Therefore, the detectable BAT volumes shown here should be considered absolute changes above a baseline of 0 mL. Finally, ^{18}F-FDG uptake may not consistently correlate with oxidative phosphorylation of glucose, but rather glucose may be taken into BAT to serve as a substrate for anaplerosis, fatty acid synthesis, or other metabolic pathways[24]. The use of dynamic ^{18}F-FDG imaging, and other metabolic tracers such as ^{15}O-H_2O,^{15}O-O_2or ^{11}C-acetate may help better characterize the thermogenesis within metabolically active adipocytes. The purpose of persistent BAT glucose uptake requires further investigation in this context.

Taken together, our data demonstrate that BAT is robustly activated immediately following just 20 minutes of pre-injection cold exposure, and BAT activation declines, but may persist, even hours following removal of a thermogenesis-provoking stimulus. These observations have implications for protocols in understanding BAT physiology, therapeutic provocations of BAT in obesity prevention and/or treatment, and also for diagnostic ^{18}F-FDG PET/CT assessments in a variety of clinical settings. In designing treatments that maximize efficacy while minimizing patient discomfort, the kinetics of BAT activation and deactivation highlight a unique window into the use of BAT thermogenesis for the treatment of obesity and associated metabolic diseases.

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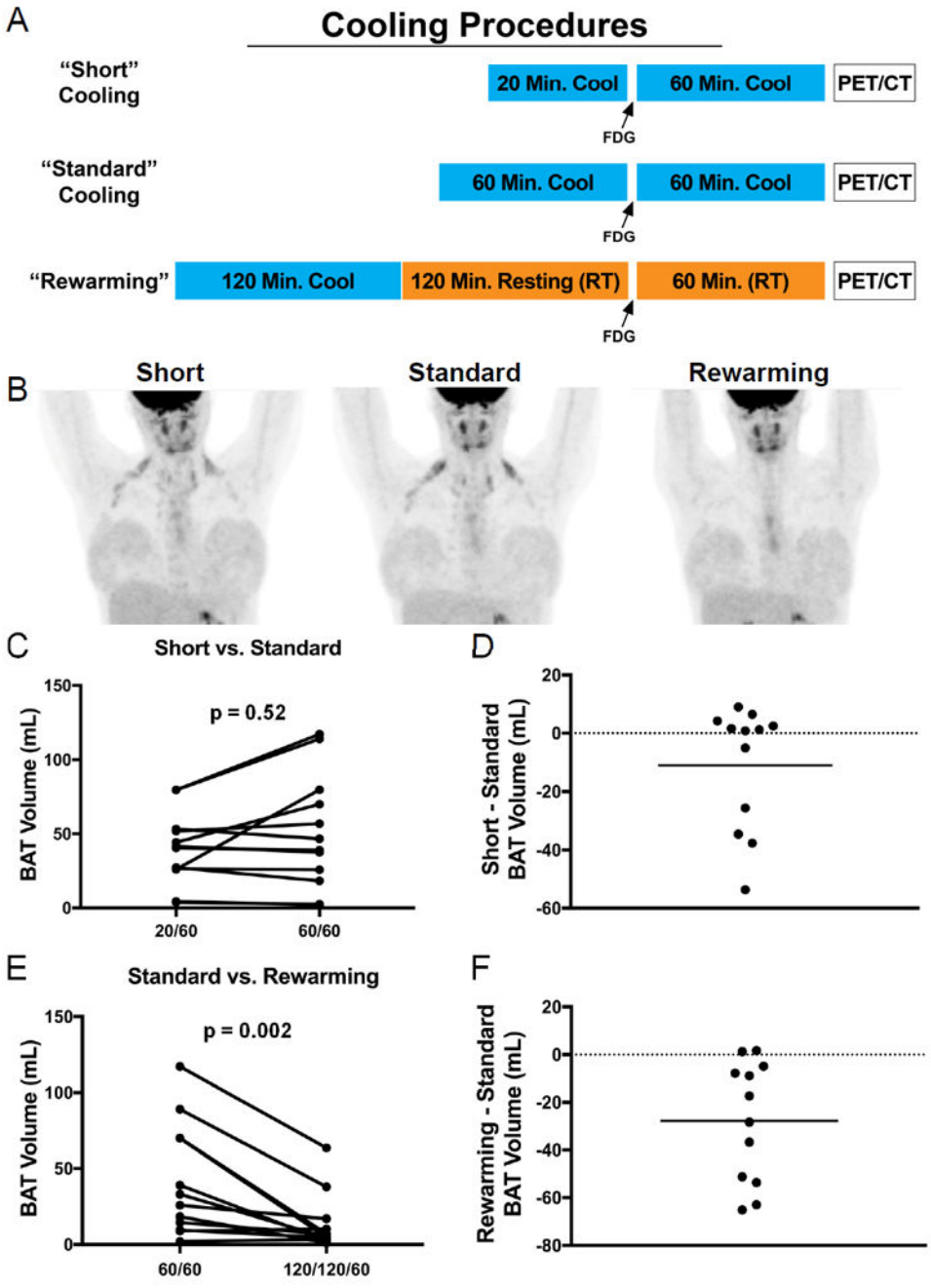

Figure 1.

Procedures and results for cold-induced PET/CT imaging studies. Three different cooling protocols are shown, with cooling in blue, room temperature (RT) in orange, and PET/CT acquisition in clear box (A). Representative image of one subject on three different imaging days (B). Results for BAT Volumes in Standard vs. Short Cooling Days (C, D) and Standard vs. Rewarming Days (E, F). Probability values were determined by Wilcoxon Signed Rank Tests.

Table 1

Subject Characteristics; Mean ± Standard Deviation shown. A Student's *t*-test was used to compare Women vs. Men.

Characteristic (units)	Combined	Women	Men	Women vs. Men (p)
Sex	12W / 7M	12	7	
Age (y)	31.2 ± 2.8	31.8 ± 3.5	30.1 ± 5.0	0.78
Height (cm)	169.3 ± 2.6	163.7 ± 2.5	179.0 ± 3.5	< 0.01
Weight (kg)	68.4 ± 3.3	62.8 ± 3.6	77.9 ± 4.9	0.02
Body mass index (kg/m^2)	23.7 ± 0.7	23.3 ± 0.9	24.2 ± 1.0	0.52
Body surface area (m^2)	1.8 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	< 0.01
Waist-hip ratio	0.79 ± 0.02	0.75 ± 0.01	0.85 ± 0.02	< 0.01
Body fat (%)	23.6 ± 1.7	27.0 ± 6.6	17.8 ± 1.8	< 0.01
Percent Lean (%)	73.0 ± 1.7	69.6 ± 1.8	78.8 ± 1.7	< 0.01