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Chronic high-fat feeding impairs adaptive induction of mitochondrial fatty acid combustion-associated proteins in brown adipose tissue of mice

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ABSTRACT

Since brown adipose tissue (BAT) is involved in thermogenesis using fatty acids as a fuel, BAT activation is a potential strategy for treating obesity and diabetes. However, whether BAT fatty acid combusting capacity is preserved in these conditions has remained unclear. We therefore evaluated expression levels of fatty acid oxidation-associated enzymes and uncoupling protein 1 (Ucp1) in BAT by western blot using a diet-induced obesity C57BL/6J mouse model. In C57BL/6J mice fed a high-fat diet (HFD) over 2–4 weeks, carnitine palmitoyltransferase 2 (Cpt2), acyl-CoA thioesterase (Acot) 2, Acot11 and Ucp1 levels were significantly increased compared with baseline and control low-fat diet (LFD)-fed mice. Similar results were obtained in other mouse strains, including ddY, ICR and KK-Ay, but the magnitudes of the increase in Ucp1 level were much smaller than in C57BL/6J mice, with decreased Acot11 levels after HFD-feeding. In C57BL/6J mice, increased levels of these mitochondrial proteins declined to near baseline levels after a longer-term HFD-feeding (20 weeks), concurrent with the accumulation of unilocular, large lipid droplets in brown adipocytes. Extramitochondrial Acot11 and acyl-CoA oxidase remained elevated. Treatment of mice with Wy-14,643 also increased these proteins, but was less effective than 4 week-HFD, suggesting that mechanisms other than peroxisome proliferator-activated receptor α were also involved in the upregulation. These results suggest that BAT enhances its fatty acid combusting capacity in response to fat overload, however profound obesity deprives BAT of the responsiveness to fat, possibly via mitochondrial alteration.

1. Introduction

Adult mammals have at least two types of adipocytes in the body: white adipocytes that accumulate fat as a triglyceride (TG) in cells in preparation for starvation, and brown adipocytes that combust fat to maintain body temperature. Brown adipocytes possess a high density of mitochondria in which energy derived from fatty acid degradation is dissipated as heat by the action of uncoupling protein 1 (Ucp1), which is exclusively expressed in this cell type [1]. For this reason, activation of brown adipocytes leads to an increase in calorie consumption and is expected to improve overweight conditions, providing a potential strategy for treating obesity and its related metabolic disorders [1–5]. In practice, metabolic activation of brown adipose tissue (BAT) has been shown in human subjects exposed to cold temperature by means of positron emission tomography with ^{18}F-fluorodeoxyglucose (^{18}F-FDG PET), and the ^{18}F-FDG uptake is inversely correlated with body mass index and body fat percentage [5,6]. Therefore, agents that could effectively stimulate BAT metabolic activity would be useful. However, whether BAT fatty acid combusting capacity is preserved in obese and diabetic conditions remains unclear.

To estimate BAT metabolic activity, non-invasive ^{18}F-FDG PET is often used in humans, but ^{18}F-FDG uptake is not an indicator specialized in fatty acid combustion. In rodent experiments, Ucp1 expression level has primarily been measured, because Ucp1 is central in the mechanism for energy expenditure in brown adipocytes. However, Ucp1 level does not exactly reflect fatty acid combusting capacity, and results reported in previous studies have not been consistent. For example, while BAT Ucp1 expression level was lower in obese ob/ob mice compared with normal mice [7], it was higher in obese mice fed a high-fat diet (HFD) than in control low-fat diet (LFD)-

Abbreviations: Acot, acyl-CoA thioesterase; Acox, acyl-CoA oxidase; BAT, brown adipose tissue; Cpt, carnitine palmitoyltransferase; ETC, electron transport chain; HFD and LFD, high- and low-fat diet; Ppar, peroxisome proliferator-activated receptor; SNS, sympathetic nervous system; TCA, tricarboxylic acid; TG, triglyceride; Ucp, uncoupling protein; WAT, white adipose tissue

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fed mice in most cases, with a huge variation in magnitude from negligible to remarkable levels [2,8,9]. In some cases, HFD had no effect or even lowered Ucp1 expression [8]. These differences are possibly due to differences in duration of HFD-feeding period and/or Ucp1 estimation based on protein or mRNA levels, as well as variations in genetic- or diet-induced obesity. Moreover, few studies have evaluated fatty acid oxidation along with Ucp1 in BAT of obese or diabetic animals [9].

In this study, we examined the changes in fatty acid combusting capacity by measuring protein expression levels of fatty acid oxidation-associated enzymes, including carnitine palmitoyltransferase 2 (Cpt2), acyl-CoA thioesterase 2 (Acot2), Acot11 and acyl-CoA oxidase 1 (Acox1), as well as Ucp1 and peroxisome proliferator-activated receptor (Ppar) γ in BAT, using a diet-induced obesity mouse model established by HFD-feeding. Our results suggest that profound obesity induced by chronic high-fat feeding could impair the adaptive induction of fatty acid combustion in BAT mitochondria.

2. Materials and methods

This study included adult male C57BL/6J mice (the strain which is most widely used in obesity research), ddY mice (as a model of postprandial hypertriglyceridemia) [10], KK-A^y (a model of type 2 diabetes) [11] and ICR (control) mice. All animals were conditioned to an environment at 23 ± 1 °C with constant humidity of 55 ± 5% and a 12 h light/12 h dark cycle and given free access to food and tap water according to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). The protocol of this study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Sciences.

2.1. Animals and treatment

Male C57BL/6J, ddY and ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan) and KK-A^y mice (CLEA Japan, Tokyo, Japan) were acclimatized at the age of 9 weeks for 1 week on a LFD (D12450B; Research Diets, New Brunswick, NJ, U.S.A) and subsequently fed a LFD or HFD (D12492; Research Diets) for 2, 4 or 20 weeks ad libitum. The LFD contained 3.85 kcal/g, with percentages of carbohydrate, fat and protein of 70%, 10% and 20%, respectively. The HFD contained 5.24 kcal/g with percentages of 20%, 60% and 20%, respectively. The carbohydrate was a combination of cornstarch, maltodextrin and sucrose, while the fat was soybean oil and lard. A group of C57BL/6J mice were orally administered Wy-14,643 (Tokyo Chemical Industry, Tokyo, Japan) at 50 mg/kg, once a day for 2 weeks after acclimatization, and were maintained on a LFD. After an overnight fast, the mice were anesthetized by intraperitoneal injection of a mixture of medetomidine (0.75 mg/kg), midazolam (4 mg/kg) and butorphanol (5 mg/kg), and sacrificed by decapitation. The serum was collected, and interscapular BAT and other tissues were excised, snap-frozen and stored at −80 °C until analysis.

2.2. Preparation of BAT homogenates

BAT homogenates were prepared in 250 mM sucrose containing 1 mM EDTA, 10 mM Tris-HCl (pH 7.5) and Complete protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) using a Potter-Elvehjem glass homogenizer with a glass pestle. After the homogenates were centrifuged at 25,000*g* for 10 min at 4 °C, the semisolid fat cake floating on the surface was removed on ice and the remaining portion was rehomogenized for use. The protein concentrations were determined using a DC protein assay kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as the standard.

2.3. Western blotting

Proteins were resolved in 10% SDS-polyacrylamide gels and immunoreactivity was detected by ImmunoStar LD reagent (Wako Pure Chemical Industries, Osaka, Japan) using a luminoimaging analyzer, as described previously [12]. For quantitative analyses, the signal intensities of the bands detected on the membranes were measured and transformed into relative values using a calibration curve generated with known amounts of protein. To detect Acot11, a rabbit polyclonal antibody against a polypeptide corresponding to mouse Acot11 amino acids 601–614 (H_2N-CLDNRNDLAPSLQTL-$CONH_2$) was raised and affinity-purified as described previously [12]. Rabbit polyclonal antibodies against Cpt2, Acot1 (which cross-reacts with Acot2) and Acox1 were described in our previous studies [13], and those against Ucp1 (AnaSpec, Fremont, CA, USA) and Pparγ (Santa Cruz Biotechnology, Dallas, TX, USA) were obtained from commercial sources.

2.4. Oil Red O staining

BAT specimens were fixed with 4% paraformaldehyde, rinsed with water, transferred into 30% sucrose in PBS at 4 °C until specimens sunk, and embedded in Optimal Cutting Temperature (OCT) matrix compound. OTC-embedded specimens were cut into 4 μm-thick sections and stained for 60 min at 37 °C in Oil red O solution followed by counterstaining with hematoxylin and eosin. Oil red O staining was carried out by Biopathology Institute Co., Ltd. (Oita, Japan). Microscopic examinations were performed using a BZ-8100 microscope (Keyence, Osaka, Japan).

2.5. Statistical analysis

The statistical significance of differences among values was examined by two-way factorial analysis of variance followed by Bonferroni's multiple comparison tests. Values of $p < 0.05$ were considered to indicate statistical significance. Statistical analyses were performed with Prism 7.0 for Mac (GraphPad, La Jolla, CA, USA).

3. Results

We first examined the effect of short-term HFD-feeding (for up to 4 weeks) on fatty acid combustion-associated proteins in BAT of C57BL/6J mice (Fig. 1). These protein levels were already elevated after 2 weeks, and significantly higher ($p < 0.05$) than the respective baseline and control levels seen in BAT of LFD-fed mice after 4 weeks. Body weight gain and white adipose tissue (WAT) weights, BAT weight, and serum levels of alanine aminotransferase (ALT) and glucose of HFD-fed mice were higher than those of LFD-fed mice, confirming establishment of the HFD-induced obesity model (Table S1).

Similar results were obtained for Cpt2, Acot2 and Ucp1 in BAT of ddY, ICR and genetically diabetic KK-A^y mice, although the extent of increase in the protein levels varied among mouse strains, with the most consistent induction observed in C57BL/6J mice (Fig. 1). Greater body weight gains in HFD-fed ddY, ICR and KK-A^y mice and higher serum glucose level in HFD-fed KK-A^y mice than in the respective LFD-fed control mice were also confirmed (Tables S2–4). However, Acot11 was increased in C57BL/6J mice, but decreased in the other three mouse strains. Moreover, basal levels of Ucp1 and Acot11 were 2–4 times higher in BAT of KK-A^y mice compared with the other strains (Fig. S1).

We next examined the effect of long-term HFD-feeding in C57BL/6 J mice. In mice fed HFD for 20 weeks, body weight gain, WAT weights, BAT weight, liver weight, and serum levels of ALT and glucose were significantly higher ($p < 0.05$) than LFD-fed control mice and baseline levels (Table S5). In BAT examined by microscopy after Oil Red O staining, unilocular large lipid droplets accumulated within brown adipocytes of these mice (Fig. 2D), whereas multimolecular large lipid