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FABP3 and brown adipocyte-characteristic mitochondrial fatty acid oxidation enzymes are induced in beige cells in a different pathway from UCP1

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ABSTRACT

Cold exposure and β_3-adrenergic receptor agonist (CL316,243) treatment induce the production of beige cells, which express brown adipocytes(BA)-specific UCP1 protein, in white adipose tissue (WAT). It remains unclear whether the beige cells, which have different gene expression patterns from BA, express BA-characteristic fatty acid oxidation (FAO) proteins. Here we found that 5 day cold exposure and CL316,243 treatment of WAT, but not CL316,243 treatment of primary adipocytes of C57BL/6J mice, increased mRNA levels of BA-characteristic FAO proteins. These results suggest that BA-characteristic FAO proteins are induced in beige cells in a different pathway from UCP1.

1. Introduction

Two types of adipose tissue are found in mammals: white and brown. White adipose tissue (WAT) is highly adapted to store excess energy in the form of triglycerides. Conversely, brown adipose tissue (BAT) oxidizes chemical energy to produce heat in response to cold exposure. Uncoupling protein-1 (UCP1) and fatty acids play an important role in thermogenesis in BAT. UCP1 is specifically expressed in BAT and is localized to the inner membrane of the mitochondria. Its physiological role is to uncouple oxidative phosphorylation so that most of the energy is dissipated as heat rather than being converted to ATP. Fatty acids also play a key role in thermogenesis as the source of oxidative fuel in the mitochondria.

Compared to white adipocytes (WA), brown adipocytes (BA) contain different types of proteins involved in fatty acid oxidation (FAO). These differences appear to reflect functional differences in the two types of adipose tissue. For example, the expression of fatty acid binding protein 3 (FABP3) is dramatically enhanced in acute cold exposure and is thought to be essential for FAO in BAT; in contrast, FABP3 expression is negligible in WAT [1–3]. FABP3 is a member of the fatty acid binding protein family, which consists of 14–15 kDa intracellular proteins that reversibly bind to hydrophobic ligands, such as saturated and unsaturated long-chain fatty acids, with high affinity. FABPs act as lipid "chaperones" that have been implicated in fatty acid uptake, transport, and targeting [4]. Among these families, FABP3 in BA is thought to transport and deliver fatty acids to the mitochondria for oxidation. Fatty acids delivered to the mitochondria are β-oxidized by mitochondrial enzymes. Proteomic analyses of mitochondria from brown and white adipocytes revealed that their proteomes are considerably different, both qualitatively and quantitatively, and are further characterized by tissue-specific protein isoforms [5]. It has been shown that BA, compared with WA, express characteristic mitochondrial FAO enzyme isoforms such as acyl-Coenzyme A (acyl-CoA) synthetase short-chain family member 1 (ACSS1), acyl-CoA synthetase long-chain family member 5 (ACSL5), carnitine palmitoyltransferase 1b (CPT1b), long-chain acyl-CoA dehydrogenase (ACADL),

Abbreviations: WAT, white adipose tissue; BAT, brown adipose tissue; UCP1, uncoupling protein 1; WA, white adipocyte; BA, brown adipocyte; FAO, fatty acid oxidation; FABP3, fatty acid binding protein3/heart-type fatty acid binding protein; Acyl-CoA, acyl-Coenzyme A; ACSS1, acyl-CoA synthetase short-chain family member 1; ACSL5, acyl-CoA synthetase long-chain family member 5; CPT1b, carnitine palmitoyltransferase 1b; ACADL, long-chain acyl-Coenzyme A dehydrogenase; ACADM, medium-chain acyl-Coenzyme A dehydrogenase; ACADS, short-chain acyl-Coenzyme A dehydrogenase; ACAA2, 3-oxoacyl-Coenzyme A thiolase; β_3AR, β_3-adrenergic receptor; cAMP, cyclic AMP; CL, CL316,243; mRNA, messenger RNA; subWAT, subcutaneous WAT; i.p, intraperitoneal; SV, stromal-vascular; cDNA, complementary DNA; Rps18, ribosomal protein S18; PPAR, peroxisome proliferator-activated receptor; PGC-1, PPARγ coactivator 1.

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medium-chain acyl-CoA dehydrogenase (ACADM), short-chain acyl-CoA dehydrogenase (ACADS), and 3-oxoacyl-CoA thiolase (ACAA2). Defects in the *Acadl* or *Acads* gene of mice resulted in an inability to maintain body temperature under cold conditions [6], which suggests that mitochondrial FAO enzymes play a vital role in thermogenesis. Fatty acids delivered to the mitochondria are activated to fatty acyl-CoAs by acyl-CoA synthetases such as ACSS1 and ACSL5. Once activated, long-chain fatty acids require carnitine palmitoyltransferase, including CPT1b, to be transported into mitochondrial matrix. In the matrix space, acyl-CoA dehydrogenases such as ACADL, ACADM and ACADS, and ACAA2 catabolize acyl-CoAs, which are ultimately processed to produce acetyl-CoAs. Thereafter, acetyl-CoAs enter the citric acid cycle and electron transport chain.

Recently, it has been reported that brown fat-like adipocytes having a multilocular morphology and expressing the BA-specific UCP1 protein exist within certain WATs in mice and rats [7]. These cells have been called recruitable brown fat cells, brown in white (brite) cells, or beige cells [8], and they become more prominent upon prolonged stimulation by cold or β_3-adrenergic receptor (β_3AR) agonists such as CL316,243 (CL) that elevate intracellular cyclic AMP (cAMP) [9]. This brown-like transformation of WAT is the most notable in the inguinal subcutaneous depot [10]. The gene expression pattern and origin of beige cells have been reported to be distinct from those of BA [11,12]. However, it has not been well documented whether BA-characteristic FAO proteins are up-regulated in beige cells.

In this study, using C57BL/6J mice, we demonstrated that cold exposure or β_3AR agonist treatment increased messenger RNA (mRNA) and protein expression of FABP3 and increased mRNA levels of several BA-characteristic mitochondrial FAO enzymes in subcutaneous WAT (subWAT). In addition, using primary adipocytes isolated from subWAT, we examined the effect of a β_3AR agonist or cAMP enhancer on the expression of these proteins in adipocytes. Unexpectedly, our results suggest that these BA-characteristic FAO proteins are induced in a different pathway from UCP1.

2. Materials and methods

2.1. Animals

C57BL/6J mice (4 or 8 weeks old, CLEA Japan, Tokyo, Japan) were fed standard rodent chow pellets and water *ad libitum* and were housed at 23 ± 1 °C on a 12 h light/dark cycle. All experimental procedures were conducted in compliance with protocols approved by the Ethical Committee for the Research of Life Science in Kurume University.

2.2. Cold exposure and β_3-adrenergic receptor agonist (CL316,243) treatment in vivo

Eight-week-old male mice were fed standard rodent chow pellets and water *ad libitum*. The mice were housed individually in plastic cages and divided into two groups that were counterbalanced by body mass. For the cold exposure studies, control groups were maintained at 23 ± 1 °C, whereas cold exposure groups were maintained at 4 °C for 5 days. For the CL treatment studies, control groups were injected intraperitoneally (i.p) once daily with saline (200 μl) for 5 days, whereas CL treatment groups were injected i.p once daily with CL (1 mg/kg; Tocris Bioscience, Bristol, UK) in saline (200 μl) for 5 days. After the mice were killed by decapitation, their posterior subcutaneous fat pads (inguinal-dorsolumbar portion) were separated immediately and used for subsequent processing and analyses.

2.3. Adipose tissue fractionation

Adipose tissue was divided into adipocyte and stromal-vascular (SV) fractions. Freshly excised subcutaneous fat pads from 8-week-old male C57BL/6J mice were rinsed in PBS, minced with scissors, and digested with 3 mg/ml collagenase type II (Worthington, Lakewood, NJ, USA) in isolation buffer (123 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 5 mM glucose, 100 mM HEPES, and 4% BSA, pH 7.4) for 1 h at 37 °C. The digested tissue was filtered through a 200 μm nylon mesh to remove undigested tissue and centrifuged at 210×*g* for 1 min. The mature adipocytes floated to the surface, and the SV cells (capillary, endothelial, mast, macrophage, and epithelial cells) were deposited. The floating cells and the SV cells were washed twice with the isolation buffer, recentrifuged at 210×*g*, and collected as the mature adipocytes and SV cells, respectively. Total RNA from the mature adipocytes and SV cells was isolated using TRIzol (Life Technologies Corporation, Carlsbad, CA) reagent. Adequate separation of adipocytes and SV cells was confirmed by RT-PCR for the adipocyte markers *adiponectin* and *Ucp1* and the SV cell marker *Ucp2* (data not shown).

2.4. Quantitative RT-PCR analysis

Total RNA from mouse tissues or cultured cells was isolated using the TRIzol method combined with RNeasy mini columns (QIAGEN, Valencia, CA) according to the manufacturer's instructions. For quantitative RT-PCR, 0.5–1 μg of total RNA was used to synthesize complementary DNA (cDNA). Target cDNA levels were quantified by real-time PCR by using the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) with SYBR Green (Applied Biosystems). Relative mRNA expression levels were calculated using mouse ribosomal protein S18 (*Rps18*). The primer sequences were as follows: *Acaa2* (forward: 5′-ggctctggtttccagtccatc-3′ ; reverse: 5′-gaagcgcacatttctgacacagta-3′), *Acadl* (forward: 5′-ctacctcatgcaagagcttccaca-3′; reverse: 5′-cttcaaacatgaactcacaggcaga-3′), *Acadm* (forward: 5′-tgatgtggcggccattaaga-3′; reverse: 5′-gggttagaacgtgccaacaagaa-3′), *Acads* (forward: 5′-aagtttggatccgcacagcag-3′; reverse: 5′-caagctttggtgccgttgag-3′), *Acsl5* (forward: 5′-cattcggcgggacagtttg-3′; reverse: 5′-atcccattgcagccctgaag-3′), *Acss1* (forward: 5′-agatcctgaagactctgcctgtcc-3′; reverse: 5′-ttgcatcactcaccaatgtcca-3′), *Cpt1b* (forward: 5′-gagacaggacactgtgtgggtga-3′; reverse: 5′-tggtacgagttctcgatggcttc-3′), *Fabp3* (forward: 5′-tggctagcatgaccaagcctactac-3′; reverse: 5′-gttccacttctgcacatggatga-3′), *Rps18* (forward: 5′-ttctggccaacggtctagacaac-3′; reverse: 5′-ccagtggtcttggtgtgctga-3′), and *Ucp1* (forward: 5′-gggcattcagaggcaaatcag-3′; reverse: 5′-ctgccacacctccagtcattaag-3′).

2.5. ELISA

Tissue concentrations of FABP3 protein were measured using a sandwich-type ELISA (Hycult Biotechnology, Uden. The Netherlands) according to the manufacturer's protocol. The values were normalized to the total protein concentrations determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL).

2.6. Primary cell culture and treatment

For the culture of primary subcutaneous white adipocytes, posterior subcutaneous fat pads (inguinal-dorsolumbar portion) were isolated from 4-week-old C57BL/6J male mice. The isolated tissues were rinsed in PBS, minced with scissors, and digested with 1 mg/ml collagenase type II (Worthington) in isolation buffer at 37 °C for 30 min. Cell suspensions were filtered through a 100 μm filter and centrifuged at 210×*g* for 10 min. The pellet consisting of preadipocytes was resuspended in 1 mL of red blood cell lysis buffer (IBL, Gunma, Japan). After incubation for 3 min at