Biochemical and Biophysical Research Communications xxx (2016) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Castration induced browning in subcutaneous white adipose tissue in male mice

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ARTICLE INFO

Article history:
Received 26 August 2016
Accepted 3 September 2016
Available online xxx

Keywords:
Beige adipocyte
High-fat diet
Non-shivering thermogenesis
Obesity
Orchidectomy

ABSTRACT

We demonstrated that castration enhanced the expression of uncoupling protein 1 (Ucp1), a thermogenic protein, in brown adipose tissue (BAT) and subcutaneous (sc) white adipose tissue (WAT) in male mice. Castration of male mice increased body temperature and reduced body weight gain compared with those of sham-operated mice. BAT *Ucp1* mRNA expression in castrated male mice was significantly higher than that in sham-operated mice. Histologically, cells with multilocular fat droplets were observed in the castrated inguinal scWAT. Immunohistochemical staining revealed that these cells positively reacted with the *anti*-Ucp1 antibody. The Ucp1-positive area near the inguinal lymph node in the castrated WAT was extensive compared with that of the sham-operated WAT. Castration-induced Ucp1 up-regulation in scWAT was suppressed by high-fat diet feeding. These findings suggest that thermogenesis by BAT activation and scWAT browning contribute to castration-induced inhibition of body weight gain. However, considering that the effect of castration was blunted by high-fat diet consumption, thermogenesis stimulation in response to castration is inhibited by chronic over-nutrition.

1. Introduction

In contrast to white adipose tissue (WAT), which is involved in the storage and mobilisation of energy in the form of triglycerides, brown adipose tissue (BAT) specialises in dissipating energy as heat during cold- or diet-induced thermogenesis. BAT thermogenic function results from the expression of a series of genes related to high mitochondrial content and elevated cellular respiration that is largely uncoupled from ATP synthesis. The uncoupling occurs through expression of uncoupling protein 1 (Ucp1), a brown adipocyte-specific mitochondrial protein that promotes proton leakage across the inner mitochondrial membrane in mammals [1].

Browning results from Ucp1-positive adipocyte induction within WAT following cold exposure or β3 agonist administration in mice. Although these inducible brown fat cells, called beige/brite adipocytes, can contribute to thermogenesis, their cell lineage is distinct from that of classical brown adipocytes [2]. Specifically, brown adipocytes differentiate from skeletal muscle precursors, whereas beige/brite cells are derived from smooth muscle-like origins [3,4].

In humans, brown adipocytes were discovered using ^{18}F-labelled glucose analogue, fluorodeoxyglucose and positron emission tomography-computed tomography [5–8] and possess molecular signatures resembling those of mouse beige adipocytes [9]. Brown and beige adipocyte thermogenesis, accompanied by enhanced insulin sensitivity, may have useful therapeutic potential for combating obesity and diabetes.

Obesity, defined as excess accumulation of fat depots, is associated with metabolic disorders, including diabetes, arteriosclerosis and hypertension. Obesity prevalence in pets as well as in humans has increased, particularly in developed countries. Neutered cats and dogs are more likely to be overweight than intact animals [10]. Furthermore, there are sex-related differences in post-neutering energy expenditure changes and body weight gain. Neutered female dogs are approximately twice as likely to be overweight than intact female dogs, whereas neutered male dog weights were not significantly different from those of intact male dogs [11]. In general, orchiectomised mice displayed reduced body weight gain

Abbreviations: BAT, brown adipose tissue; sc, subcutaneous; CAST, castrated mice; HFD, high-fat diet; LN, lymph node; ND, normal diet; SHAM, sham-operated mice; Ucp1, uncoupling protein 1; WAT, white adipose tissue.

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http://dx.doi.org/10.1016/j.bbrc.2016.09.017
0006-291X/

compared with that of sham-operated mice [12,13]. Total and resting energy expenditures adjusted for total or lean body weight were modestly increased following gonadectomy in male cats [14], although this finding remains controversial. Furthermore, subcutaneous (sc) WAT from castrated male but not female mice exhibited enhanced insulin-induced glucose uptake compared with that of sham-operated mice, indicating enhanced insulin sensitivity in scWAT of castrated male mice [15]. These observations led us to the hypothesis that enhanced BAT Ucp1 expression and WAT browning occur following castration in males. In the present study, we investigated Ucp1 expression in BAT and scWAT in castrated male mice. Our study revealed that WAT browning occurs following male castration. This browning was mitigated by high-fat diet feeding.

2. Materials and methods

2.1. Mice and treatments

C57BL/6J male mice (6 weeks old) were obtained from CLEA Japan Inc. (Tokyo, Japan). Mice were castrated or sham operated at 7 weeks of age and housed individually. All operative procedures were performed under pentobarbital anaesthesia (50 mg/kg body weight, intra peritoneal injection). An incision was made in the wall of the abdomen. The testis with epididymis was removed following seminal duct ligation. Mice were maintained in a 12-h light–dark cycle at 24 ± 2 °C and given a normal diet (ND: CE-2; CLEA Japan Inc.) and water *ad libitum*. In some experiments, mice received a high-fat diet (HFD: D12492; Research Diets Inc., New Brunswick, NJ, USA) starting at 8 weeks of age. Body weight and rectal temperature were monitored weekly. Temperature was measured using a microprobe thermometer system equipped with a rectal probe (Model BAT-12; Muromachi Kikai Co., Tokyo, Japan).

All experiments were performed between 13:00 and 16:30 during the light cycle. Experimental procedures and animal care were performed in accordance with the requirements of the Institutional Animal Care Committee at Kitasato University, in compliance with National Institutes of Health guidelines.

2.2. Histological analysis

Mouse interscapular BAT and scWAT from the inguinal region were fixed in Bouin's fluid and embedded in paraffin. Four micrometer sections were affixed to slides and stained with haematoxylin and eosin. For immunohistochemistry, deparaffinised sections were incubated with H_2O_2, blocked with 10% normal goat serum, incubated with a rabbit polyclonal to Ucp1 antibody (3 μg/mL, No. ab10983; Abcam, Cambridge, UK) overnight at 4 °C, and then visualised with 3,3′-diaminobenzidine tetrahydrochloride using the Histofine Simple Stain MAX-PO kit (Nichirei, Tokyo, Japan), as previously described [16]. Subsequently, the rate of Ucp1 positive adipocytes in inguinal scWAT was calculated. Arbitrary fields of view observed under a 4 × objective lens (approximately 2×10^6 μm^2/tissue) were analysed using the NIH image J 1.48 Mac OS X (National Institutes of Health, Maryland, USA) for estimation of Ucp1-positive adipocytes in an area near the inguinal lymph nodes [17].

2.3. RNA isolation and RT-quantitative PCR (RT-qPCR)

Adipose tissue *Ucp1* expression was analysed by RT-qPCR as described previously [18]. Briefly, total RNA from adipose tissues was extracted using Isogen (Nippon Gene Co. Ltd., Tokyo, Japan). cDNA was reverse transcribed from 5 ng of total RNA and used as a template. The primer sets used were as follows: 5′-ctttgcctcactcaggattgg-3′ and 5′-actgccacacctccagtcatt-3′ (for *Ucp1*, NM_009463) and 5′-ccaatgactcctatgaccccta-3′ and 5′-cagccaagattcacggtagat-3′ (for *Tbp*, NM_013684). The Ct value was determined, and *Ucp1* gene transcript abundance was analysed using the ΔΔCt method with *Tbp* as the reference gene [19].

2.4. Statistical analyses

Results are expressed as means ± SEM. Gene expression data were log-transformed to provide an approximation of a normal distribution before analyses. Student's t tests were performed using Prism5 software (GraphPad Software Inc., San Diego, CA, USA) to compare results between two sets of data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of castration on Ucp1 expression in inguinal WAT

Consistent with previous reports [12,13], castration resulted in reduced body weight gain in male mice compared with that of sham-operated mice (Fig. 1A). The body temperature of castrated mice was higher than that of sham-operated mice (Fig. 1B). BAT *Ucp1* gene transcript levels were significantly higher in castrated mice than those in sham-operated mice (Fig. 2A). *Ucp1* mRNA expression in scWAT tended to increase following castration. Histologically, cells with multilocular fat droplets were observed in

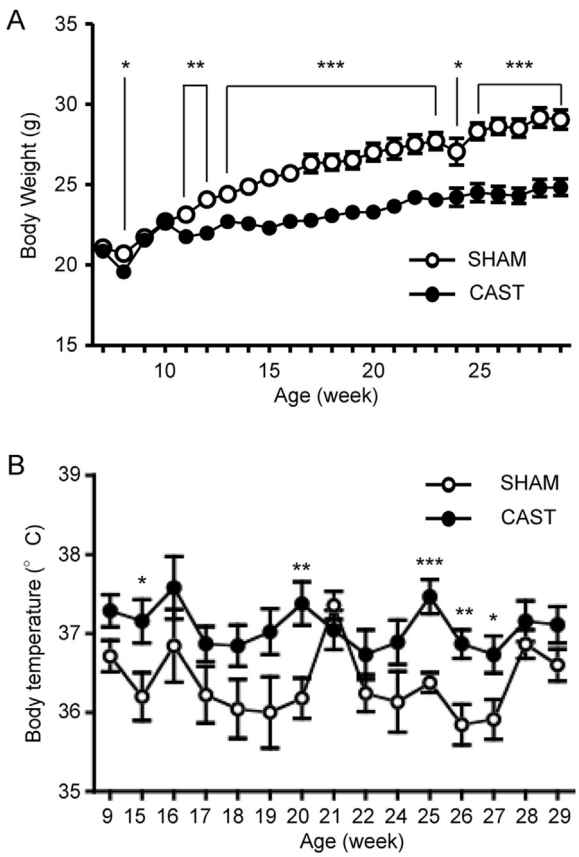

Fig. 1. Effect of castration on body weight and body temperature in male mice. C57BL/6J male mice (7 weeks old, $n = 9$) were castrated or sham operated, and body weight (A) and rectal temperature (B) were measured weekly as indicated. Values are mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. SHAM, sham-operated mice. CAST, castrated mice.

Please cite this article in press as: O. Hashimoto, et al., Castration induced browning in subcutaneous white adipose tissue in male mice, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.09.017