Supporting Information

Lactate induces the development of beige adipocytes via an increase in the level of reactive oxygen species.

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1. Supporting materials and methods

The antibodies and assay ID numbers of the TaqMan gene expression assays used in this study are shown in the tables below.

List of antibodies

Antibody	Manufacturer	Product No.
COXIV	Cell Signaling Technology, Tokyo, Japan	#4844
GAPDH	Fujifilm Wako Pure Chemical Corporation, Osaka, Japan	016-25523
LDHA	Proteintech, Tokyo, Japan	19987-1-AP
LDHB	Proteintech, Tokyo, Japan	19988-1-AP
MCT1	Proteintech, Tokyo, Japan	20139-1A-AP
PGC-1α	Abcam, Tokyo, Japan	ab54481
UCP1	Abcam, Tokyo, Japan	ab209483

List of ID numbers of TaqMan gene expression assays

UCP1	Mm01244861_m1
Cidea	Mm00432554_m1
Elovl3	Mm00468164_m1
Cox8b	Mm00432648_m1
TBP	Mm00446971_m1

Cidea, cell death-inducing DNA fragmentation factor, alpha subunit-like effector A

Elovl3, elongation of very long chain fatty acids-like 3

Cox8b, cytochrome C oxidase subunit VIII b

Immunoblot analysis. The tissue samples were homogenized, centrifuged and the total protein concentrations of the obtained supernatant were determined using a Protein Assay System (Bio-Rad, Richmond, CA, USA) with bovine γ-globulin employed as a standard. Aliquots of the supernatant were treated with Laemmli sample buffer for 5 min at 100 °C. The samples were then loaded onto an SDS-PAGE system. The resulting gel was

transblotted onto a PVDF membrane, which was blocked with 5% skim milk for 1 h at room temperature. After a washing with 20 mM Tris-HCl-buffered saline containing 0.05% (w/v) Tween 20 (TTBS), the membrane sheets were reacted with various antibodies for 16 h at 4 °C. After a washing with TTBS, the membranes were reacted with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibodies (1:2000 dilution; Cell Signaling Technology, Tokyo, Japan) for 1 h at room temperature. After a washing, immunoreactivity was visualized using the ECL reagent (Thermo Fisher Scientific, Yokohama, Japan), and the relative signal intensity was evaluated with iBright CL1500 Imaging System (Thermo Fisher Scientific).

2. Supporting Table.

Table S1. Body weight, food intake, and relative tissue weights in the control and lactate groups.[1]

	Control	Lactate
Initial body weight, *g*	18.7 ± 0.4	18.6 ± 0.3
Final body weight, *g*	26.3 ± 0.6	27.2 ± 0.5
Food intake, *g/(4 weeks · mouse)*	98.9 ± 1.7	105.3 ± 1.9*
Water intake, *g/(4 weeks · mouse)*	94.1 ± 4.2	111.2 ± 4.6*
iWAT, *g/100 g body*	1.84 ± 0.07	1.91 ± 0.08
eWAT, *g/100 g body*	2.00 ± 0.13	2.16 ± 0.09
Interscapular BAT, *g/100 g body*	0.48 ± 0.03	0.53 ± 0.01

[1] Values are presented as means ± SEM (n = 10).

*, Significantly different from the control group (p < 0.05).

3. Supporting Fig.

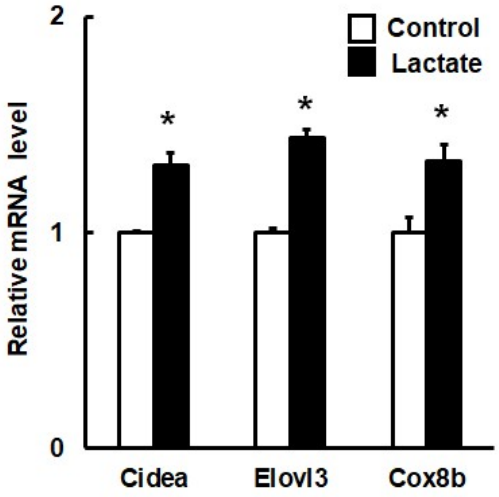

Fig. S1. mRNA levels of browning markers in C3H10T1/2 cells treated with vehicle (control) or 20 mM lactate for 16 h. The mRNA levels of each target genes are expressed as fold-change relative to the control (= 1) after normalization to the mRNA levels of TBP. Data are presented as means ± SEM (n = 3). *Mean values are significantly different from those of the control group ($p < 0.05$). Cidea, cell death-inducing DNA fragmentation factor, alpha subunit-like effector A; Elovl3, elongation of very long chain fatty acids-like 3; Cox8b, cytochrome C oxidase subunit VIII b.