Materials and Methods

Mouse Studies

Wild-type C57BL/6 N mice were obtained from Charles River Laboratories. All experiments were performed in adult male mice kept on an inverted 12-h:12-h dark:light cycle, fed ad libitum with a chow diet. All animal studies were approved by the Veterinäramt Zürich.

Pump implantation

Micro-osmotic pumps (1007D, Alzet) were filled with 100 ul of sodium acetate (30 mM, pH 7.4), or CFMB (10 uM, Calbiochem 371725), or saline solution as control. A catheter (MIP-01, Alzet) was connected to the pump and the pump was immersed in saline at 37°C for 16 hours. Mice were anesthetized with Isoflurane. Fur on the interscapular area was removed with Veet cream, an “I-shaped” cutaneous incision was made to expose the brown adipose tissue. The pump was inserted underneath the skin and the catheter was inserted in the fat pad. The skin flap was closed with stitches and tissue adhesive solution. The catheter was tethered to the skin for stabilization. Carprofen (5mg/kg) injection was applied after surgery as analgesia.

Tissue harvest

Mice were euthanized in a CO_2 atmosphere. Blood was collected by cardiac puncture, and serum was obtained by centrifuging coagulated blood at 3,000 rpm for 15 min at 4 °C.

Acetate abundance determination

Acetate concentration was measured as previously established (1, 2) using the Acetate Colorimetric Assay Kit (MAK086, Sigma). Brown adipose tissue or serum were minced in lysis buffer and incubated with the Acetate Enzyme Mixture and Acetate Substrate Mix for 40 minutes at room temperature. The absorbance of the final solution was measured using a Gen5 plate reader (BioTek) at 450 nm.

Histology and image analysis

Histology was performed according to a previously established protocol (3). Brown adipose tissues were excised, fixed in fresh 4% paraformaldehyde (Sigma) in PBS (Gibco, pH 7.4) for 24 h at 4 °C and then embedded with paraffin. 4-μm-thick paraffin sections were subjected to histological staining. Heat-induced antigen retrieval was applied on rehydrated paraffin sections. After blocking with 5% BSA for 1 h, primary antibody (1:200 anti-UCP1; ab10983, Abcam) diluted in 5% BSA was applied to sections overnight at 4 °C. After washing with PBS, a secondary antibody (Signal Stain Boost IHC, Cell Signaling) was applied according to Cell Signaling’s manual, the sections were washed three times, and the signals were detected using the DAB method (Cell Signaling). Standard H&E staining was performed on rehydrated fat-paraffin sections. Slides were dehydrated and covered with coverslip by resin-based mounting. All images were acquired by an Axioscope A1 instrument.

RNA analysis

Total RNA was extracted from tissues or cells using Trizol reagent (15596018, Invitrogen) according to the manufacturer’s instructions. Quality of the RNA from BAT was determined using a TapeStation instrument (Anglient). All samples had an RNA integrity number (RIN) > 8. The rRNA was depleted, and purified RNA was used for the preparation of libraries using the TruSeq Stranded Total RNA (20020596, Illumina); samples were sequenced on a Novaseq 6000 instrument. Fastq reads were mapped to GENCODE Release M26 (GRCm39). Transcripts were defined using the Ensemble annotations over protein-coding mRNAs and long non-coding RNA. Differential expression analysis of mapped RNA-seq data was performed using DESeq2, MetaCore (Clarivate) was applied for pathway analysis. All RNA sequencing data that support the finding of this study have been deposited in ArrayExpress, with the accession code E-MTAB-10680. Primers applied for RT-PCR are *Adipoq* FW: TGTTCCTCTTAATCCTGCCCA, *Adipoq* RV: TGTTCCTCTTAATCCTGCCCA; *Cidea* FW: GCAGCCTGCAGGAACTTATCAGC, *Cidea* RV: GATCATGAAATGCGTGTTGTCC; *Ucp1* FW: GATCATGAAATGCGTGTTGTCC, *Ucp1* RV: CAATGAACACTGCCACACCTC.

Western blot analysis

Protein samples were isolated from adipose tissue with RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS and 10% glycerol) supplemented with protease inhibitor cocktail (Roche) and Halt phosphatase inhibitor (Thermo Scientific). Homogenized protein lysates were obtained by rotation at 4 °C for 30 min, followed by centrifugation at 14,000 rpm for 30 min. Protein amounts were quantified using the DC Protein Assay (Bio-Rad). Protein samples were heated at 95 °C for 5 minutes, separated by SDS-PAGE on 12% polyacrylamide gels and transferred onto nitrocellulose membrane. Membranes were probed using the indicated antibodies (1:1000 anti-UCP1, ab10983, Abcam; 1:1000 anti-HSP90, 4877S, CST), and chemiluminescent signals were detected by a LAS 4000 Mini Image Quant system (GE Healthcare). Band intensity was quantified using ImageJ.

Analysis of adipocyte differentiation

For cellular separation, dissected adipose tissues were minced with a scalpel blade and incubated in 2.0 ml per mg wet tissue 0.2% collagenase type II in collagenase buffer (25 mM $KHCO_3$, 12 mM KH_2PO_4, 1.2 mM $MgSO_4$, 4.8 mM KCl, 120 NaCl, 1.2 mM $CaCl_2$, 5 mM glucose, 2.5% BSA, 1% penicillin–streptomycin, pH 7.4) for 50 min at 37 °C with occasional resuspension. 10 ml centrifugation buffer (70% PBS, 15% FBS, 15% HistoPaque 1119) was added, and samples were centrifuged for 5 min at 200 g. The SVF pellet from the initial centrifugation was resuspended in 2 ml erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.4) and incubated for 4 min on ice. Samples were filtered through 40-μm cell strainers and then centrifuged for 5 min at 200 g. The supernatant was removed, and the pellets were resuspended in culture medium (10% FBS and 1% P/S in DMEM); SVF cells were plated in a plate that was precoated with collagen I and differentiated. Cells were re-fed every 48 h with 1 μM rosiglitazone and 0.5 μg/ml insulin. Differentiated adipocytes at day 10 were used for differentiation analysis. Briefly, cells in a 96-well optical plate were fixed with 5% formaldehyde at 4 °C for 10 min, followed by three washes with PBS. Cells were stained with LD540 for lipid droplets and Hoechst 33342. 33 images per well were taken with an automated microscope imaging system (Operetta, Perkin Elmer). Images were analyzed using the Operetta imaging software as described previously (4).

Extracellular respiration

Primary brown preadipocytes were counted and plated at a density of 7,000 cells per well of a Seahorse plate and differentiated. At day 10 post-differentiation induction, mature brown adipocytes were loaded to an XF96 Extracellular Flux Analyzer (Seahorse Bioscience).

Indirect calorimetry

Indirect calorimetry measurements were performed with the Phenomaster (TSE Systems) according to the manufacturer’s guidelines and protocols. Mice were single-caged and acclimated to the metabolic cages for 48 h before metabolic recording.

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