SUPPLEMENTAL METHODS

Western blot

Proteins were extracted using PRO-PREP (Intron Biotechnology, Seongnam, Korea) and quantified using the Bradford method. The quantified proteins were mixed with sodium dodecyl sulfate (SDS) sample buffer (Biosesang, Seongnam, Korea) and boiled for about 10 minutes. After cooling in ice, the proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and moved to a 0.45-µm nitrocellulose membrane (Amersham Bioscience, Watertown MA, USA). The membranes were reacted sequentially with primary antibody, secondary antibody conjugated with horseradish peroxidase, and ECL Western blotting solution (Bio-Rad Laboratories, Hercules, CA, USA). The band densities were measured using ImageJ software (National Institute of Mental Health, Bethesda, MD, USA). The abundance of each protein was calculated by target protein band density per beta actin band density and phosphorylated-form band density per total-form band density.

CL treatment

CL 316,243 (CL, 1 mg per kg, daily) was administered to mice via intraperitoneal (IP) injection for 14 days, and then fat and muscle tissues were harvested. CL was dissolved in phosphate buffered saline (PBS) (1 mg/mL, stock solution) and stored at –20°C. The stock solution was diluted 1/10 using PBS (0.1 mg/mL, working solution). The volume of working solution required for the IP injection was as follows: volume of IP glucose injection (µL)=10× body weight (g). Therefore, animals were injected with 1 mg of CL per kg, with the vehicle being PBS.

Antibodies

We used the following antibodies: anti-beta actin rabbit antibody (sc-130656, 1:2,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-phosphorylated adenosine monophosphate-activated protein kinase (AMPK) rabbit antibody (#2535, 1:2,000 dilution), anti-total AMPK rabbit antibody (#2532, 1:2,000 dilution), anti-phosphorylated p38 rabbit antibody (#9211, 1:2,000 dilution), anti-total p38 rabbit antibody (#9212, 1:2,000 dilution), anti-hormone-sensitive lipase rabbit antibody (#4107, 1:2,000 dilution, Cell Signaling Technology, Danvers, MA, USA), anti-sestrin2 rabbit antibody (ab178518, 1:2,000 dilution), anti-peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) rabbit antibody (ab54481, 1:2,000 dilution), anti-uncoupling protein 1 (UCP1) rabbit antibody (ab10983, 1:2,000 dilution), anti-phosphorylated forkhead box O1 (FOXO1) rabbit antibody (ab38501, 1:2,000 dilution), anti-total FOXO1 rabbit antibody (ab34406, 1:2,000 dilution, Abcam, Cambridge, MA, USA), anti-F-box protein-32 (atrogin1) goat antibody (PAB15627, 1:2,000 dilution), and anti-tripartite motif containing 63 (muscle ring-finger protein-1 [MurF1]) goat antibody (PAB7497, 1:2,000 dilution, Abnova, Taipei, Taiwan).

Quantitative real-time polymerase chain reaction

Total RNA from adipose and muscle tissue was extracted using Qiazol Lysis Reagent (Qiagen, MD, USA) according to the user manual and used for cDNA synthesis. Each cDNA was analyzed for gene expression by quantitative real-time polymerase chain reaction (PCR). All transcript levels were normalized to mouse beta-actin.

Primers

The primer sets used for polymerase chain reaction polymerase chain reaction (qPCR) were as follows: *UCP1*, 5′-ACT GCC ACA CCT CCA GTC ATT-3′ (forward) and 5′-CTT TGC CTC ACT CAG GAT TGG-3′ (reverse); PR domain containing 16 (*PRDM16*), 5′-CAG CAC GGT GAA GCC ATT C-3′ (forward) and 5′-GCG TGC ATC CGC TTG TG-3′ (reverse); elongation of very long chain fatty acids protein 3 (*Elovl3*), 5′-TCC GCG TTC TCA TGT AGG TCT-3′ (forward) and 5′-GGA CCT GAT GCA ACC CTA TGA-3′ (reverse); cell death activator CIDE-A (*Cidea*), 5′-TGC TCT TCT GTA TCG CCC AGT-3′ (forward) and 5′-GCC GTG TTA AGG AAT CTG CTG-3′ (reverse) [1]; mitochondrial transcription factor A (*Tfam*), 5′-CCC AAA TTT AAA GCT AAA CAC CCA GAT GC-3′ (forward) and 5′-CCC ATC AGC TGA CTT GGA GTT AGC-3′ (reverse); nuclear respiratory factor 1 (*NRF1*), 5′-GGC TGC TGC AGG TCC TGT GGG-3′ (forward) and 5′-GGT GCT GCG CCA AAC ACC-3′ (reverse) [2]; myoblast determination protein 1 (*MyoD*), 5′-AGT GAA TGA GGC CTT CGA GA-3′ (forward) and 5′-GCA TCT GAG TCG CCA CTG TA-3′ (reverse); myocyte enhancer factor 2C (*Mef2c*), 5′-CGG TGT CGT CAG TTG TAT GG-3′ (forward) and 5′-TGC AGT AGA TAT GCG GCT TG-3′ (reverse); and myogenic factor 5 (*Myf5*), 5′-AGG AAA AGA AGC CCT GAA GC-3′ (forward) and 5′-GCA AAA AGA ACA GGC AGA GG-3′ (reverse) [3].

Hematoxylin and eosin staining

The fixed adipose tissues were embedded and cut into 4-µm sections. The sections were deparaffinized by xylene and sequentially rehydrated by 100%, 90%, 80%, and 70% ethanol.

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The rehydrated sections were stained with hematoxylin and eosin, sequentially dehydrated by 70%, 80%, 90%, and 100% ethanol, and soaked in xylene for clearing. The samples were mounted and observed under a microscope. Quantification of lipid droplet diameter was done in five randomly selected views using ImageJ software.

Mitochondrial DNA measurement

Genomic DNA (gDNA) and mitochondrial DNA (mtDNA) were extracted using lysis buffer (consisting of 100 mM Tric-HCl; pH 8.5, 5 mM ethylene-diamine-tetraacetic acid (EDTA), 0.2% SDS, 200 mM NaCl, 100 ug proteinase K/mL) and used for quantitative real-time PCR. mtDNA content was calculated using the ratio of nicotinamide adenine dinucleotidedehydrogenase subunit I (Nd1) to lipoprotein lipase (Lpl) [4].

The primer sets used for qPCR were as follows: Nd1, 5′-CCC ATT CGC GTT ATT CTT-3′ (forward) and 5′-AAG TTG ATC GTA ACG GAA GC-3′ (reverse); and Lpl, 5′-GGA TGG ACG GTA AGA GTG ATT C-3′ (forward) and 5′-ATC CAA GGG TAG CAG ACA GGT-3′ (reverse) [4].

Serum free fatty acid measurement

The level of serum free fatty acid was calculated using the Free Fatty Acid Assay Kit (BioMax, Seoul, Korea) according to the user manual.

Statistical analysis

Values are presented as the mean±standard deviation and were obtained from six mice per group. The significance of the differences between groups was determined by analysis of variance (ANOVA) and the *post hoc* Tukey-Kramer test. Results were considered significant when $P<0.05$.

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