SI Text

ACC Activity Assay. ACC activity in individual hypothalamic nuclei (Arc, PVN, and LHA) was measured by $^{14}CO_2$ fixation to acid-stable products (1, 2). Freshly dissected tissue pellets were homogenized in the lysis buffer containing 20 mM Tris·HCl (pH 7.4), 50 mM sodium chloride, 250 mM sucrose, 2 mM DTT, 1% Triton X-100, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, and 1× protease inhibitors (Roche). The reaction was started by mixing homogenized sample (30-50 μg of protein) with the reaction buffer containing [^{14}C]$NaHCO_3$ (0.05μCi/μl) (1 Ci = 37 GBq). After an incubation for 20 min at 37°C, HCl was added, and the reaction mixture was dried under 55°C for 5-6 h. Water was added to the dried mixture, and the [^{14}C] activity was counted. ACC activity was expressed as picomoles of $NaHCO_3$ (incorporated into acid-stable products) per minute. The value was further normalized to the total protein content (in micrograms) in the sample. Previous studies have demonstrated that in addition to ACC, carboxylases such as pyruvate carboxylase and propionyl-CoA carboxylase also use bicarbonate as a substrate in the cellular carboxylation reaction (2). To minimize the contribution from carboxylases other than ACC, the assay was performed in the presence or absence of sodium citrate (16.7 mM), a specific allosteric activator of ACC (1, 2). ACC activity was then calculated by subtracting the activity measured in the absence of added citrate from the activity in the presence of the indicated concentration of citrate (1, 2). Added citrate stimulated the incorporation rate of $NaHCO_3$ from basal level (i.e., without added citrate) by 2.5-fold. To measure the ACC activity in the hypothalamic nuclei in response to leptin, rats were fasted overnight before ICV leptin or vehicle (PBS). The ACC activities after fasting were 0.1-0.3 pmol/min/μg. These values are comparable with the reported ACC activity from skeletal muscle (1) or heart (2) by using $^{14}CO_2$ fixation assay. In the MBH (Arc) and the PVN, the increase of the $NaHCO_3$ incorporation rate detected in the leptin-treated sample is greater in the presence of added citrate than that in the absence of added citrate, which shows that leptin specifically activates ACC, which is sensitive to citrate.

Quantification of LCFA-CoA in Brain Nuclei. Quantification of LCFA-CoA (palmitoyl-CoA, 16:0; and oleoyl-CoA, 18:1) in the dissected hypothalamic nuclei (MBH, PVN, and LHA) was performed based on established protocols (3, 4) with modifications. The samples containing LCFA-CoA were sonicated for 20 sec in KH_2PO_4 solution (100 mM, pH 4.5), and then the lysates were extracted with equal volume of 2-propanol. Appropriate volume of ammonium sulfate and acetonitrile were added successively to the mixture. After centrifugation, the organic layers containing LCFA-CoA were evaporated under a stream of nitrogen. The solid residue was mixed well with 0.5 M chloroacetaldehyde reagent and centrifuged. The supernatant was heated at 85°C for 20 min in a Teflon-capped glass HPLC vial and subjected to HPLC analysis. HPLC analysis was accomplished by Beckman System Gold 126 Solvent Module and Beckman 32 Karat Software for acquisition and analysis of data. A Luna 150 × 2 mm column of phenylhexyl-coated 5-μm silica particles was used along with a C18 guard cartridge (Phenomenex) maintained at 40°C for the separation of individual LCFA-CoA. The mobile phase consisted of (*i*) 0.25% triethyl amine/0.1% tetrahydrofuran and (*ii*) 90% acetonitrile. Samples of 20 μl were injected under initial conditions of 20% *ii* and a flow rate of 0.4 ml/min. A gradient of 20-35% *ii* in 30 min followed by increase to 100% *ii* over 2 min at 40 min was applied. Then, at 47min, the flow rate was increased to 0.75 ml/min over 30 sec. At 52 min, the mobile phase was brought back to 20% *ii* over 2 min, and at 57 min, the flow rate was reduced to 0.4 ml/min over 1 min, thus reestablishing the initial conditions. The eluent was monitored by a PerkinElmer LS-5 Fluorescence Spectrophotometer (excitation, 230 nm; emission, 420 nm). The reproducibility was essentially 95% when the heptadecanoyl-CoA (17:0; Sigma) was used as the internal standard. The detection range was from 10 fmol to 10,000 fmol. To calculate the LCFA-CoA amount in the samples, the same LCFA-CoA (i.e., palmitoyl-CoA and oleoyl-CoA; Sigma) was used as the standard. The LCFA-CoA level was normalized to the protein content in the sample. The LCFA-CoA levels that we detected are comparable with the reported values of brain LCFA-CoA (3).

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