SUPPLEMENTRAY METHODS

INTRAPERITONEAL PYRUVATE TOLERANCE TEST

Mice fasted overnight were injected intraperitoneally with 2 g/kg sodium pyruvate. Tail blood was collected at 0, 15, 30, 45, 60, 90 and 120 min and glucose levels were determined using a glucometer (Elite; Bayer, Pittsburgh, PA).

HYPERINSULINEMIC/EUGLYCEMIC CLAMP AND TISSUE UPTAKE OF 2-DEOXYGLUCOSE

Catheterization of jugular vein and carotid artery was performed in Sirt1-LKO and littermate control mice 5 days before the insulin clamp to allow for post-operative recovery. Insulin clamps and glucose tracer analyses in conscious, cannulated mice were performed as described (1). Glucose uptake into specific tissues was also measured by the bolus administration of 2-deoxy-d-[1-^{14}C] glucose (Perkin Elmer, Wellesley, MA) at the end of the clamp procedure. The mice were euthanized 25 min later and tissue samples were collected, weighed, and digested in NCSII solubilizer (Amersham, Piscataway, NJ). Radioactivity in tissue and plasma samples was determined by liquid scintillation spectrometry.

SERUM INSULIN LEVEL

Serum insulin levels were measured using the Ultrasensitive Mouse Insulin ELISA Assay following the manufacturer's instructions (Crystal Chem, Elk Grove Village, IL).

HEPATIC FATTY ACID PROFILE AND LIVER TRIGLYCERIDE MEASUREMENTS

Snap-frozen liver samples were weighed, total lipids were extracted and fatty acids in mouse liver were measured as described in (2). A 500 µL aliquot of the combined organic fractions was

mixed with 1 mL of TRITON X-100 5% in chloroform and evaporated under a stream of nitrogen. Samples were reconstituted by adding 1 mL of water and triglycerides were measured using the EnzyChrom Triglyceride Assay kit (BioAssay Systems, Hayward, CA).

QUANTITATIVE REAL-TIME PCR

We used a model StepOne™ Real-Time PCR System (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA) and iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories, Hercules, CA). Predesigned mouse and human *β-actin*, *Sirt1* and *Rictor* primers were from Qiagen (Valencia, CA). Gene expression values were calculated based on the ΔΔCt method.

WESTERN BLOTTING AND IMMUNOPRECIPITATION

Protein was extracted from frozen liver samples or from cultured mouse primary hepatocytes or HepG2 cells. Western blotting was performed as described (3), and immunoprecipitation was performed using the Dynabeads® Protein G magnetic beads (Invitrogen, Gaithersburg, MD). Phospho-mTOR$_{S2448}$, Phospho-mTOR$_{S2481}$, mTOR, Phospho-p70S6K$_{T389}$, Phospho-S6$_{S235/236}$, Phospho-4EBP1$_{T37/46}$, Phospho-4EBP1$_{S65}$, Sirt1, Rictor, phospho-Akt$_{S473}$, phospho-FOXO1$_{S256}$ and phospho-AMPK$_{T172}$ antibodies were from Cell Signaling Technology (Danvers, MA). Sirt1 and Rictor antibodies for immunoprecipitation were from Santa Cruz Biotechnology (Dallas, TX). β-actin antibody was from Abcam (Cambridge, MA). 4-HNE antibody was from NOF Corporation (White Plains, NY).

SIRT1 ACTIVITY ASSAY

Sirt 1 activity was measured by Sirt1 immunoprecipitation followed by HDAC colorimetric activity assay using COLOR DE LYS® HDAC colorimetric activity assay kit (Enzo Life Sciences, Farmingdale, NY).

FATTY ACID OXIDATION

Fatty acid β-oxidation was measured as previously described (4) with modifications, using the same liver lobe from each animal. Tissue homogenates (2.5 mg of liver) were used to measure total (both mitochondrial and peroxisomal) fatty acid oxidation activities in buffer containing KCl (30 mM), Tris/HCl (75 mM), EGTA (0.7 mM), $MgCl_2$ (5 mM), NAD (1 mM), ATP (5 mM), CoA (100 μM), l-carnitine (0.4 mM), l-malate (0.5 mM) and cytochrome c (25 μM), pH 7.4; or to measure peroxisomal activity only, in the same buffer but lacking l-carnitine, l-malate and cytochrome c, and including antimycin (73 μM) and rotenone (10 μM) to block the respiratory chain. Fatty acid β-oxidation was initiated by addition of 120 μM [1-^{14}C] oleic acid (0.9 μCi/assay, 59 mCi/mmol) as potassium salt, bound to BSA in a 5:1 molar ratio. The end products were filtered for radioactivity measurements. All chemicals were from Sigma-Aldrich (St. Louis, MO) whereas [1-^{14}C] oleic acid was from Perkin Elmer (Wellesley, MA).

MITOCHONDRIA COMPLEX ACTIVITY MEASUREMENTS

Mitochondrial Complex I enzyme activity was determined using a microplate colorimetric assay kit (ab109721, Abcam, Cambridge, MA) with purified liver mitochondria (ab110168) according to the manufacturer's protocol. Data were expressed as fold change relative to control.

INDIRECT CALORIMETRY

O_2 consumption and CO_2 production were monitored by indirect calorimetry (Oxymax, Columbus Instruments) in metabolic chambers as described in (1). The chambers were equipped with two-dimensional infrared beam sensors (Opto-M3; Columbus Instruments, USA) to measure locomotor activity. The animals were acclimated to the chambers for 2 days, and VO_2 and VCO_2 measurements taken every 18 minutes were collected and recorded on a computer over the next 24 hours. During the 12-hour dark/12-hour light phases, mice had free access to food and water. Net oxidation rates of fat and carbohydrates were calculated

according to the formulae by Simonson and DeFronzo (5): Fat oxidation = 1.69 × (VO_2–VCO_2); Carbohydrate oxidation = 4.57 × VCO_2–3.23 × VO_2. Values were normalized with respect to the body weight and adjusted to an effective metabolic body size ($kg^{0.75}$).

TOTAL ANTIOXIDANT CAPACITY

Total antioxidant capacity in the circulation were measure using serum samples by a colorimetric kit from Abcam (ab65329) according to manufacture protocol.

FATTY ACID OXIDATION ASSAY IN LIVE CELLS

Fatty acid oxidation rate was measured by cellular energy flux analysis using a fluorescence FAO kit from Abcam (ab217602).

TISSUE HISTOLOGY AND STAINING

Formalin-fixed liver samples were processed, and 4-μm–thick paraffin sections were stained with hematoxylin and eosin, Gills Formula (H&E, Thermo Fisher Scientific, Waltham, MA) and analyzed using an Olympus BX41 microscope (USA).

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