Supporting Information

Vatner et al. 10.1073/pnas.1423952112

SI Methods

Short Hyperinsulinemic-Euglycemic Clamp Studies. To assess classical hepatic insulin signaling in rats treated with insulin receptor ASO vs. control ASO, insulin infusions were performed to attain a hyperinsulinemic-euglycemic state for 20 min. A primed/continuous infusion of human insulin (40 mU/kg·min for 3 min/[4 mU/kg·min; Novo Nordisk) and a variable infusion of ∼20% (wt/vol) dextrose to maintain euglycemia were used. At the end of the clamp, rats were anesthetized with sodium pentobarbital injection (75 mg/kg), and all tissues were taken within 1 min, frozen immediately using cooled aluminum tongs in liquid N_2, and stored at −80 °C.

In Vivo de Novo Lipogenesis Studies. Four groups of rats were studied: regular chow-fed controls were compared with high fat diet-fed rats and control ASO-injected controls were compared with IRASO-injected rats. Three days before death, total body water deuterium enrichment was increased by s.c. injection of a bolus of normal saline made with 99% ^{2}H-enriched water (Cambridge Isotope Laboratories), at a dose of 23.4 mL/kg. The rats were given 6% (vol/vol) ^2H_2O-enriched drinking water for the ensuing 3 d. Liver and plasma were taken during euthanasia under isoflurane. Hepatic palmitate isotope enrichment was determined by GC/MS (described below). The plasma ^2H_2O pool was assessed by synthesis of acetylene from plasma treated with calcium carbide. Deuterium enrichment of acetylene was analyzed by GC/MS.

Calculation of de novo hepatic palmitate synthesis from isotopic data has been previously described (1–3). Fraction hepatic palmitate pool newly synthesized over the 3 d (F) was calculated by the following equation: $F = ME/(N \times p)$, where molar enrichment (ME) equals the sum of $m1 + (2 \times m2)$, where m1 and m2 are the atom percent enrichments of singly and doubly deuterium labeled palmitate; N is the number of exchangeable hydrogens, previously reported to be 22; and p is the plasma atom percentage of enrichment of deuterium in water.

Biochemical Analysis. Glucose concentrations were determined using an YSI (2700 select), plasma fatty acids and triglycerides were determined using standard kits (Wako and Sekisui, respectively), and insulin and C-peptide concentrations were measured using an RIA kit.

Liver triglycerides were extracted using ∼100 mg tissue. Tissues were homogenized in ice cold 2:1 chloroform:methanol, and lipids were extracted with shaking at room temperature for 3–4 h. Sulfuric acid was added to ∼100 mM, and samples were vortexed and then centrifuged to achieve phase separation. The organic phase was collected for analysis of triglyceride content (Sekisui Triglyceride-SL kit; Sekisui Diagnostics) and analysis of $U^{13}C$ palmitate enrichment.

Hepatic Gene Expression. Total RNA was extracted from ∼20 mg liver, using the RNeasy mini kit (Qiagen). RNA was reverse-transcribed into cDNA with the use of the QuantiTect Reverse Transcription Kit (Qiagen). The abundance of transcripts was assessed by real-time PCR on an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems) with a SYBR Green detection system (Stratagene). The expression data for each gene of interest were against β-actin as the invariant control, and relative expression was determined using amplification efficiencies (4). Primer sequences are available on request.

Western Blotting. Tissue (∼100 mg) was homogenized in 1 mL ice-cold homogenization buffer (20 mM Tris·HCl, pH 7.4, 5 mM EDTA, 0.25 mM EGTA, 10 mM $Na_4P_2O_7$, 1% Nonidet P-40, and protease and phosphatase inhibitor mixtures; Roche Diagnostics) and centrifuged at 6,000 × *g* at 4 °C for 15 min. The supernatant was taken, and protein concentration was determined by the Bradford method (Thermo Scientific). One hundred micrograms of protein was loaded and resolved by SDS/PAGE using a 4–12% (Novex by Life Technologies) gradient gel and electroblotted onto a polyvinylidene difluoride membrane (DuPont) using a wet-transfer cell. The membrane was then blocked for 60 min at room temperature in wash buffer containing 5% (wt/vol) nonfat dried milk and incubated overnight with primary antibody. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology) for 60 min. Detection was performed with enhanced chemiluminescence.

Akt, phosphorylated Akt (Ser473), insulin receptor β, ACC, and phosphorylated ACC antibodies were purchased from Cell Signaling Technology. β-actin antibody was purchased from Sigma.

Statistical Analysis. Statistical analysis of the data was performed using GraphPad Prism 6. When two groups were compared, the Student unpaired *t* test was used. When three or more groups were compared, a one-way ANOVA analysis was used followed by Tukey's multiple comparison test. All data are expressed as mean ± SEM unless otherwise indicated. $P < 0.05$ was considered significant.

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2. Lee WN, et al. (1994) In vivo measurement of fatty acids and cholesterol synthesis using D2O and mass isotopomer analysis. *Am J Physiol* 266(5 Pt 1):E699–E708.
3. Wadke M, Brunengraber H, Lowenstein JM, Dolhun JJ, Arsenault GP (1973) Fatty acid synthesis by liver perfused with deuterated and tritiated water. *Biochemistry* 12(14): 2619–2624.
4. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29(9):e45.

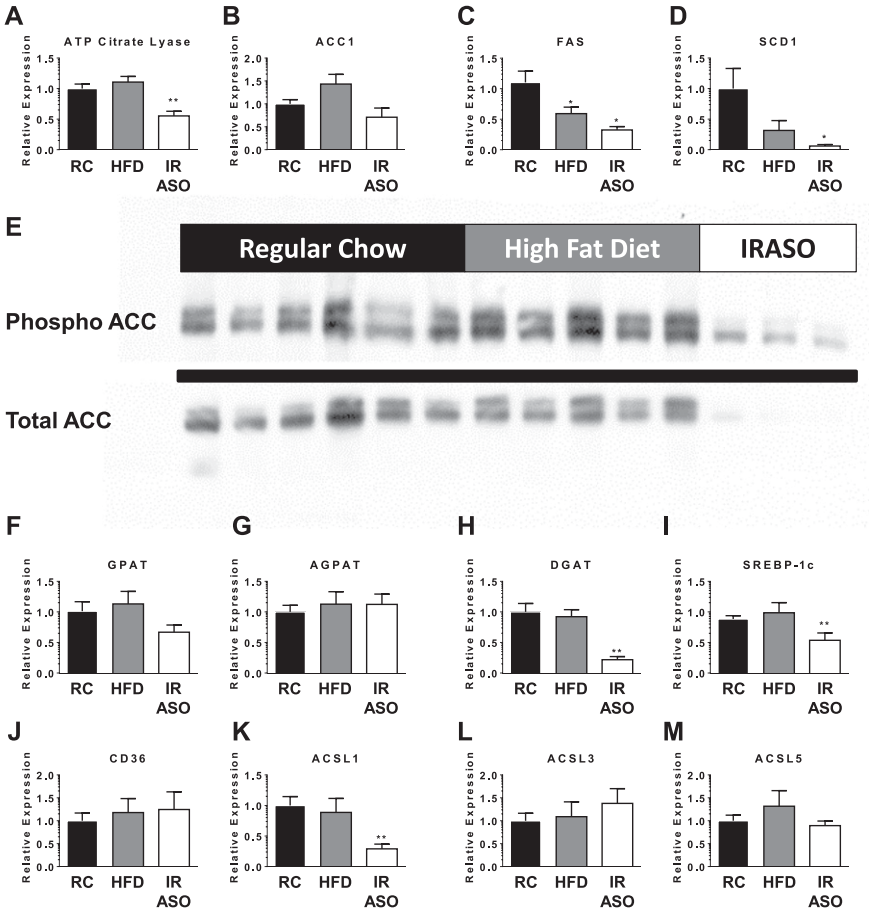

Fig. S1. Molecular evaluation of lipogenic pathways. Gene expression assessed by qPCR (*A–D* and *F–M*) compared in animals treated with basal insulin and saline, divided into three groups: black bar, regular chow-fed rats; gray bar, high fat diet-fed rats; white bar, IRASO-treated rats. Genes assessed: (*A*) ATP citrate lyase; (*B*) acetyl CoA carboxylase 1 (ACC1); (*C*) fatty acid synthase (FAS); (*D*) stearoyl CoA desaturase 1 (SCD1); (*F*) mitochondrial glycerol-3-phosphate acyltransferase (GPAT); (*G*) acylglycerolphosphate acyltransferase 2 (AGPAT); (*H*) diacylglycerol acyltransferase 2 (DGAT); (*I*) sterol regulatory element binding protein 1c (SREBP-1c); (*J*) fatty acid translocase/CD36 (CD36); (*K–M*) acyl CoA synthetase long chain family members 1, 3, and 5 (ACSL1, ACSL3, and ACSL5, respectively). (*E*) Immunoblot of hepatic acetyl CoA carboxylase protein, comparing phosphorylated ACC with total ACC abundance. ACC1 is the lower-molecular-weight band and ACC2 is the higher-molecular-weight band. $*P < 0.04$ and $**P < 0.01$ compared with regular chow group.