## Supplemental Methods

**Genotyping.** Genomic DNA obtained from the tail clippings was PCR amplified for 30 cycles (annealing at 60 $^{o}$C for 1 min; extension at 72 $^{o}$C for 40 sec.) using Platinum Taq DNA polymerase (Invitrogen) and the following three primers (5' to 3'):

| | | |
|---|---|---|
| 1-Fwd (0.5 μM) | complementary to nt 723-742 of intron 1 | GGCGGGGAAACTCTCCACAT |
| 2-Rev (0.38 μM) | complementary to nt 1192-1172 of intron 1 | AGGGAATGGATTGCCCTCTTG |
| 3-Rev (0.12 μM) | complementary to the gene-trap vector | CCCTGGGGTTCGTGTCCTAC |

**Blood and tissue analyses.** For fasting samples, mice were deprived of food (but not water) at 9 AM and tails were nicked at 3 PM and bled into heparinized capillary tubes (Fisher, Inc.). After clamp studies, blood was obtained by exsanguination. Plasma was separated by centrifugation at 2000 *g* for 30 sec. and stored at –80$^{o}$C. For SDS-PAGE, plasma was diluted 40 fold into SDS sample buffer and heated at 95$^{o}$C for 10 min. prior to immunoblotting (40 µl / lane). Commercial kits used include a mouse C-peptide ELISA kit (Shibayagi Co.), a mouse leptin EIA kit, an ultrasensitive insulin EIA kit (both from Alpco Diagnostics), a total ketone kit and an L-Type TG H Kit (both from Wako Diagnostics). The latter was also used to determine the TG content of liver homogenized in buffer A (3) without glycerol. The molar insulin/C-peptide ratio is shown in arbitrary units in Fig. 4C because the numerical values exceed the physiologically plausible range. A likely explanation for the systematic overestimation of this ratio is that the reference solutions of insulin and C-peptide were from different vendors. Tissue glycogen content was determined by acid hydrolysis (1N HCl, 100$^{o}$C, 2 hr) followed by glucose analysis using an oxidase kit (Thermo Scientific, Inc.) (34). The same kit was applied to a set of plasma samples containing various glucose levels. These samples were in turn used to calibrate the glucometer (OneTouch Ultra2) used in Figs. 4B, S2, S3 and S4.

**Real-time quantitative PCR (qPCR).** Tissues were extracted using the appropriate RNeasy Mini Kit (Qiagen). RNA (1 µg, quantified based on $OD_{260}$) was reverse-transcribed using the High-Capacity cDNA Kit (Applied Biosystems). 2% of the cDNA product was subjected to qPCR analysis (25 µl reactions) using iTaq SYBR Green Supermix in the Chromo4 detection system (both from Bio-Rad) with a threshold set at 0.05. All reactions had $C_T$ values less than 25. For each cDNA sample, the $C_T$ value of the reference gene GAPDH (average of quadruplicate) was subtracted from the $C_T$ value of each target gene (average of duplicate) to obtain $\Delta C_T$, which was then used to calculate the SEM for each genotype and the $P$ values using Student's two-tailed $t$ test. The fold difference between genotypes was calculated using the REST algorithm (35), where PCR efficiencies were determined by comparing reactions containing 2X, 1X, 0.5X and 0.25X input cDNA. For each gene, the PCR product was verified by melting-curve analysis in every reaction and by agarose gel electrophoresis at least once. Primer sequences (5' to 3') are as follows:

| | | |
|---|---|---|
| ACO1 (acyl-CoA oxidase 1) | CATGCACCCGCCCATGAC | CAGGCAACGGCCGAAAGC |
| AdipoQ (adiponectin) | AGAGAAGGGAGAGAAAGGAGATGC | TGAGCGATACACATAAGCGGC |
| FAT/CD36 (fatty acid translocase) | GGCTAAATGAGACTGGGACC | CATCACCACTCCAATCCCAAG |
| CPT-1α (carnitine palmitoyltransferase 1, liver isoform) | CACTCCTGGAAGAAGAAGTTCA | GTATCTTTGACAGCTGGGAC |
| CPT-1β (carnitine palmitoyltransferase 1, muscle isoform) | TGATCATGTATCGCCGCAAACT | CATCTGGTAGGAGCACATGG |
| Ero1-Lα (endoplasmic oxidoreductin-1-like α) | CGATATACAGTCCCCCGATG | TACTTTTTCCTCGCCCAGAAG |
| ERp44 (44-kDa endoplasmic reticulum resident protein) | AGCCAGATGTTGCATCCAATTT | AAGGCACAGTCATCATGCAAAAT |
| FABP3 (fatty-acid binding-protein 3) | CCCCTCAGCTCAGCACCAT | CAGAAAAATCCCAACCCAAGAAT |
| GAPDH (glyceraldehyde phosphate dehydrogenase) | AATGTGTCCGTCGTGGATCT | CCCTGTTGCTGTAGCCGTAT |
| GLUT1 (glucose transporter 1) | TGTGGTGTCGCTGTTTGTTGTAG | CAATGAAGTTTGAGGTCCAGTTGG |
| GLUT4 (glucose transporter 4) | AGAGTCTAAAGCGCCT | CCGAGACCAACGTGAA |
| LPL (lipoprotein lipase) | TCTGGGACTGAGGATGGCAA | TGAGCAGTTCTCCGATGTCC |
| MCAD (medium-chain acyl-CoA dehydrogenase) | GAAAGCGGCTCACAAGCAG | CCGCAGCTTTCCGGAATGT |
| PGC1α (PPARγ coactivator 1α) | CTGGGTGGATTGAAGTGGTG | TCAGTGCATCAAATGAGGGC |
| RBP4 (retinol binding-protein 4) | TGAGCGCCACAGCCAAGGGAC | CCAGTTGCTCAGAAGACGGAC |
| TNKS (tankyrase) | TGGAATTGGAGGAGGAACAG | TCATGGTGCTGAATTGCAGG |
| Ubc9 (a SUMO-conjugating enzyme) | GTGACAAAAGCGTGTCCAG | TCCTCCAGGCTTTCCTTTC |
| UCP1 (uncoupling protein 1) | CGTACCAAGCTGTGCGATGT | TAGAAGCCCAATGATGTTCAGT |
| UCP2 (uncoupling protein 2) | CCGGGCTGGTGGTGGTC | ACACCTTTCCAGAGGCCC |
| UCP3 (uncoupling protein 3) | CATCGCCAGGGAGGAAGGA | GTTGACAATGGCATTTCTTGTGA |

**Figure S1**. Growth curve of males on normal chow and weighed between 3 and 4 PM without fasting. n=17-27 per group at each time point.

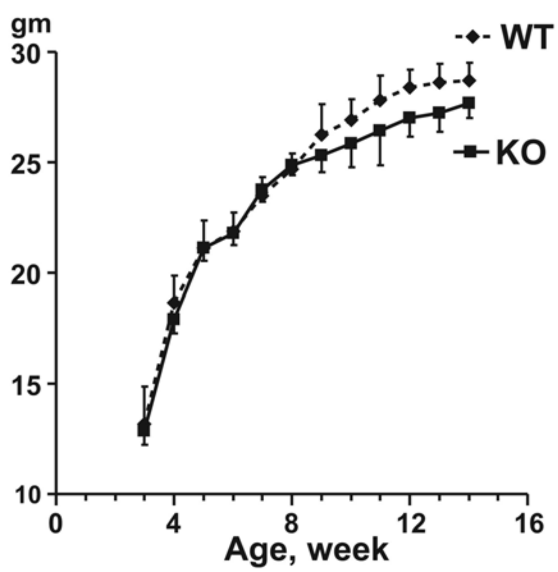

**Figure S2**. Plasma from 2-month-old males obtained at 3 PM after a 6-hr fast was analyzed for insulin and glucose (28 WT, 28 KO) and immunoblotted for adiponectin (AdipoQ) and RBP4 (23 WT, 18 KO) as described in Methods.

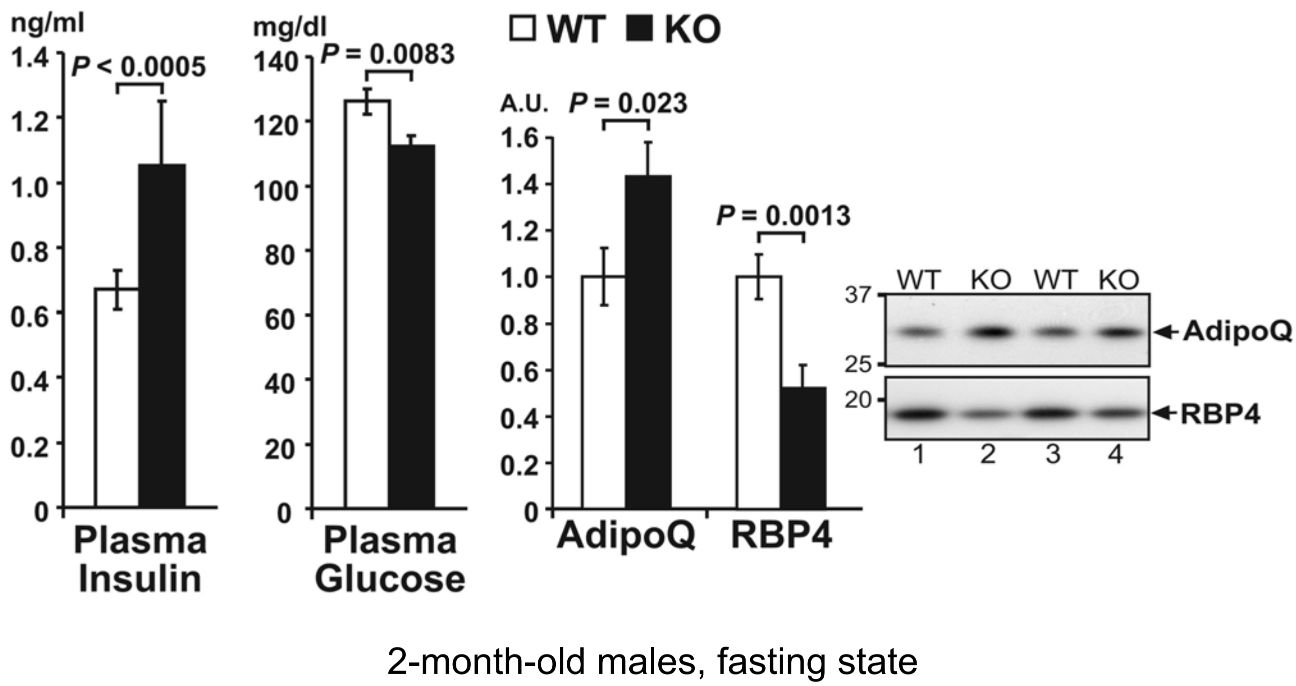


**Figure S3**. Plasma from 8-month-old females obtained at 3 PM after a 6-hr fast was analyzed for insulin (6 WT, 9 KO) and glucose (4 WT, 4 KO) and immunoblotted for adiponectin (AdipoQ), RBP4, and transthyretin (TTR; 6 WT, 9 KO) as described in Methods.

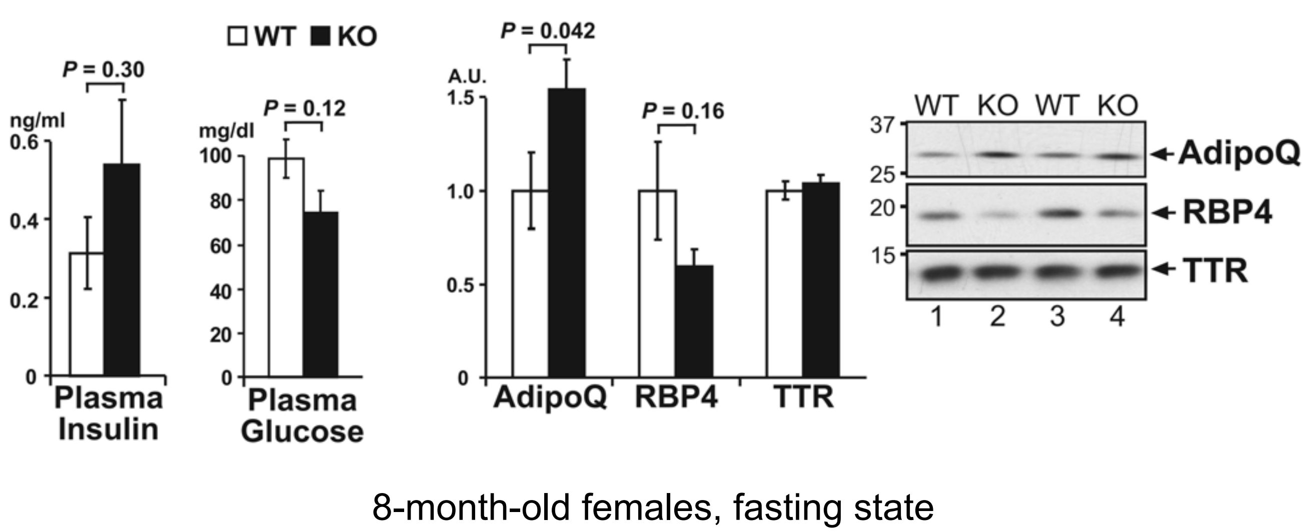

**Figure S4**. Plasma from 3-month-old males (5 WT, 11 KO) obtained at 9 AM without fasting was analyzed for insulin and glucose as described in Methods.

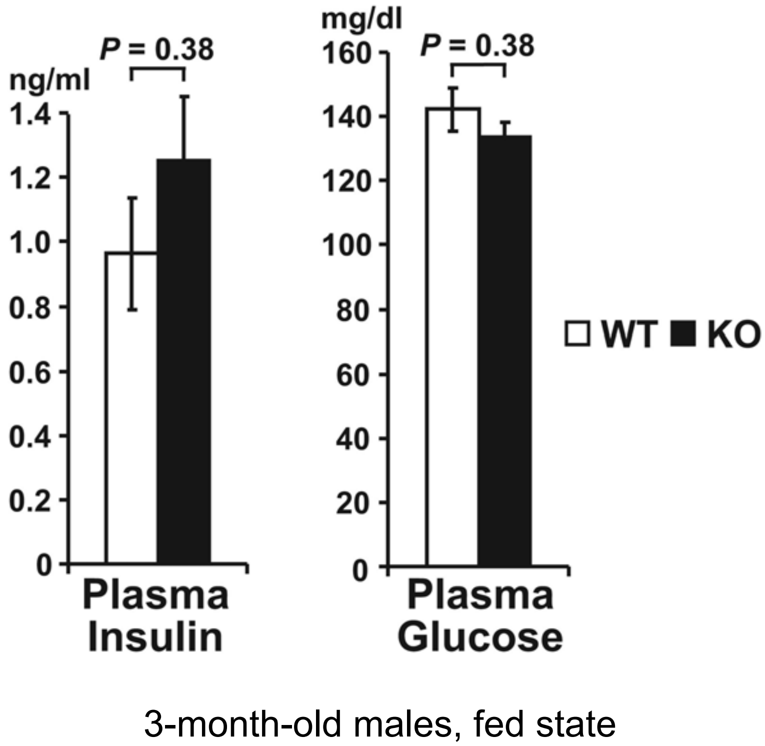


**Figure S5**. Adipose tissue NAD content. Periovarian fat pads from 8 month-old mice (WT=6, KO=10) were homogenized in 1M $HClO_4$ using a Duall 20 glass grinder. NAD content was determined as described in (15) and normalized to protein content.