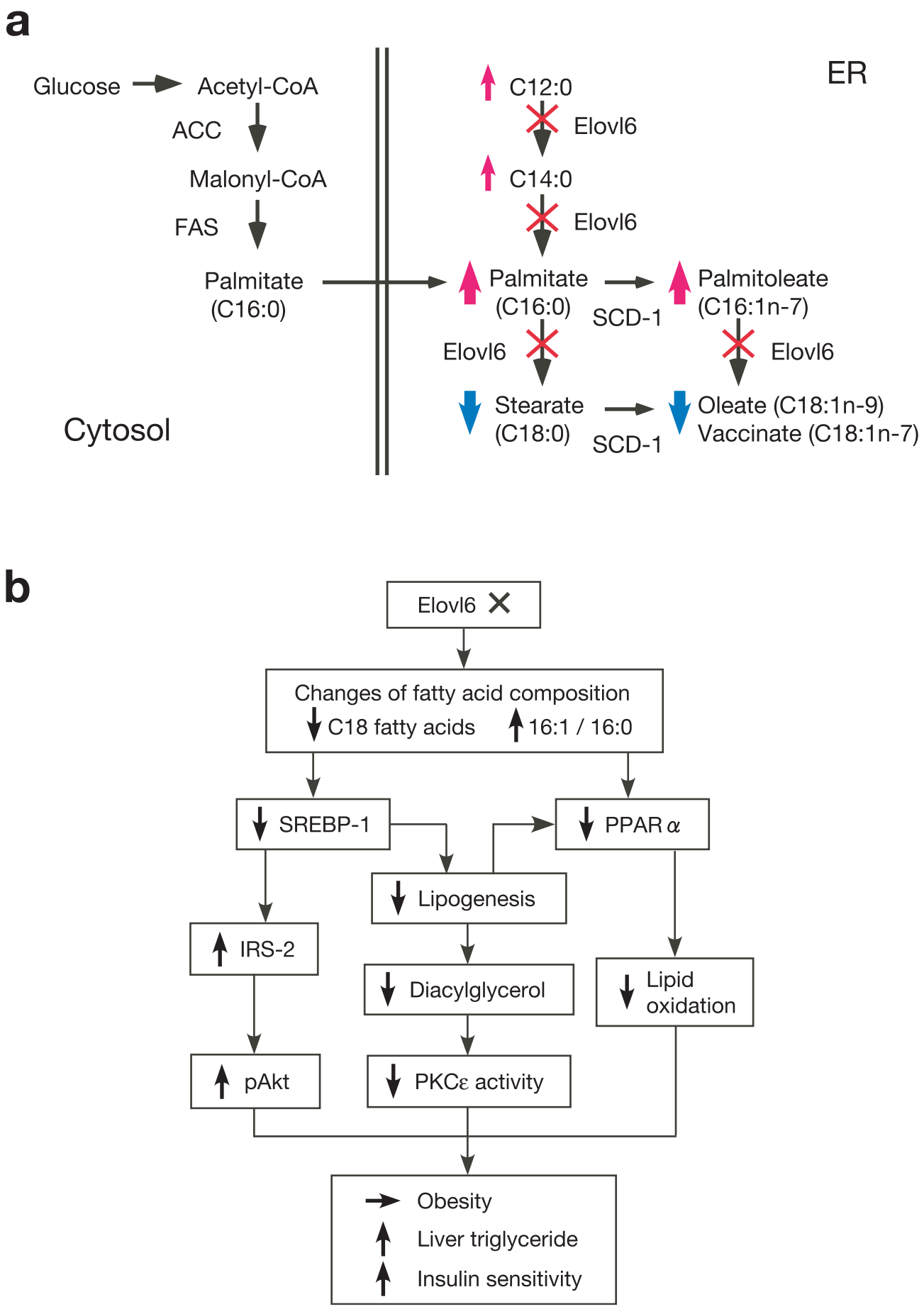
Supplementary Figure 1

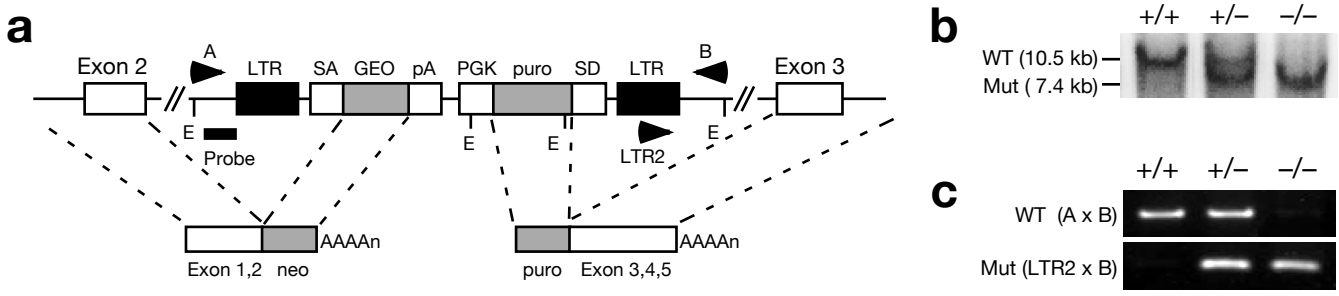


Supplementary Figure 1

Role of Elovl6 in mammalian fatty acid synthesis and hepatic insulin sensitivity.

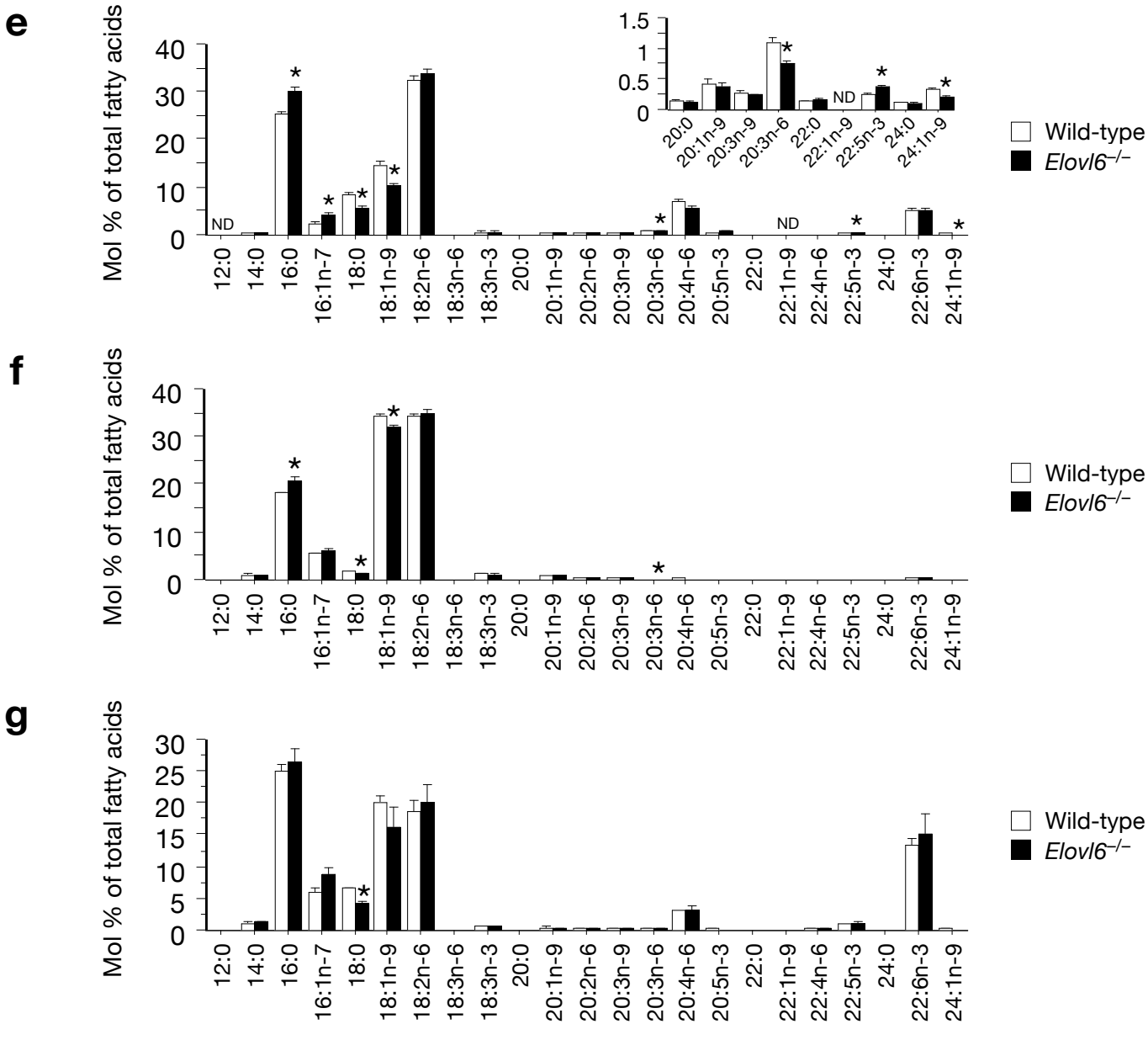
(**a**) Schematic representation of the pathways controlling long chain fatty acid synthesis. Elovl6 has a specific activity for the elongation of C12–16 saturated- and monounsaturated- fatty acids. During fatty acid synthesis, palmitate (16:0), produced by fatty acid synthase (FAS) in the cytosol, is transferred to endoplasmic reticulum (ER) membranes, where Elovl6 and stearoyl-CoA desaturase (SCD) are sequentially involved to produce the main final product, 18:1. (**b**) Proposed molecular basis for *Elovl6* deficiency-mediated metabolic effects in mice. *Elovl6* deficiency altered hepatic fatty acid composition; the decreased levels of 18:0 and 18:1, and increased levels of 16:0 and 16:1. The changes of a fatty cid chain length (decrease C18 fatty acids) and ratio of fatty acids (increase 16:1/16:0 ratio) could reduce SREBP-1 and PPARα in the liver. The reduction of SREBP-1 leads to increase IRS-2 levels and insulin sensitivity, and decrease fatty acid synthesis by the reduction of lipogenic gene expression. The reduction of lipogenesis could lead to decreased hepatic diacylglycerol contents, which leads to decrease PKCε activity and increase insulin sensitivity. The reduction of *de novo* lipogenesis could also reduce PPARα activity and expression. The reduction of PPARα leads to decrease lipid oxidation by reduction of gene expression of fatty acid oxidation enzymes. *Elovl6* deficiency suppressed both synthesis and degradation of fatty acids, resulting in slightly increased triglycerides in the liver. Taken together, *Elovl6* deficiency improves hepatic insulin sensitivity without amelioration of obesity and hepatosteatosis.

Supplementary Figure 2



d

Mating	Number of litters	Number of genotype		
		+/+	+/–	–/–
+/– M x +/– F	85	158	303	50
–/– M x +/– F	52	NA	273	127

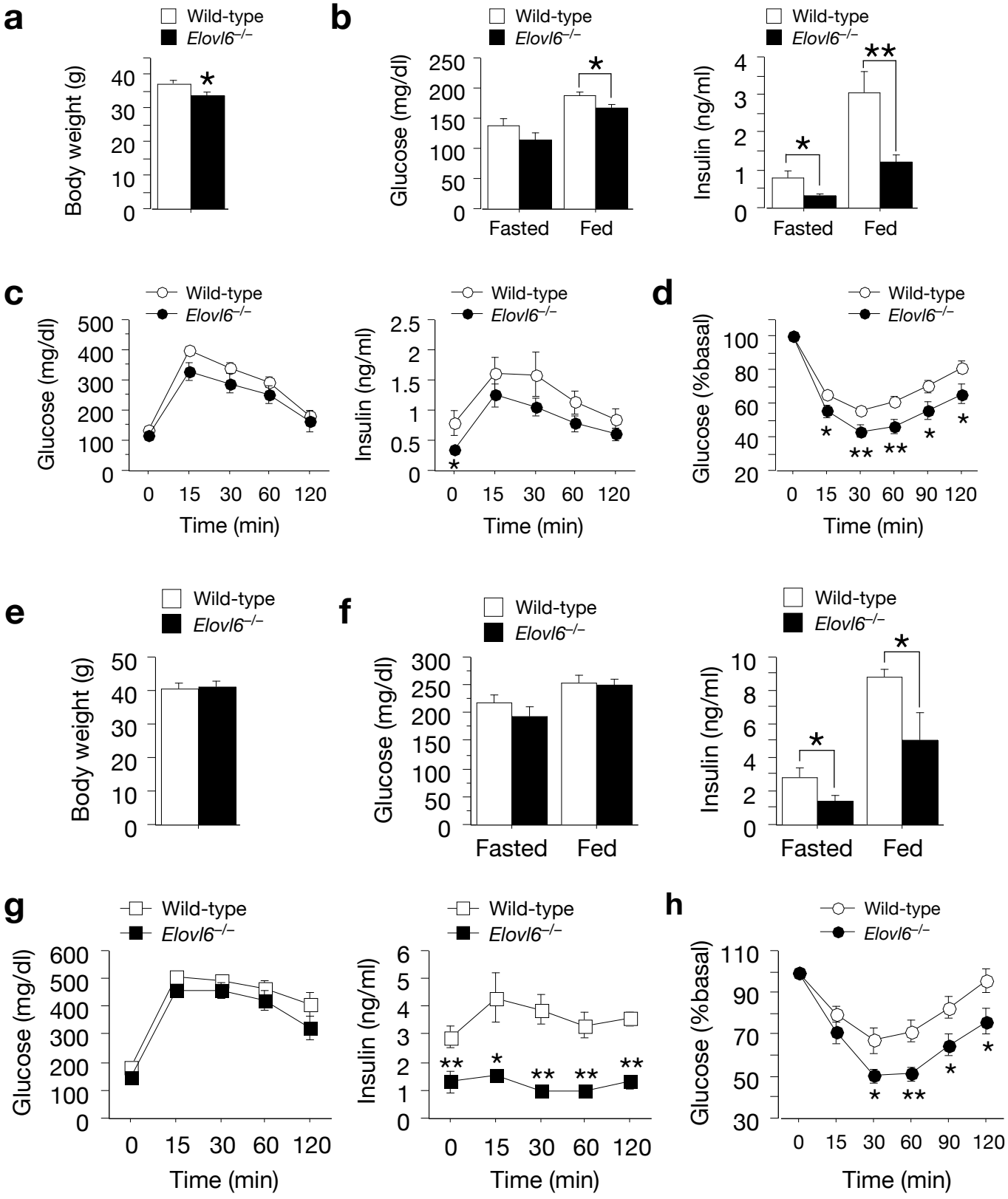


Supplementary Figure 2

Generation of *Elovl6*$^{-/-}$ mice.

(**a**) Schematic representation of the *Elovl6* locus after insertion of the retroviral gene trap. Expression of the trapped gene is disrupted because the inserted sequence causes the endogenous transcript to be divided into two separated transcripts, neither of which can produce the endogenous protein. LTR, long terminal repeat; SA, splice acceptor sequence; neo, neomycin resistance cassette; pA, polyadenylation sequence; PGK, phosphoglycerate kinase-1 promoter; puro, puromycin resistance cassette; SD, splice donor sequence; E, *Eco*RI. Primers used for genotyping by PCR are primer A, primer B, and primer LTR2. The location of the probe used for Southern blot analysis is denoted by the horizontal filled rectangle labeled "probe". (**b**) Southern blot analysis of tail DNA from wild-type (+/+), heterozygous (+/–), and homozygous (–/–) mice. Genomic DNA was digested with *Eco*RI and detected using probe to yield the expected fragments of 10.5 kb (wild-type allele) and 7.4 kb (mutated allele). (**c**) PCR analysis of genomic DNA prepared from mouse tail. (**d**) *Elovl6*$^{-/-}$ mouse survival chart. Genotype was determined by PCR analysis of DNA prepared from tail. M, male; F, female; NA, not applicable. (**e**–**g**) Fatty acid composition (mol % of total) of plasma (**e**), white adipose tissue (**f**), and skeletal muscle (**g**) in wild-type and *Elovl6*$^{-/-}$ mice. Total lipids were extracted from livers of mice fed a normal chow diet, and lipid fractions were quantified by gas chromatography. Values are means ± SEM for n = 3–5 mice. *, $p < 0.05$ as compared with their respective wild-type. ND, not detectable.

Supplementary Figure 3

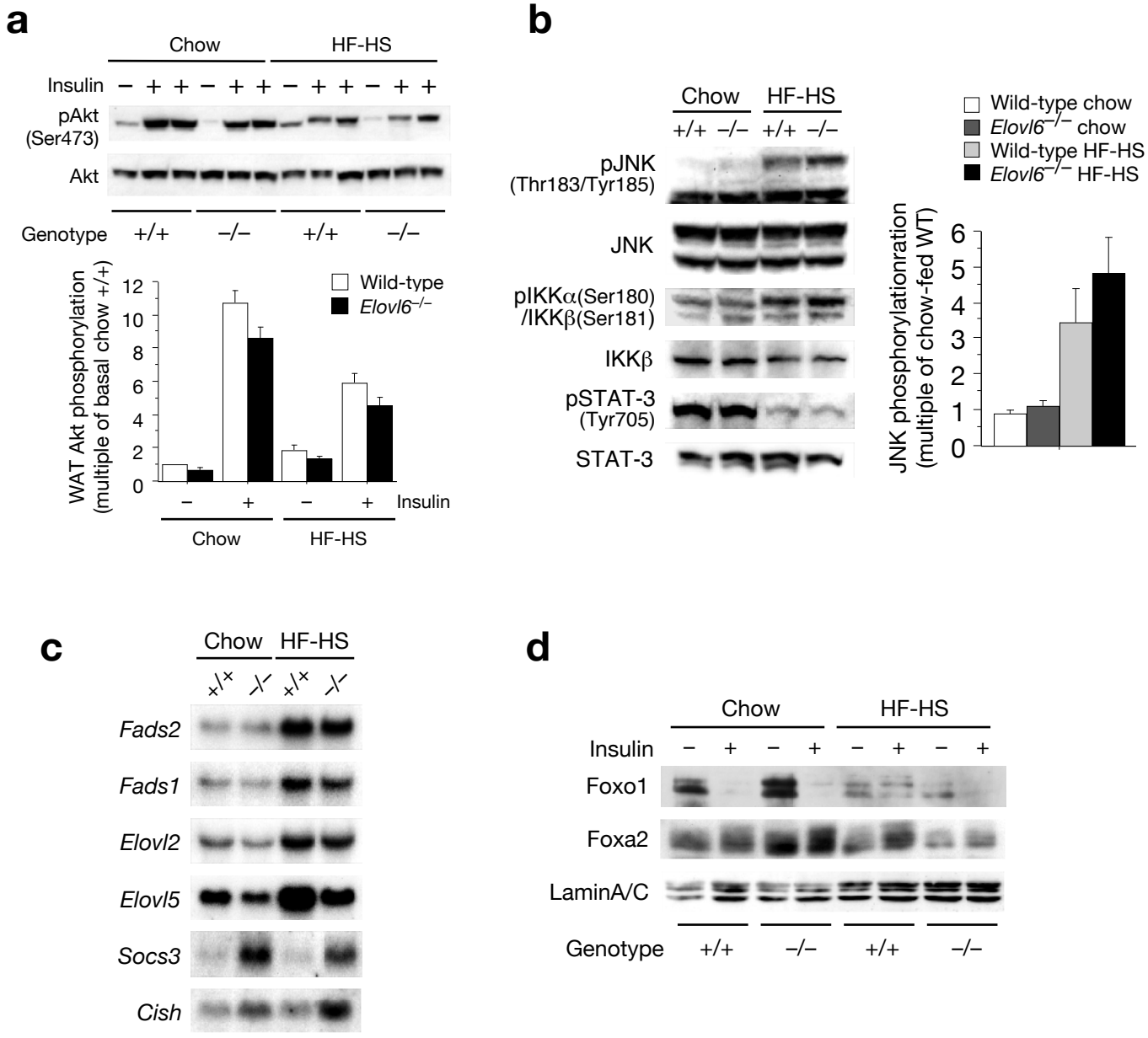


Supplementary Figure 3

Effects of the aging and body weight differences on the insulin sensitivity of *Elovl6*$^{-/-}$ mice.

(**a**–**d**) Protection from age-associated insulin resistance in chow-fed *Elovl6*$^{-/-}$ mice. (**a**) Body weight of 6–8 month old wild-type and *Elovl6*$^{-/-}$ mice fed a chow diet. (**b**) Plasma glucose (left) and insulin (right) concentrations in 6–8 month old wild-type and *Elovl6*$^{-/-}$ mice on fasted or fed state. (**c**) Plasma glucose (left) and insulin (right) levels during an intraperitoneal glucose tolerance test. (**d**) Insulin tolerance tests in 6–8 month old wild-type and *Elovl6*$^{-/-}$ mice (0.5 U/kg of body weight). (**e**–**h**) Protection from insulin resistance in HF-HS–fed *Elovl6*$^{-/-}$ mice were not caused by the body weight difference. Six-week-old wild-type and *Elovl6*$^{-/-}$ mice were fed HF-HS diet for 8-12 weeks, and body weight-matched wild-type and *Elovl6*$^{-/-}$ mice were used for each experiment. (**e**) Body weight of HF-HS–fed wild-type and *Elovl6*$^{-/-}$ mice used in this experiments. (**f**) Plasma glucose (left) and insulin (right) levels in fasted or fed state. (**g**) Plasma glucose (left) and insulin (right) levels during an intraperitoneal glucose tolerance test. (**h**) Insulin tolerance tests in body weight-matched wild-type and *Elovl6*$^{-/-}$ mice fed HF-HS diet (1.0 U/kg of body weight). n = 8–10 per group in a–d and n = 10–12 per group in e–h. *, $p < 0.05$; **, $p < 0.01$ as compared with their respective wild-type.

Supplementary Figure 4

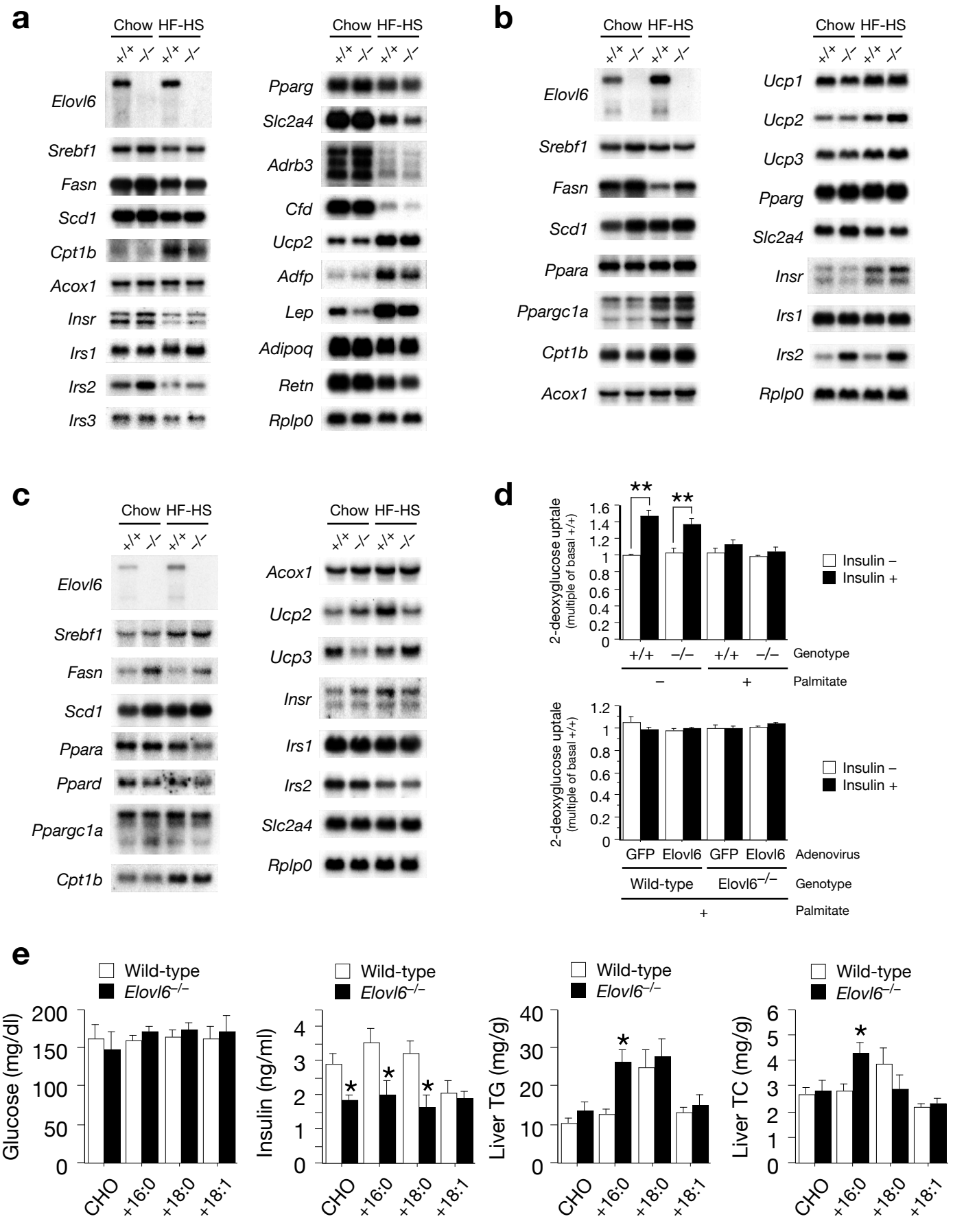


Supplementary Figure 4

Effects of HF-HS diet on insulin signaling and inflammation in the white adipose tissue (WAT) and livers of wild-type and *Elovl6*$^{-/-}$ mice.

(**a**) Immunoblot analysis (top) and densitometric quantification (bottom) of Ser473-phosphoylated Akt (pAkt) and total Akt in response to a bolus injection of insulin in WAT. The blot is representative of three independent experiments. Data represent means ± SEM (*n* = 6 per group). (**b**–**d**) Examination of protein and mRNA levels in the livers of wild-type and *Elovl6*$^{-/-}$ mice fed a standard chow or HF-HS diet. (**b**) Protein levels of inflammatory and cytokine signaling molecules were examined by immunoblotting in livers of wild-type and *Elovl6*$^{-/-}$ mice fed a chow or HF-HS diet for 12 weeks (left). Densitometric quantification of the JNK was also shown (*n* = 8–11 per group, right). (**c**) Northern blot analysis of various mRNA levels in the liver of wild-type and *Elovl6*$^{-/-}$ mice fed a standard chow or HF-HS diet for 12 weeks. *Fads2*, Δ6 desaturase; *Fads1*, Δ5 desturase; *Socs3*, suppressor of cytokine signaling 3; *Cish*, cytokine inducible SH2-containing protein. (**d**) Immunoblot analysis of nuclear abundance of Foxo1 and Foxa2 in the livers of wild-type and *Elovl6*$^{-/-}$ mice fed on a standard chow or HF-HS diet. Mice in 24 h fasted state were injected intraperitoneally with PBS or human regular insulin (1.0 U/kg of body weight). After 60 min, livers were rapidly excised and nuclear extracts were prepared.

Supplementary Figure 5



Supplementary Figure 5

Effects of *Elovl6* deficiency on mRNA levels in the white adipose tissue (WAT), brown adipose tissue (BAT) and skeletal muscle, and 2-deoxyglucose uptake in the skeletal muscle cells, and effects of dietary fatty acid supplementation on plasma and liver metabolic parameters in wild-type and *Elovl6*$^{-/-}$ mice.

(**a**–**c**) Northern blot analysis of various mRNA levels in WAT (**a**), BAT (**b**) and skeletal muscle (**c**) from wild-type and *Elovl6*$^{-/-}$ mice fed on a standard chow diet or HF-HS diet for 12 weeks. *Cpt1b*, carnitine palmitoyltransferase 1b, muscle; *Insr*, insulin receptor; *Pparg*, peroxisome proliferator-activated receptor gamma; *Slc2a4*, glucose transporter 4; *Adrb3*, b3-adorenergic receptor; *Cfd*, adipsin; *Adfp*, adipose differentiation-related protein; *Lep*, leptin; *Adipoq*, adiponectin; *Retn*, resistin; *Ppargc1a*, peroxisome proliferator-activated receptor gamma coactivator-1a; *Ppard*, peroxisome proliferator-activated receptor delta. (**d**) Basal and insulin-stimulated 2-deoxyglucose uptake was assayed in myotubes prepared from wild-type and *Elovl6*$^{-/-}$ mice following incubation in the absence or presence of palmitate (0.5 mM) for 16 h (top), and infection with adenovirus expressing GFP or Elovl6 (MOI 100) with palmitate (0.5 mM) for 16 h (bottom), followed by incubation in the absence or presence of insulin (100 nM) for 15 min. Values are represented as means ± SEM (n = 6 per group). **, $p < 0.01$ as compared with their respective wild-type controls. (**e**) Effects of tripalmitin (16:0), tristearin (18:0), or triolein (18:1) supplementation on plasma glucose, plasma insulin, liver triglyceride and liver total cholesterol levels in wild-type and *Elovl6*$^{-/-}$ mice. Mice were fed high carbohydrate fat-free diet (CHO), tripalmitin, tristearin, or triolein-supplemented diets (20% by weight) for 2 weeks. n = 8–10 per group. *, $p < 0.05$ as compared with their respective wild-type.

Crucial role of long chain fatty acid elongase (Elovl6) in obesity-induced insulin resistance

Takashi Matsuzaka[1,2], Hitoshi Shimano[1,2,*], Naoya Yahagi[2,3], Toyonori Kato[1], Ayaka Atsumi[1], Takashi Yamamoto[1], Noriyuki Inoue[1], Mayumi Ishikawa[1], Sumiyo Okada[1], Naomi Ishigaki[1], Hitoshi Iwasaki[1], Yuko Iwasaki[1], Tadayoshi Karasawa[1], Shin Kumadaki[1], Toshiyuki Matsui[1], Motohiro Sekiya[3], Ken Ohashi[3], Alyssa H Hasty[4], Yoshimi Nakagawa[1,2], Akimitsu Takahashi[1], Hiroaki Suzuki[1], Sigeru Yatoh[1], Hirohito Sone[1], Hideo Toyoshima[1], Jun-ichi Osuga[3], and Nobuhiro Yamada[1]

Supplementary Methods

Materials. We purchased antibodies to IRβ, phospho-IRβ (Tyr972), IRS-1, IRS-2, and phosphotyrosine 4G10 from Upstate Biotechnology, and antibodies to SREBP-1, Foxa2, PKCε, and PKCβII from Santa Cruz Biotechnology. We obtained antibodies to Akt, phospho-Akt (Ser473), Foxo1, AMPK, phospo-AMPKα (Ser485), JNK, Phospho-JNK (Thr183/Tyr185), IKK, phospho-IKK, STAT-3, phospho-STAT-3 (Tyr705), PKCα, and PKCδ from Cell Signaling Technology. 2-Deoxy-D-[1-^{14}C]glucose and [1-^{3}H]mannitol were purchased from PerkinElmer Life Sciences. Tripalmitin, tristearin, and triolein were purchased from Wako Pure

Chemicals. Other chemical compounds were obtained from Sigma.

PCR genotyping. Routine genotyping was performed on tail DNA by PCR. We used a single antisense primer (primer A: 5'- GGATTCCCCACCTATTTCCTTCAG -3') corresponding to intron sequence downstream of the insertion site and two sense primers, one corresponding to intron sequence upstream of the insertion (primer B: 5'-AACCCTTTCCTCCCCAACTTGCTC-3') and the other within the 3' end of the Lexicon insertion (LTR2: 5'- AAATGGCGTTACTTAAGCTAGCTTGC -3'). The primer pair A/B amplifies a fragment of 300 bp, and primer pair LTR2/B amplifies a fragment of 173 bp.

Generation of *ob*/*ob*-*Elovl6*$^{-/-}$ Mice. Mice deficient in both *Elovl6* and *Lep* were obtained by breeding *Elovl6*$^{-/-}$ mice to C57BL/6 *ob*/+ mice (The Jackson Laboratory, Bar Harbor, Maine). To generate *ob*/*ob* mice lacking the *Elovl6* gene, *Elovl6*$^{-/-}$ males were first bred to *ob*/+ females to create compound heterozygous (*ob*/+-*Elovl6*$^{+/-}$). In a second cross, compound heterozygous were bred with *Elovl6*$^{-/-}$ mice, and ob/+-*Elovl6*$^{-/-}$ offspring were identified. In the third set of crosses, ob/+-*Elovl6*$^{-/-}$ mice were bred to each other to produce *ob*/*ob*-*Elovl6*$^{-/-}$ animals. In parallel, *ob*/+ mice were bred to each other to produce *ob*/*ob* as well as wild-type animals. The *Lep* gene genotyping was determined as described previously[51].

Assays. Plasma concentrations of IL-6 and TNF-α were assayed by enzyme-linked

immunosorbent assay kit (PIERCE).

Histology. Tissue specimens were fixed in 4% paraformaldehyde, and embedded in paraffin. Thin sections were subjected to standard hematoxylin and eosin staining.

Primers for quantitative RT-PCR. The primer sets were as follows; *Elovl6* forward, 5'-CCCGAACTAGGTGACACGAT-3', *Elovl6* reverse, 5'-CCAGCGACCATGTCT TTGTA-3'; *Srebf1* forward, 5'- CGGCGCGGAAGCTGT-3', *Srebf1* reverse, 5'-TGCA ATCCATGGCTCCGT-3'; *Ppara* forward, 5'-CCTCAGGGTACCACTACGGAGT-3', *Ppara* reverse, 5'- GCCGAATAGTTCGCCGAA-3', *Ppia* forward, 5'-CCTGAAGTG CTCGACATCACA-3', *Ppia* reverse, 5'- GCGCTTGTACCCATTGATGA-3'.

Primary cultures from muscle cells and measurement of 2-deoxyglucose uptake. Primary myotube cultures were prepared by isolating satellite cells from hind-limb muscles of 8 week-old wild-type and *Elovl6*$^{-/-}$ mice, and cultured as previously described[52], with some modifications. Briefly, myoblasts were proliferated for 5-6 days in Dulbecco's minimum essential medium (DMEM) supplemented with 20% fetal bovine serum (FBS) and antibiotics. For myotube culture, myoblasts were cultured for 6-7 days in DMEM with 2.5% FBS and antibiotics. Specific 2-deoxyglucose (2DG) uptake was determined by subtracing the non-specific uptake of mannitol from the total uptake of 2DG as described previously[53], with some modifications. The cells were incubated for 15 min in Krebs-Ringer phosphate buffer containing 1 mM 2DG, 0.1

μCi/ml [1-^{14}C]2DG and 0.5 μCi/ml [1-^{3}H]mannitol. After the incubation, the cells were washed three times with ice-cold PBS and then dissolved in 1 N NaOH. The sample solution was neutralized with 1 N HCl and counted by a liquid scintillation.

Supplementary References

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Supplementary Table

Phenotypic characteristics of wild-type and *Elovl6*$^{-/-}$ mice fed a standard chow or high-fat, high-sucrose (HF-HS) diet.

Diet	Standard chow		HF-HS	
Parameter measured	Wild-type	*Elovl6*$^{-/-}$	Wild-type	*Elovl6*$^{-/-}$
Body weight (g)	28.1±0.42	24.6±1.2 *	45.5±1.25	41.0±1.32 *
Liver (% body weight)	3.93±0.13	4.29±0.15	4.09±0.18	4.60±0.21
Epididymal fat (%body weight)	1.53±0.16	1.04±0.05 *	3.44±0.12	3.22±0.18
Brown fat (% body weight)	0.31±0.02	0.25±0.03	0.70±0.03	0.62±0.06
Liver triglyceride (mg/g)	5.2±0.49	6.4±0.59	28.4±4.3	35.5±2.6
Liver cholesterol (mg/g)	1.65±0.10	1.79±0.12	2.70±0.26	3.47±0.37
Plasma FFA (mM)	0.38±0.04	0.49±0.06	0.37±0.02	0.38± 0.04
Plasma triglyceride (mg/dl)	123±10.3	114±6.6	105±5.8	112±15.4
Plasma cholesterol (mg/dl)	77.1±2.2	73.9±2.8	129.9±8.4	138.7±14.5
Plasma glucose (mg/dl)	177±6.9	161±6.2	209±7.7	190±9.7
Plasma glucose (fasted,mg/dl)	118±11.1	120±8.2	167±9.2	143±8.9
Plasma insulin (ng/ml)	2.45±0.23	1.10±0.18 *	8.05±0.54	4.15±0.67 *
Plasma insulin (fasted, ng/ml)	0.57±0.11	0.44±0.09	2.56±0.31	1.36±0.36 *
Plasma leptin (ng/ml)	3.45±0.59	1.04±0.46 *	32.0±3.1	12.5±2.9 *
Plasma adiponectin (μg/ml)	45.1±3.6	51.9±5.4	30.3±3.9	29.4±2.7
Plasma TNF-α (pg/ml)	4.11±1.00	5.93±1.92	9.07±3.06	9.81±2.34
Plasma IL-6 (pg/ml)	4.78±0.33	4.24±1.01	4.55±2.48	4.64±1.83
Plasma ALT (IU/l)	11.1±0.8	12.2±1.5	21.8±2.4	13.4±1.9 *

Six-week-old male mice were fed a chow or HF-HS diet for 12 weeks. Physiologic and metabolic parameters were determined throughout standard chow diet or at the end of 12 weeks of HF-HS diet feeding. Mice were killed at non-fasted state otherwise noted. Values are mean ± SEM for n = 15–18 mice. Asterisks represent significant difference of at least $p < 0.05$.