Supplementary Methods

Animals and diets

The high-fat diet (HFD) used in the long-term diet-induced obesity studies was from Research Diets (RD12492) and the high-carbohydrate (HC) diet used in the fasting-refeeding study was from Harlan Teklad, Inc. (TD03045). The caloric contributions (% fat: % carbohydrate: % protein; kcal/g) for the chow, HF, and HC diets as indicated by the manufacturer are (chow, 16.7: 56.4: 26.8; 3.50 kcal/g), (HF, 59.9: 20.1: 20.0; 5.24 kcal/g), and (HC, 2.6: 76.7: 20.7: 3.53 kcal/g). For all diet studies, mice were individually caged 1-week prior to the start of the study. For the diet-induced obesity studies, 8-week old male mice were fed chow or HFD for 8-weeks. Oral glucose tolerance tests were performed after a 4-hour fast after 7-weeks of feeding. Mice were sacrificed at the end of 8-weeks of feeding without fasting. For the fasting-refeeding studies, 10-12 week old male mice were either fasted for 12 hours (fasted group) or fasted for 12 hours followed by refeeding the HC diet for 12 hours (refed group). For cold-tolerance tests, 10- to 12-week old male and female mice were individually caged 1-week prior to cold exposure and allowed *ad libitum* access to food through the course of the cold challenge. The high-fat diets used in the cold tolerance experiments were specially formulated in order to be able to control the composition of the fat source. These diets were designed to contain 20% fat by weight from either fully- hydrogenated coconut oil (saturated fat -- data not shown) or high-oleic safflower oil (monounsaturated fat) as the predominant fat source, supplemented to a 20% fat-free base (TD88232 from Harlan Teklad).

Lipid analyses

Skin surface neutral lipids were separated along with authentic standards by thin-layer chromatography using heptane:isopropyl ether:acetic acid (60:40:3, v/v/v) as a solvent system (1). For better resolution of wax esters, wax diesters and cholesterol esters, a benzene:hexane (65:35, v/v) solvent system was utilized (2). For separation of ceramides, plates were washed with chloroform: methanol (9:1 v/v) and heat activated for 1 hour at 110 deg C; ceramides were resolved by sequential development in chloroform/methanol/acetic acid (190:9:1, v/v/v), twice (3). Plates were sprayed uniformly with 10% cupric sulfate in 8% aqueous phosphoric acid, allowed to dry at room temperature, and then charred at 110 deg C for 30 minutes for visualization of lipid species (4). For fatty acid analysis by gas chromatography, lipids were visualized by spraying with 5 mM 2'7'-dichlorofluorescein in ethanol. Sebaceous lipids including triglycerides, wax esters, wax diesters and cholesterol esters were scraped and methylated as previously described (2). Hepatic lipids were extracted by the Folch method (5) and separated by TLC as previously described (1). Fatty acid composition of skin and hepatic lipids was quantified by gas chromatography as previously described, with penta- and heptadecanoic acids added as internal standards (1).

REFERENCES

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Legends to Supplementary tables and figures

Supplementary Table 1. Fatty acid composition of hepatic lipids.

(A-C) Hepatic lipids were extracted from 30 mg of frozen liver from 16-week old male mice fed chow or HFD for 8 weeks. Lipids were separated by thin-layer chromatography, and bands corresponding to hepatic TG (A), CE (B) and FFA (C) were scraped, methylated, and quantified by gas chromatography with penta- and hepta-decanoic acids as internal standards. Results are presented as mean $\pm$ SEM and represent at least five animals in each group.*, *$p<0.05$ vs. Lox counterparts.*

Supplementary Figure 1. Cutaneous phenotype of SKO mice.

Alopecia and closed eye fissures of SKO mice: SKO mice developed dry scaly skin with hair loss and closed eye fissures, as has been reported in GKO mice. 12-week old male littermates are shown here.

Supplementary Figure 2. Energy expenditure in SKO mice is increased to the same extent as in GKO mice and hepatic lipid accumulation is significantly decreased in SKO mice

(A,B) O_2 consumption (A) and CO_2 production (B) were measured in individually caged 12-week old male in indirect calorimetry chambers. There were no significant differences in body weights of animals (Lox, 26.55 $\pm$ 0.45; GKO, 26.72 $\pm$ 0.28 and SKO, 27.05 $\pm$ 0.61 g), and data is expressed as ml/kg body weight/minute of (A) O_2 consumed or (B) CO_2 produced.

(C) Heat and (D) respiratory quotient were calculated from VO_2 and VCO_2 measurements. Six mice of each genotype were measured over two consecutive 24-hour periods consisting of alternating 12-hour dark and light cycles.

(E) Hepatic lipids were extracted from frozen liver samples of chow- and HFD-fed Lox and SKO mice and separated by thin-layer chromatography with heptane:isopropyl ether:acetic acid (60:40:3, v/v/v) as the developing solvent.
*, *$p<0.05$ vs. Lox counterparts*

Supplementary Figure 3. Hepatic SREBP-1 and lipogenic gene expression are differentially regulated in SKO and GKO mice

(A, B) Nuclear levels of SREBP-1, SREBP-2 and histone H_3 (A) and hepatic lipogenic gene expression (B) were measured in Lox and GKO mice after 8-weeks of Chow- or HFD-feeding.

(C, D) Induction of nuclear levels of SREBP-1 (C) and lipogenic genes (D) were measured in livers of mice fasted for 12-hours or fasted and refed a high-carbohydrate lipogenic diet for 12-hours. Data are presented as mean $\pm$ SEM. *, *$p<0.05$ vs. Lox counterparts*
Acc, acetyl-CoA carboxylase; F, fasted; Fas, fatty acid synthase; Pgc-1β, PPAR-gamma co-activator-1 beta; RF ,fasted-refed; Scd1, stearoyl-CoA desaturase 1; Srebp- 1c, sterol regulatory element binding protein-1c

SUPPLEMENTARY TABLES

Supplementary Table 1A. Fatty acid composition of hepatic triglycerides.

TG (μmol/g)	Lox Chow	SKO Chow	Lox HFD	SKO HFD
12:0	0.07 ± 0.04	0.08 ± 0.01	0.13 ± 0.02	0.08 ± 0.03*
12:1	ND	ND	0.20 ± 0.19	0.06 ± 0.02
14:0	0.25 ± 0.12	0.16 ± 0.02	0.75 ± 0.11	0.32 ± 0.16*
14:1	ND	ND	0.45 ± 0.07	0.35 ± 0.08*
16:0	5.28 ± 2.00	3.65 ± 0.18	30.34 ± 7.87	9.21 ± 3.14*
16:1	0.59 ± 0.22	0.27 ± 0.01	1.70 ± 0.33	0.47 ± 0.36*
18:0	2.33 ± 0.62	2.11 ± 0.24	5.34 ± 1.28	4.25 ± 2.64
18:1(n-9)	5.23 ± 1.50	3.52 ± 0.18	30.63 ± 5.36	10.24 ± 4.23*
18:1 (n-7)	0.44 ± 0.18	0.24 ± 0.02	1.70 ± 0.43	0.57 ± 0.21*
18:2 (n-6)	2.44 ± 0.75	1.75 ± 0.15	17.88 ± 4.12	5.32 ± 2.36*
18:3 (n-6)	0.06 ± 0.02	0.04 ± 0.00	0.05 ± 0.01	0.03 ± 0.01*
18:3 (n-3)	0.11 ± 0.03	0.07 ± 0.01	0.69 ± 0.12	0.19 ± 0.09*
20:0	0.10 ± 0.03	0.07 ± 0.01	0.64 ± 0.13	0.28 ± 0.10*
20:1 (n-9)	0.04 ± 0.01	0.01 ± 0.00	0.08± 0.02	0.04 ± 0.03
20:3 (n-9)	0.08 ± 0.02	0.06 ± 0.00	0.48 ± 0.09	0.19 ± 0.07*
20:4	0.07 ± 0.03	0.06 ± 0.00	0.19 ± 0.04	0.12 ± 0.02*
20:5 (n-3)	0.04 ± 0.01	0.04 ± 0.01	0.14 ± 0.04	0.02 ± 0.01*
22:0	0.03 ± 0.02	0.02 ± 0.00	0.05 ± 0.02	0.02 ± 0.00*
22:1 (n-9)	0.01 ± 0.00	0.01 ± 0.00	0.09 ± 0.10	0.03 ± 0.03
22:6	0.21 ± 0.05	0.17 ± 0.06	0.61 ± 0.16	0.17 ± 0.09*
Total	**18.35 ± 1.92**	**12.70 ± 0.67**	**98.68 ± 14.23**	**33.92 ± 11.27***

**, p<0.05 vs. Lox counterparts*

Supplementary Table 1B. Fatty acid composition of hepatic cholesterol esters.

CE (μmol/g)	Lox chow	SKO chow	Lox HFD	SKO HFD
12:0	0.07 ± 0.03	0.04 ± 0.00	0.06 ± 0.03	0.07 ± 0.03
14:0	0.08 ± 0.02	0.05 ± 0.01	0.14 ± 0.05	0.13 ± 0.05
14:1	0.23 ± 0.07	0.15 ± 0.02	0.37 ± 0.08	0.37 ± 0.05
16:0	1.09 ± 0.21	0.79 ± 0.06	2.10 ± 0.73	1.80 ± 0.73
16:1 (n-9)	0.18 ± 0.08	0.10 ± 0.01	0.31 ± 0.05	0.19 ± 0.04*
18:0	1.85 ± 0.25	1.39 ± 0.15	3.55 ± 2.02	3.30 ± 1.74
18:1 (n-9)	0.52 ± 0.17	0.22 ± 0.02	1.66 ± 0.24	1.03 ± 0.29
18:1 (n-7)	0.02 ± 0.01	0.01 ± 0.00	0.05 ± 0.01	0.03 ± 0.02
18:2 (n-6)	0.09 ± 0.03	0.05 ± 0.00	0.43 ± 0.14	0.21 ± 0.10*
18:3 (n-6)	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
18:3 (n-3)	0.02 ± 0.01	0.02 ± 0.01	0.07 ± 0.04	0.06 ± 0.03
20:0	0.05 ± 0.01	0.04 ± 0.00	0.10 ± 0.04	0.09 ± 0.04
20:1 (n-9)	0.01 ± 0.00	0.14 ± 0.27	0.02 ± 0.01	0.11 ± 0.22
20:4	0.05 ± 0.02	0.04 ± 0.00	0.10 ± 0.02	0.10 ± 0.01
22:0	0.01 ± 0.00	0.02 ± 0.02	0.02 ± 0.00	0.02 ± 0.02
Total	**4.26 ± 0.74**	**3.19 ± 0.48**	**10.52 ± 1.07**	**8.69 ± 0.26***

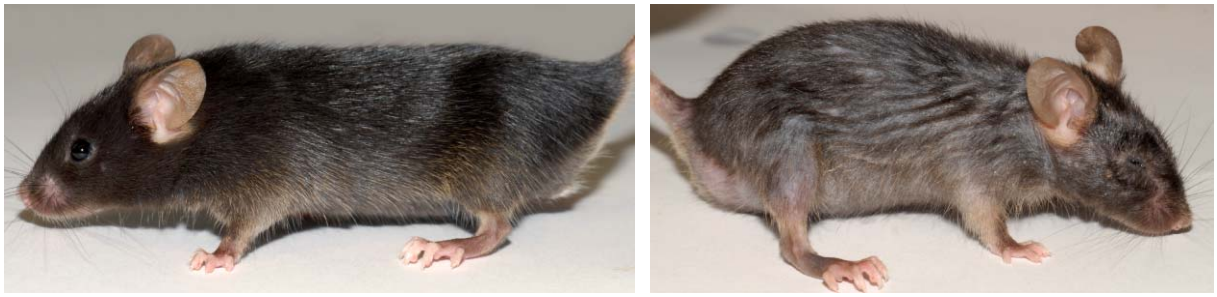
**, p<0.05 vs. Lox counterparts*

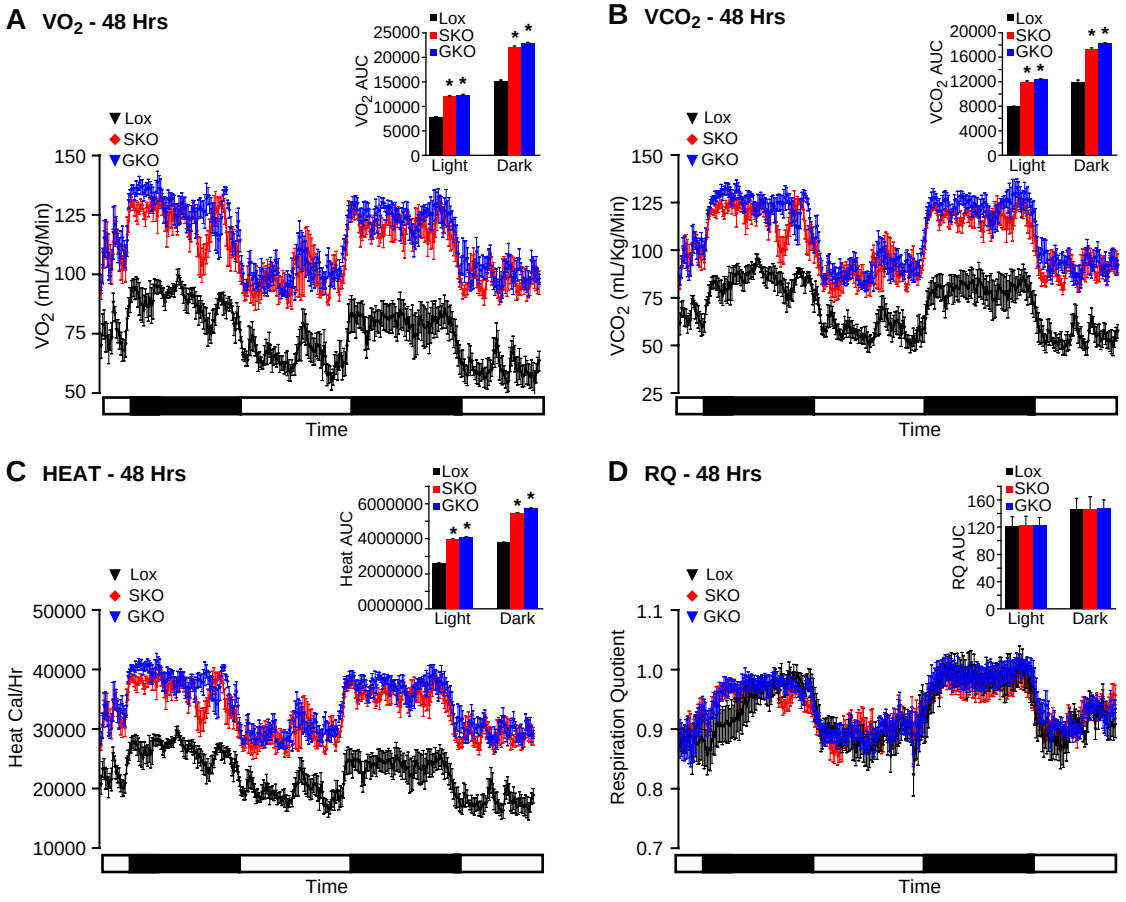
Supplementary Table 1C. Fatty acid composition of hepatic free fatty acids.

FFA (nmol/g)	Lox Chow	SKO Chow	Lox HFD	SKO HFD
12:0	13.48 ± 4.27	18.31 ± 6.00	19.64 ± 5.87	21.26 ± 3.14
12:1	0.71 ± 1.58	3.05 ± 2.93	ND	2.12 ± 2.92
14:0	18.72 ± 4.00	17.28 ± 1.63	18.99 ± 3.48	18.16 ± 2.48
14:1	40.07 ± 8.78	40.36 ± 3.17	44.05 ± 4.83	41.32 ± 5.03
16:0	1169.79 ± 63.18	1233.32 ± 158.87	649.58 ± 142.02	565.28 ± 137.99
16:1	109.25 ± 17.58	82.97 ± 5.54*	119.17 ± 20.75	98.54 ± 10.88*
18:0	183.64 ± 43.30	159.92 ± 5.91	166.30 ± 25.58	168.77 ± 15.70
18:1 (n-9)	215.32 ± 68.57	144.08 ± 17.20*	404.76 ± 96.39	236.37 ± 84.39*
18:1 (n-7)	16.49 ± 3.65	10.50 ± 1.65*	22.78 ± 5.75	15.56 ± 3.95*
18:2 (n-6)	49.55 ± 11.50	31.05 ± 4.03*	219.40 ± 72.48	107.75 ± 58.48*
18:3 (n-3)	2.92 ± 2.20	0.60 ± 0.83*	6.06 ± 8.45	1.32 ± 0.94*
20:0	4.14 ± 1.12	2.35 ± 0.48	7.40 ± 1.78	7.88 ± 0.45
20:1 (n-9)	6.69 ± 0.70	6.85 ± 2.14	11.09 ± 3.03	10.99 ± 3.70
20:3 (n-9)	2.51 ± 1.62	2.07 ± 0.55	9.09 ± 3.35	6.09 ± 2.03
20:3 (n-6)	5.53 ± 1.48	6.19 ± 2.72	31.02 ± 11.53	19.50 ± 6.96
20:4	19.59 ± 4.73	17.23 ± 1.82	21.23 ± 3.12	20.86 ± 0.96
20:5	2.68 ± 2.07	0.00 ± 0.00*	1.35 ± 1.17	1.98 ± 1.95
22:0	2.49 ± 0.46	1.56 ± 0.32*	4.12 ± 1.47	4.89 ± 1.66
22:1 (n-9)	ND	ND	ND	0.64 ± 1.44
22:5 (n-3)	ND	ND	3.38 ± 0.73	2.89 ± 0.57
22:6	ND	ND	11.19 ± 5.41	5.91 ± 3.55
Totals	**1875.67 ± 180.24**	**1788.52 ± 166.57**	**1962.64 ± 163.73**	**1282.85 ± 165.16***

**, p<0.05 vs. Lox counterparts*

Lox **SKO**

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VO2 (mL/Kg/Min)
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B VCO2 - 48 Hrs
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C HEAT - 48 Hrs
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D RQ - 48 Hrs
Respiration Quotient
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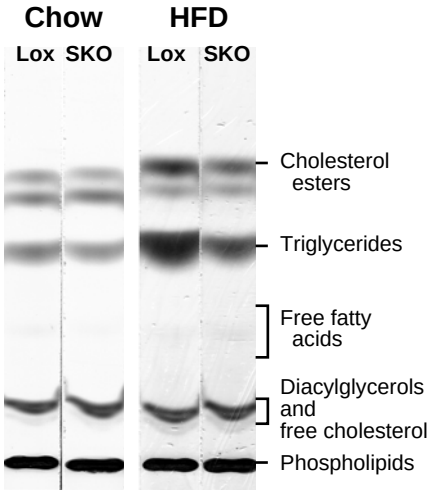
Chow
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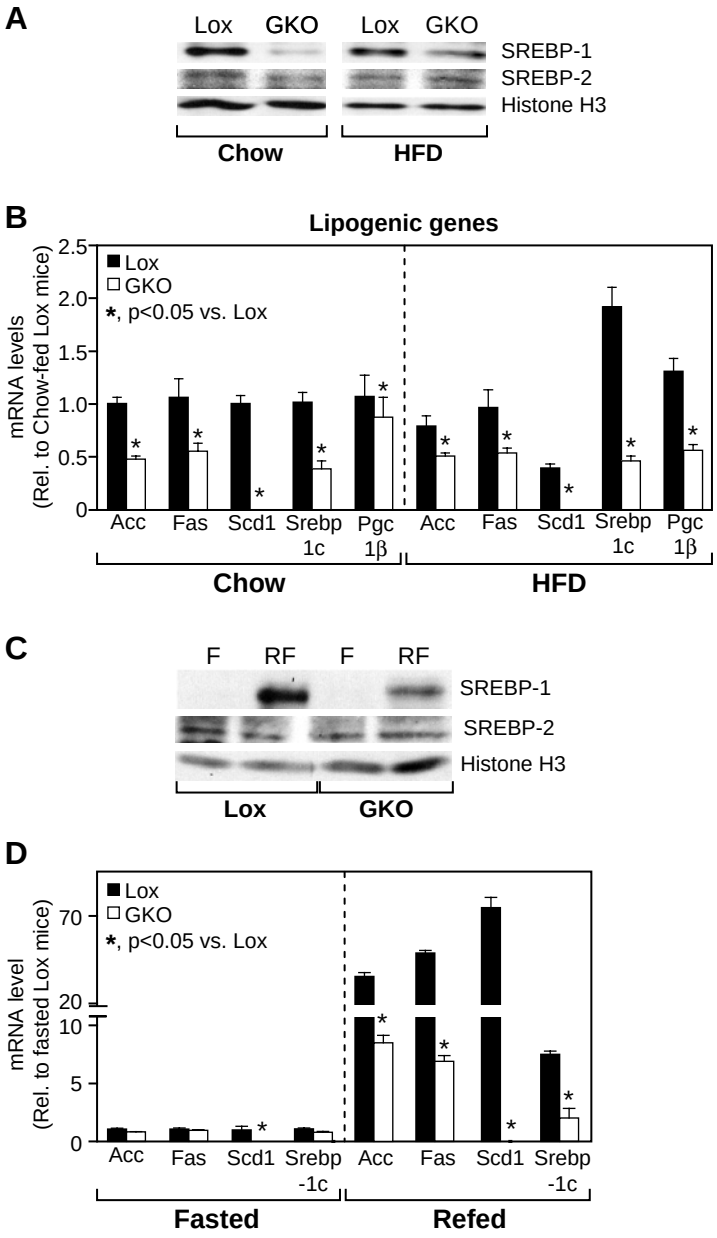
Cholesterol esters
Triglycerides
Free fatty acids
Diacylglycerols and free cholesterol
Phospholipids


A
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(Rel. to Chow-fed Lox mice)
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(Rel. to fasted Lox mice)
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