Online Supplement

Supplemental Methods

Mouse model
Generation of tissue-specific SOAT2 knockouts has been described in detail previously[1]. Floxed mice (LoxP sites flanked exons 11 through 13 of the SOAT2 gene on chromosome 15) are designated as $SOAT2^{fl/fl}$. After introducing Cre recombinase driven either by the albumin or villin promoter, SOAT2 was specifically deleted in the liver ($SOAT2^{fl/fl}Alb^{Cre+}$) or small intestine ($SOAT2^{fl/fl}Vil^{Cre+}$), which are designated as $SOAT2^{L-/L-}$ or $SOAT2^{SI-/SI-}$. $SOAT2^{fl/fl}$ mice were maintained on a mixed background (strains of C57BL/6, 129S6, 129SvEv). To create the conditional knockouts on $LDLr^{-/-}$ background, $SOAT2^{L-/L-}$ and $SOAT2^{SI-/SI-}$ mice were bred with $SOAT2^{+/+}LDLr^{-/-}$ (strain of C57BL/6) or $SOAT2^{-/-}LDLr^{-/-}$ (strain of C57BL/6). Genotypes of litters were screened by PCR. Animals used in the study were from breeders set up as follows: heterozygotes of $SOAT2^{fl/+}LDLr^{-/-}$ with or without Alb^{Cre} (or Vil^{Cre}), $SOAT2^{fl/-}LDLr^{-/-}$ with or without Alb^{Cre} (or Vil^{Cre}). Essentially equal numbers of both male and female mice were included in the study. At the age of 8 to 9 weeks, the experiments were started by feeding the mice a semi-synthetic diet containing 20% of energy as lard with added cholesterol (0.1% wt/wt) for a total of 16 weeks.

Plasma lipoprotein analyses
Cholesterol concentrations were measured using a colorimetric enzymatic assay as previously described [2-4]. For LDL isolation, an aliquot of plasma containing about twenty µg of total plasma cholesterol was diluted in phosphate buffered saline into a final volume of 400 µL. After centrifugation to remove any protein precipitates, samples were injected onto a Superose 6 HR 10/30 chromatography column (Amersham Pharmacia), which was subsequently run at 0.4 mL/min with PBS. The signal was obtained as cholesterol using the enzymatic assay of the effluent, which was continuously monitored spectrophotometrically using Chrom Perfect Spirit Software (Justice Laboratory Software). LDL was collected for each plasma sample. Total lipid was extracted with chloroform/methanol (2:1) and phases were split with H_2O. The chloroform phase was recovered and dried down under nitrogen and then dissolved in 1 mL chloroform/methanol (1:1). Fifty µL of LDL lipid extract was diluted in 500 µL methanol containing 500 pg/µL of cholesterol heptadecanoate (Nu-Chek Prep) as an internal standard and 1 ng/µL of sodium formate. After standing for 30 min, the solution was analyzed by direct infusion into a Waters Quattro II tandem mass spectrometer operated at a flow rate of 10 µL/min in the positive ion mode. Cholesterol ester species were quantified with a response curve against 0.78 µM internal standard as described elsewhere[5].

Biliary lipid analysis
Gallbladder bile was collected at necropsy from fasted mice. Biliary lipids were assayed enzymatically as previously described[2,4]. For analysis of biliary lipid concentrations, a measured volume (5 to 10 uL) of bile was placed into a glass tube and the lipids were extracted in chloroform/methanol (2:1). An aliquot of chloroform phase was used for enzymatic quantification of cholesterol and phospholipids in a similar fashion to that described for liver lipid measurement. An aliquot of the aqueous phase of the extraction was analyzed for total bile acid content using a hydroxysteroid dehydrogenase-based enzymatic assay.

Quantification of cholesterol in aortae
Lipids of the fixed entire aorta were extracted into chloroform/methanol (2:1) overnight after the addition of 20 µg of 5-alpha cholestane as an internal standard[2]. Aortic protein was then washed twice with chloroform/methanol (2:1) and pooled solvent containing lipids was evaporated under

nitrogen. Dried lipids were dissolved in 250 µL hexane and 1 µL of hexane phase was injected onto ZB-50 GLC column to measure free cholesterol (FC). After the FC quantification, the remaining samples were saponified and total cholesterol (TC) of aortas was then determined by GLC. Aortic CE was calculated using the equation (TC – FC) × 1.67.

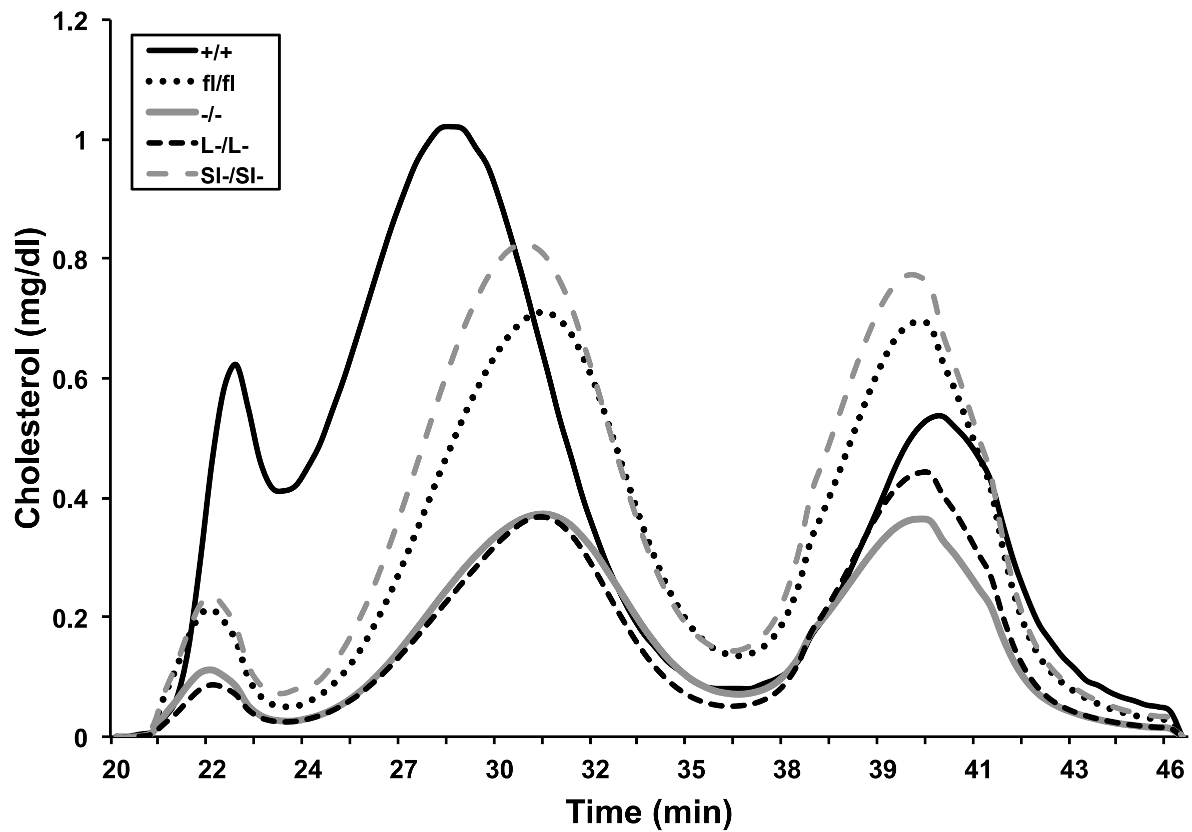
Real-time PCR analysis of intestinal and hepatic mRNA levels

Total RNA was extracted from ~100 mg of liver and proximal small intestine with Trizol (Invitrogen Life Technologies) using the protocol provided by the manufacturer. The RNA was resuspended in 300 µl of diethyl pyrocarbonate water and 1 µg of RNA was reverse transcribed to cDNA using qScript reverse transcriptase (Quanta) under the following conditions: 25°C 5 min, 42°C 30 min and 85°°C 5min. The cDNA was diluted 1:10 using diethyl pyrocarbonate water and real-time PCR was done in duplicate with 5µl of cDNA, 10 µl of SYBR GREEN PCR mastermix (Roche), 3 µl of diethyl pyrocarbonate water and 1 µl of forward and reverse primer (20 pmol) for a final reaction volume of 20 µl. Primer sequences are as presented in the following Table. PCR was then run on the Sequence Detection System 7500 (Applied Biosystems) using the following conditions: 94°C for 10 min, 94°C 10 sec and 60°C for 1 min. The fluorescence measurement used to calculate threshold cycle (Ct) was made at the 60°C point. A dissociation curve was run at the end of the reaction to ensure a single amplification product. Ct values were entered into the following equation to determine the arbitrary unit value: $1 \times 10^9 \times e(-0.6931 \times Ct)$. All values were then normalized to cyclophilin mRNA concentration of the sample to take total RNA concentration into account.

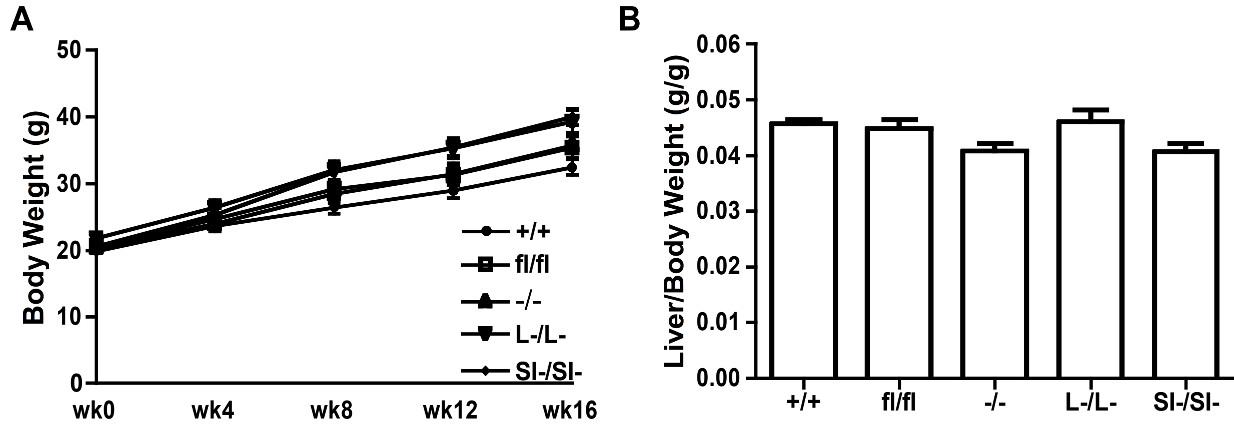
Primer Table

Cyclophilin	*F*	GCG	GCA	GGT	CCA	TCT	ACG		
	R	GCC	ATC	CAG	CCA	TTC	AGT	C	
SCD1	*F*	TTC	CCT	CCT	GCA	AGC	TCT	AC	
	R	CAG	AGC	GCT	GGT	CAT	GTA	GT	
Srebp 1c	*F*	GGC	TCT	GGA	ACA	GAC	ACT	GG	
	R	TGG	TTG	TTG	ATG	AGC	TGG	AG	
DGAT1	*F*	GAG	GCC	TCT	CTG	CCC	CTA	TG	
	R	GCC	CCT	GGA	CAA	CAC	AGA	CT	
DGAT2	*F*	CCG	CAA	AGG	CTT	TGT	GAA	G	
	R	GGA	ATA	AGT	GGG	AAC	CAG	ATC	A
mFAS	*F*	GCT	GCG	GAA	ACT	TCA	GGA	AAT	
	R	AGA	GAC	GTG	TCA	CTC	CTG	GAC	TT
mACC1	*F*	TGG	ACA	GAC	TGA	TCG	CAG	AGA	AAG
	R	TGG	AGA	GCC	CCA	CAC	ACA		

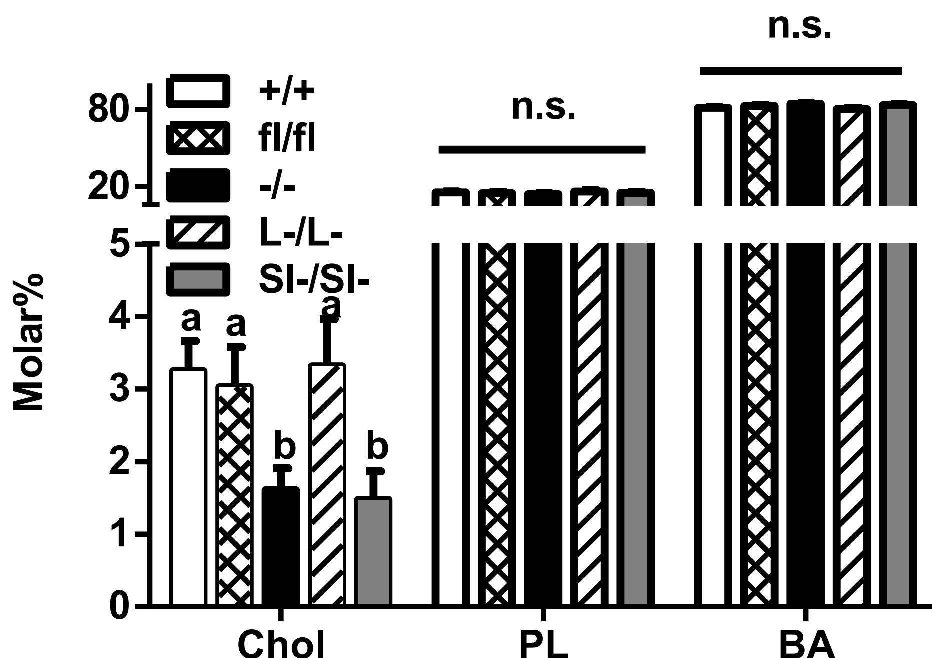
Supplemental Figures



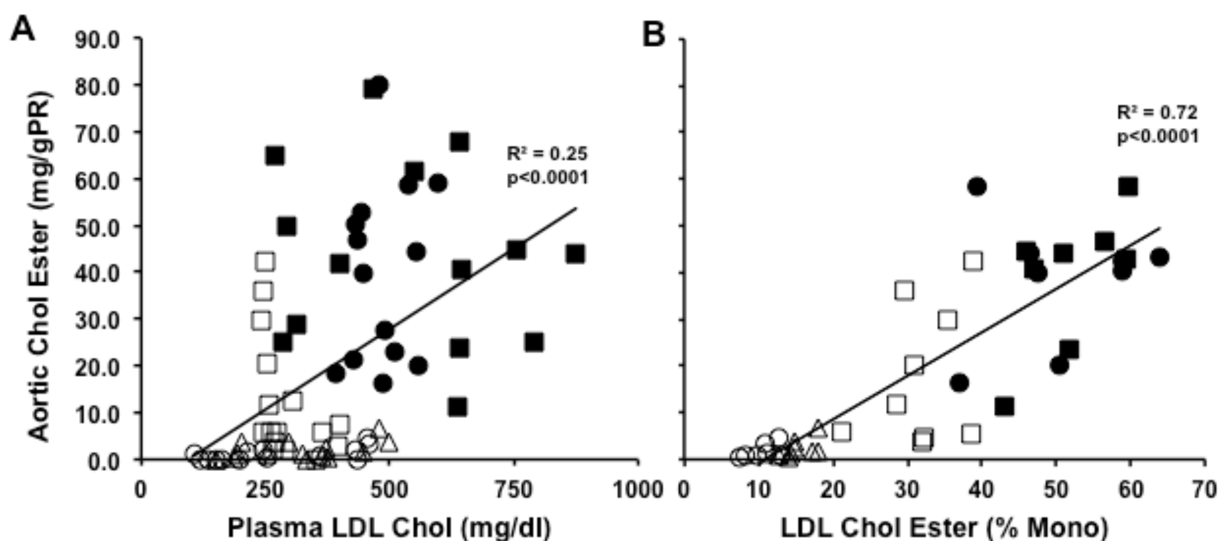
Online Figure I: HPLC profiles of plasma lipoprotein cholesterol distribution. A representative profile for each experimental group is plotted**.**



Online Figure II. Body weights and liver to body weight ratios for all groups of mice. (A) All mice were fed a semi-synthetic diet containing 0.1% cholesterol for 16wks. Body weight was measured every 4wks. (**A**) No significant differences among groups in body weights at each time point were present. Liver weights and body weights were measured at necropsy at week16 (**B**). Data is expressed as ratio of liver weight to body weight. There was no significant difference among genotypes. Data (mean ± SEM) are for 18 to 20 animals per group.



Online Figure III. Total body SOAT2 knockout and intestine-specific, but not liver-specific, deletion of SOAT2 reduces biliary cholesterol levels. A total of 5 to 10uL gall bladder bile were used for the analysis. Lipids were extracted with CHCl3/MeOH (1:2). Biliary cholesterol (Chol), phospholipids (PL) were measured by enzymatic assay kits. Biliary bile acids (BA) were quantified by enzymatic assay using 3alpha HSD. Values are expressed as percentage molar of individual lipid class to total biliary lipids (sum of Chol, PL and BA). Data represent the mean ± SEM from 16 to 18 mice per group. Bars not sharing common letters differ with $P < 0.05$. ns: not significantly different.



Online Figure IV. Relationship between aortic atherosclerosis, measured as CE concentration in aorta and LDL cholesterol concentration in plasma (**A**) and LDL cholesterol ester composition **(B**) measured as percentage of cholesterol esters containing monounsaturated fatty acids. Individual experimental groups are indicated with different symbols**,** as designated in the legend for Figure 5, i.e. filled boxes, SOAT2$^{+/+}$; filled circles, SOAT2$^{fl/fl}$; open circles, SOAT2$^{-/-}$; open triangles, SOAT2 $^{L-/L-}$; open boxes, SOAT2 $^{SI-/SI}$. The regression coefficient and significance level is shown together with the least squares best fit regression line.

Online Table I. Liver and intestinal gene expression levels among SOAT2 genotypes of mice

A. Liver

Gene	SOAT2 Genotype (AU)				
	+/+	fl/fl	-/-	L^-/L^-	SI^-/SI^-
SCD-1	3.4±0.4	3.9±0.4	3.5±0.4	5.0±0.3	4.4±0.5
ACC (*1000)	28.3±5.1	32.0±3.4	30.1±1.7	36.9±3.3	37.3±2.9
FAS (*100)	9.6±3.7	12.5±2.6	7.4±1.2	15.5±2.4	17.1±1.9
SREBP-1c (*1000)	10.9±0.9	11.4±0.8	11.2±0.6	11.1±0.7	10.1±0.4
DGAT1 (*100)	12.7±0.4	10.9±0.6	12.1±0.7	12.6±0.5	11.2±0.5
DGAT2 (*100)	23.7±0.2	26.7±3.3	30.6±1.9	32.0±3.8	31.5±3.0

B. Small Intestine

Gene	SOAT2 Genotype (AU)				
	+/+	fl/fl	-/-	L^-/L^-	SI^-/SI^-
SCD-1	1.9±0.1 [a]	1.9±0.5 [a]	22.9±3.9 [b]	1.3±0.4 [a]	24.5±2.1 [b]
ACC (*1000)	9.6±0.5	9.6±0.4	11.4±0.6	10.6±0.5	11.1±0.7
FAS (*100)	16.7±1.5	15.2±0.9	15.6±1.0	16.7±0.7	15.5±1.1
SREBP-1c (*1000)	17.8±0.5	17.6±0.5	18.2±0.9	16.8±0.6	18.2±0.9
DGAT1 (*100)	36.0±1.5	34.4±1.4	33.0±1.0	36.6±1.4	36.8±0.6
DGAT2 (*100)	19.8±1.7	18.4±2.5	18.6±2.3	15.8±1.4	18.1±1.0

Online References

1. Zhang J, Kelley KL, Marshall SM, Davis MA, Wilson MD, Sawyer JK, Farese RV, Jr., Brown JM, Rudel LL. Tissue-specific knockouts of acat2 reveal that intestinal depletion is sufficient to prevent diet-induced cholesterol accumulation in the liver and blood. *Journal of lipid research*. 2012;53:1144-1152
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