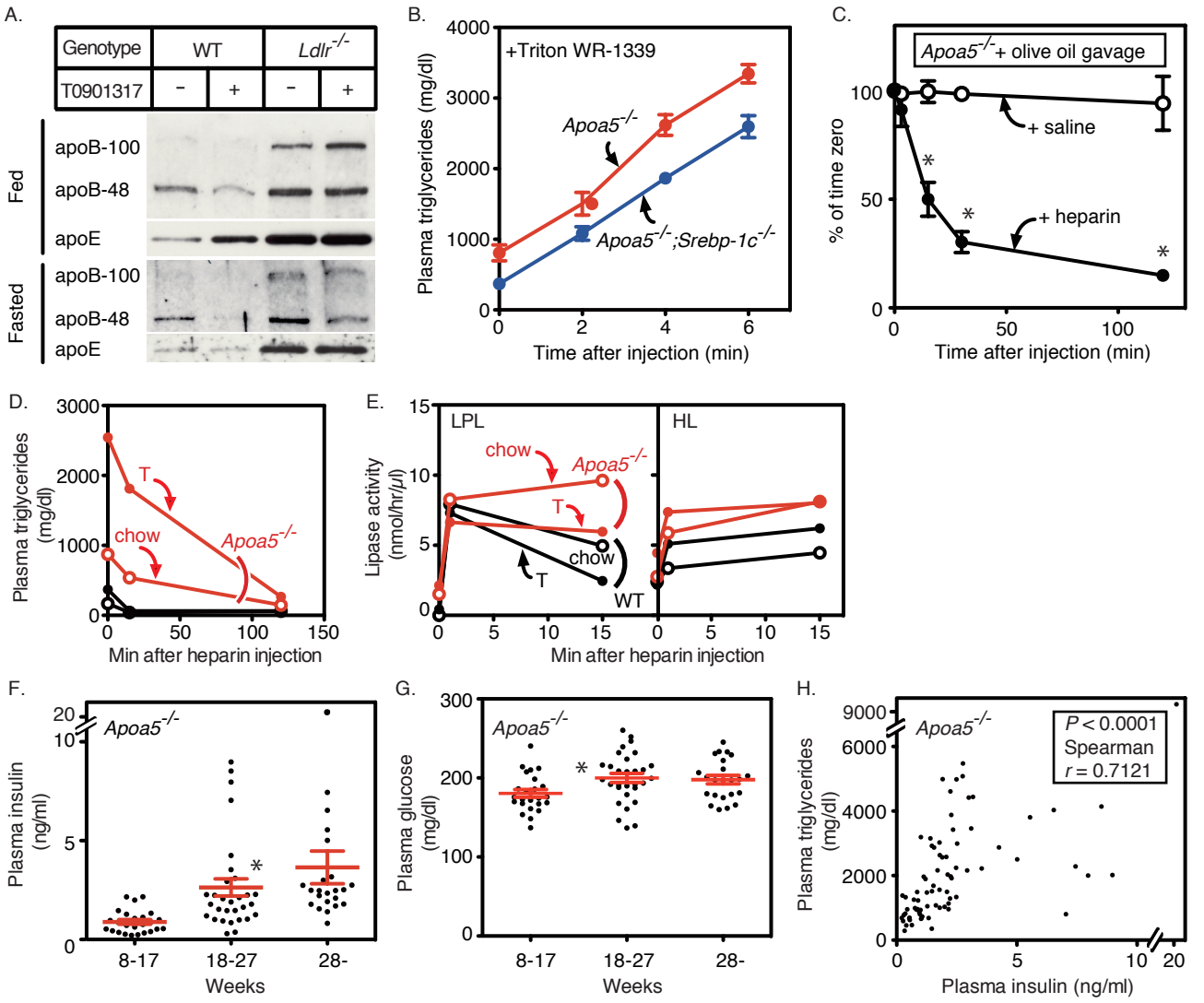
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Figure I. Related to Figures 1, 4 and 5.
(A) Western blot analyses of apolipoproteins in VLDL. VLDL was isolated from pooled plasma of male wild-type and *Ldlr*$^{-/-}$ mice on a chow diet or a chow diet containing 0.0075% T0901317 (10-18 weeks old, n=8 for each group) either fed *ad libitum* (upper panel) or fasted 4 h (lower panel). Equal amounts of VLDL protein (7.5 ng per each lane) were loaded and blotted against anti-apoB and apoE antibody as described in Methods. (B) Hepatic VLDL secretion in *Apoa5*$^{-/-}$, and *Apoa5*$^{-/-}$*;Srebp-1c*$^{-/-}$ mice. *Apoa5*$^{-/-}$ (red), and *Apoa5*$^{-/-}$*;Srebp-1c*$^{-/-}$ (blue) male littermates were fed a chow diet and used for experiments. Triton WR-1339 was injected intravenously in overnight-fasted mice, and aliquots of blood were obtained by retro-orbital bleeding at the indicated time points. Hepatic VLDL secretion was determined by the increments of plasma TG levels after the injection. Each value represents the mean ± SEM of data from 5-7 mice. (C) Effects of intravenous injection of heparin on the clearance of chylomicrons in *Apoa5*$^{-/-}$ mice. *Apoa5*$^{-/-}$ mice (11-16 weeks old, male) were fasted for 16 hours, followed by gavage with olive oil (10 μl/g-body weight). Four hours after the gavage, mice were randomly assigned to a group injected with normal saline (100 μl/body) or a group injected with heparin (50 IU/100 μl/body). Aliquots of blood were obtained by retro-orbital bleeding at the indicated time points (0, 3, 15, 30, 120 min) and used to determine the plasma levels of triglycerides. Data were expressed as % of plasma triglycerides levels at time zero (plasma triglyceride levels at time zero were: 2,052 ± 279 mg/dl for the saline group, 1,086 ± 227 mg/dl for the heparin group). Each value represents the mean ± SEM of data from 4 mice. (D and E) Effects of intravenous injection of heparin on plasma triglyceride levels and lipase activities in wild-type and *Apoa5*$^{-/-}$ mice with or without LXR-agonist treatment. Wild-type (black) and *Apoa5*$^{-/-}$ (red) male littermates (2-5 month old) were fed a chow diet (open circles) or a chow diet containing 0.015% T0901317 (T) (filled circles) for 6 days, followed by intravenous injection with heparin (50 U/mouse). Blood samples were collected *via* retro-orbital bleeding at the indicated time. Plasma was pooled for the measurement of both triglycerides levels (D) and the activities of lipoprotein lipase (LPL) (E, left) and hepatic lipase (HL) (E, right). For the measurement of lipase activities, aliquots of the pooled plasma were ultracentrifuged to remove VLDLs, thus avoiding the contamination of endogenous substrates (VLDLs) into the lipase assay reactions. (F-H) Effects of aging on metabolic parameters in *Apoa5*$^{-/-}$. *Apoa5*$^{-/-}$ (8~17 weeks old (n = 25, male), 18~27 weeks old (n = 30, male), 28~ weeks old (n = 23, male)) were fed an *ad libitum* chow diet, and aliquots of blood were obtained by retro-orbital bleeding. Plasma levels of insulin (F), glucose (G) and triglycerides (Figure 5B) were determined. Data are presented as the mean ± SEM. Correlation between plasma insulin (F) and triglycerides (Figure 5B) was analyzed to determine Spearman correlation coefficients (H). Statistical significance was determined by two-way ANOVA with post hoc test (for B and C), Kruskal-Wallis test with post hoc test (for F), and one-way ANOVA with post hoc test (for G). *$P < 0.05$ versus control (for C), versus all other groups (for F), or versus the younger age group (G).

SUPPLEMENTAL INFORMATION

Figure I.



Supplementary Table I. Relative amounts of mRNAs in livers of *Apoa5*$^{-/-}$, and *Apoa5*$^{-/-}$;*Srebp-1c*$^{-/-}$ mice fed with an ad libitum chow diet or a high-fructose diet. Related to Figure 4.

	Apoa5$^{-/-}$		*Apoa5*$^{-/-}$;*Srebp-1c*$^{-/-}$	
	Chow	High fructose	Chow	High fructose
	n=5	n=5	n=4	n=4
SREBP pathway				
SREBP-1c	1.0 ± 0.2	1.6 ± 0.3	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]
SREBP-1a	1.0 ± 0.1	1.3 ± 0.1	0.7 ± 0.0	1.2 ± 0.1 [a]
SREBP-2	1.0 ± 0.0	1.2 ± 0.1	1.8 ± 0.4	3.7 ± 0.5 [a,b]
Fatty acid and TG synthesis				
Fatty acid synthase	1.0 ± 0.3	11.8 ± 3.7 [a]	0.4 ± 0.1	3.7 ± 0.5
Long chain fatty acyl elongase-6	1.0 ± 0.1	6.8 ± 1.3 [a]	0.6 ± 0.1	2.8 ± 0.5 [b]
Stearoyl-CoA desaturase-1	1.0 ± 0.1	7.6 ± 1.4 [a]	0.2 ± 0.0	2.4 ± 0.4 [b]
Phospholipid transfer protein	1.0 ± 0.1	1.6 ± 0.2 [a]	0.7 ± 0.1	1.1 ± 0.1
Other LXR targets				
ABC-A1	1.0 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.8 ± 0.1 [b]
ABC-G5	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.9 ± 0.2 [a,b]
ABC-G8	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	1.4 ± 0.1 [b]
Lipoprotein lipase	1.0 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	1.4 ± 0.3
Apolipoproteins				
Apolipoprotein A-I	1.0 ± 0.0	1.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.1
Apolipoprotein A-II	1.0 ± 0.0	1.1 ± 0.0	0.7 ± 0.0 [b]	0.7 ± 0.0 [b]
Apolipoprotein A-IV	1.0 ± 0.1	6.4 ± 0.7 [a]	0.7 ± 0.2	4.2 ± 0.3 [a,b]
Apolipoprotein A-V	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Apolipoprotein B	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.0	1.3 ± 0.1
Apolipoprotein C-I	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.0
Apolipoprotein C-II	1.0 ± 0.1	2.4 ± 0.2 [a]	0.9 ± 0.1	2.2 ± 0.2 [a]
Apolipoprotein C-III	1.0 ± 0.1	1.5 ± 0.1 [a]	0.9 ± 0.2	1.5 ± 0.1 [a]
Apolipoprotein C-IV	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.1	1.1 ± 0.0
Apolipoprotein E	1.0 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	1.0 ± 0.1

Apoa5$^{-/-}$, and *Apoa5*$^{-/-}$;*Srebp-1c*$^{-/-}$ male littermates were fed a chow diet or a high-fructose diet for 14 days. Total liver RNA was quantified by real-time PCR. Cyclophilin was used as the invariant control. Values represent the amount of mRNA relative to that in chow-fed *Apoa5*$^{-/-}$ mice, which is arbitrarily defined as 1. Each value represents the mean ± SEM. One-way ANOVA with Tukey post hoc test was used to evaluate statistical significance [a] between dietary treatments within the same genotype ($P < 0.05$) and [b] between genotypes within the same dietary treatment ($P < 0.05$).