Loss of small heterodimer partner expression in the liver protects against dyslipidemia[S]

Helen B. Hartman, KehDih Lai, and Mark J. Evans[1]

Cardiovascular and Metabolic Disease Research, Wyeth Research, Collegeville, PA 19426

Abstract **Multiple studies suggest increased conversion of cholesterol to bile acids by cholesterol 7α-hydroxylase (CYP7A1) protects against dyslipidemia and atherosclerosis. CYP7A1 expression is repressed by the sequential activity of two nuclear hormone receptors, farnesoid X receptor (FXR) and small heterodimer partner (SHP). Here we demonstrate 129 strain SHP$^{-/-}$ mice are protected against hypercholesterolemia resulting from either a cholesterol/cholic acid (chol/CA) diet or from hypothyroidism. In a mixed 129-C57Bl/6 background, LDLR$^{-/-}$ and LDLR$^{-/-}$SHP$^{-/-}$ mice had nearly identical elevations in hepatic cholesterol content and repression of cholesterol regulated genes when fed a Western diet. However, the LDLR$^{-/-}$SHP$^{-/-}$ mice had greatly reduced elevations in serum VLDL and LDL cholesterol levels and triglyceride (TG) levels as compared with LDLR$^{-/-}$ mice. Additionally, the hepatic inflammation produced by the Western diet in the LDLR$^{-/-}$ mice was abolished in the LDLR$^{-/-}$SHP$^{-/-}$ mice. CYP7A1 expression was induced 10-fold by the Western diet in the LDLR$^{-/-}$SHP$^{-/-}$ mice but not in the LDLR$^{-/-}$ mice. Finally, hepatocyte-specific deletion of SHP expression was also protective against dyslipidemia induced by either a chol/CA diet or by hypothyroidism.** While no antagonist ligands have yet been identified for SHP, these results suggest selective inhibition of hepatic SHP expression may provide protection against dyslipidemia.—Hartman, H. B., K. Lai, and M. J. Evans. **Loss of small heterodimer partner expression in the liver protects against dyslipidemia.** ***J. Lipid Res.*** **2009.** 50: **193–203.**

Supplementary key words SHP • farnesoid X receptor • FXR • CYP7A1

Small heterodimer partner (SHP) is an orphan nuclear receptor lacking a DNA-binding domain (1). In vitro assays have suggested that SHP can bind to and either repress or enhance the activity of numerous other transcription factors (2). SHP is expressed in multiple organs including the liver, intestine, adrenals, adipose, pancreas, skeletal muscle, and heart. The function of SHP in many of these organs remains unknown. The best characterized physiological role for SHP is in the liver, where SHP functions as part of the negative feedback loop controlling bile acid synthesis. Bile acids bind to and activate the farnesoid X receptor (FXR), which subsequently induces SHP transcription (3, 4). SHP then inhibits transcription of two genes regulating the size and hydrophobicity of the bile acid pool, cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1), respectively (5, 6). Thus when bile acid levels are elevated, the FXR/SHP pathway functions as a classical negative feedback regulatory loop to return bile acid levels to appropriate levels. In either FXR$^{-/-}$ or SHP$^{-/-}$ mice, this pathway is defective, and the knockout mice have a constitutively elevated bile acid pool size (7–10).

The expression of CYP7A1 is known to correlate with plasma LDL cholesterol levels. In humans, genetic polymorphism studies have demonstrated that the CYP7A1 locus contributes to heritable variations in plasma LDL cholesterol levels within populations (11). Further, humans with a homozygous deletion of CYP7A1 have greatly reduced rates of cholesterol conversion into bile acids, hepatic steatosis, and LDL cholesterol levels increased by approximately 40% (12). Similarly, there is also an inverse correlation between CYP7A1 expression and plasma cholesterol levels in primates (13). In mice, transgenic overexpression of CYP7A1 blocks diet-induced hypercholesterolemia and atherosclerosis (14, 15). The loss of SHP repression of CYP7A1 expression could thus be predicted to have beneficial effects on LDL cholesterol levels. Further, SHP may play a role in regulating HDL cholesterol levels. Activation of FXR is known to reduce HDL cholesterol levels (16). This may be mediated in part by the direct binding of FXR to the apoAI promoter (17). Alternatively, SHP has been demonstrated to repress liver receptor homolog-1 (LRH-1) activation of the apoAI promoter, and transgenic

Abbreviations: apoE, apolipoprotein E; chol/CA, cholesterol/cholic acid; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12 α-hydroxylase; FGF-15, fibroblast growth factor-15; FPLC, Fast protein liquid chromatography; FXR, farnesoid X receptor; IBABP, ileal bile acid binding protein; LDLR, low density lipoprotein receptor; LRH-1, liver receptor homolog-1; LXR, liver X receptor; PXR, pregnane X receptor; SHP, small heterodimer partner; TG, triglyceride; WT, wild-type.

[1] To whom correspondence should be addressed.
e-mail: Evans.mark.mje@gmail.com

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of two figures and a table.

Manuscript received 20 June 2008 and in revised form 18 September 2008 and in re-revised form 25 September 2008.

Published, JLR Papers in Press, September 26, 2008.
DOI 10.1194/jlr.M800323-JLR200

overexpression of SHP significantly reduces HDL cholesterol levels (18), together suggesting a loss of SHP could also elevate HDL cholesterol levels.

Several studies have examined the role of FXR in dyslipidemia and atherosclerosis in mice. The initial studies of $FXR^{-/-}$ mice demonstrated an increase in cholesterol content of the VLDL, LDL, and HDL fractions resulting in a proatherogenic lipid profile (8, 19). FXR is expressed at high levels in the liver and intestine, but deletion of FXR expression in the liver is sufficient to increase plasma total cholesterol and triglyceride (TG) levels (20). The effects of the loss of FXR on plasma lipids differ between apolipoprotein E $(apoE)^{-/-}$ and low density lipoprotein receptor $(LDLR)^{-/-}$ mice. Male $FXR^{-/-}apoE^{-/-}$ mice had increased plasma lipid levels and atherosclerotic lesion areas as compared with $apoE^{-/-}$ mice (21). In male $LDLR^{-/-}FXR^{-/-}$ mice, plasma LDL cholesterol levels and lesion size were reduced compared with $LDLR^{-/-}$ mice, while in females there was no difference between plasma LDL cholesterol levels and lesion size between $LDLR^{-/-}FXR^{-/-}$ and $LDLR^{-/-}$ mice (22).

The effects of the loss of SHP on cholesterol levels are less well explored. $SHP^{-/-}$ mice (9, 10) do not have altered total cholesterol levels (10) and are resistant to elevation of total cholesterol levels when fed a diet containing 2% cholesterol plus 0.5% CA (23). However, recent results indicate the loss of SHP protects against steatosis in ob/ob mice by increasing VLDL secretion (24), suggesting the loss of SHP may also have negative effects on plasma cholesterol levels. Finally, SHP is expressed at significant levels in the liver and small intestine, two organs with major roles in cholesterol homeostasis (25), and the relative role of SHP expression in these two organs is undetermined. Here we demonstrate the loss of SHP can protect against elevations of VLDL or LDL cholesterol levels in multiple models of dyslipidemia in the mouse. Further, the selective loss of SHP expression in hepatocytes recapitulates the protection seen in the complete absence of SHP, demonstrating that hepatocyte SHP is critical for these effects.

MATERIALS AND METHODS

Mice

Floxed SHP mice were generated on the 129svEv-Brd background. The SHP genomic locus contains two exons, with the translation start in exon 1. A construct in which a 5′ LoxP site was placed 1.3 kb upstream from the transcription start site and a neomycin resistance cassette and 3′ LoxP site was placed 0.4 kb downstream of exon 1 was introduced into embryonic stem cells to generate a floxed SHP allele (see supplementary Fig. I). Cells confirmed to have the mutation by PCR, and genomic Southern blot were injected into mouse blastocysts, and chimeric mice were generated. To generate $SHP^{-/-}$ mice, the $SHP^{+/flox}$ mice were bred with mice transgenic for Cre recombinase under the control of the protamine promoter (Jackson Laboratories, strain #003328) to generate $SHP^{+/-}$. $SHP^{+/-}$ mice were interbred to generate $SHP^{+/+}$, $SHP^{+/-}$, and $SHP^{-/-}$ littermates for initial studies. Subsequent studies were performed using separate $SHP^{-/-}$ and 129svEv-Brd control breeding colonies. To generate hepatocyte specific deletion of SHP, $SHP^{flox/flox}$ mice were bred with transgenic C57Bl/6 mice expressing Cre recombinase under the control of the albumin promoter (Jackson Laboratories, #003574). Resulting offspring that were $SHP^{+/flox}$Alb-Cre were bred to mice lacking the transgene to generate $SHP^{flox/flox}$Alb-Cre mice ($SHP^{hep/hep}$) plus the control $SHP^{flox/flox}$ mice, both in a 129-C57Bl/6 mixed background. To generate mice lacking LDLR and SHP, $SHP^{-/-}$ mice were bred to C57Bl/6 background $LDLR^{-/-}$ mice (Jackson Laboratories, #002207) to generate $LDLR^{+/-}SHP^{+/-}$ mice on a mixed 129-C57Bl/6 background. These heterozygous mice were then interbred to generate $LDLR^{-/-}SHP^{-/-}$ mice. $FXR^{-/-}$ mice on a C57Bl/6 background were obtained from Jackson Laboratories (#004144).

Mice were genotyped with PCR reactions containing specific primers for each induced mutation. Mouse-tail snips were digested in 190 μl tail digestion buffer (50 mM Tris-HCL pH 8.0, 0.5% SDS, 50 mM EDTA pH 8.0) plus 10 μl of Proteinase K (20 mg/ml, Promega) overnight at 55°C, then inactivated for 20 min at 95°C. The inactivated digest was utilized in PCR reactions. All PCR reactions contained 12.5 μl GoTaq mixture (Promega), 1 μl tail digest, primers (25 pmole each), and water to 25 μl. Cycling conditions were 95°C for 5 min, then 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s, then 72°C for 5 min. SHP wild-type (WT), conditional, and constitutive deleted alleles were distinguished with a three-primer reaction. A forward primer GCCTTTAACTCAAGTACTAGGGAGGCAG (F1) plus two reverse primers CTACCCAGAGCGACATGGTGAGAC (R1) and AGTTGTGTCTGGTTCCTGACCTTGG (R2) were used to differentiate SHP alleles (see supplementary Fig. I). These primers produce a product of 324 base pairs for the WT allele, 409 base pairs for the conditional allele, and 496 base pairs for the deleted allele. The albumin-cre transgene was detected with cre-specific primers GTGGCAGATGGCGCGGCAACACCATT and GTGGCAGATGGCGCGGCAACACCATT to produce a 726 base pair product. All PCR reactions to detect the presence of the transgene contained primers (CCAATCTGCTCACACAGGATAGAGAGGGCAGG and CCTTGAGGCTGTCCAAGTGATTCAGGCCATCG) that produce a 500 base pair product as an internal control.

In vivo studies

All procedures involving animals were reviewed and approved by the Wyeth Institutional Animal Care and Use Committee. Age- and sex-matched 8–10-week-old mice were fed standard chow (5001; Harlan Teklad, Madison, WI) and housed in a temperature controlled virus free facility on a 12 hr light/dark cycle with free access to food and water. Where indicated, mice were fed standard chow supplemented with 2% cholesterol and 0.5% CA (Sigma Aldrich, St. Louis, MO) for 5 to 10 weeks. Mice on a Western diet were fed a high-fat diet containing 42% fat and 0.2% cholesterol (TD88317; Harlan Teklad, Madison, WI) for 7 days. For hypothyroidism studies, mice were fed a hypothyroid diet (TD95125; Harlan Teklad, Madison, WI) for 21 days. At the end of each study, animals were fasted for 4 h, euthanized, and blood samples collected by cardiac puncture for lipid analysis. Liver and ileum tissue were removed for mRNA quantification.

RNA analysis

RNA was isolated from frozen tissue as described previously (26). Gene expression was measured by real time reverse transcription-PCR using an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA). Specific real-time PCR primers and probes were used for the following mouse genes: SHP, CYP7A1, CYP8B1, CYP3A11, FGF15, FXR, GSTA1, VCAM, ICAM-1, TNFα, ileal bile acid binding protein (IBABP), and OSTα (see supplementary Fig. II). All results were normalized

to GAPDH (4308313; PE Applied Biosystems, Foster City, CA) and are the mean ± SEM. Statistical significance was determined by ANOVA. Microarray analysis of liver or ileum RNA was performed essentially as described previously (27).

Lipid analysis and liver function tests

Total serum cholesterol (Roche kit# 12217295 001), TGs (Roche kit# 12146029 216), ALT (Roche kit# 12217317 001), and AST (Roche kit# 12217309 001) levels were quantified with a Roche/Hitachi 912 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Serum VLDL, LDL, and HDL cholesterol were determined by fast performance liquid chromatography as previously described (28). Where indicated, serum LDL (Roche kit# 04714423 190) and HDL (Roche kit# 03030024 122) cholesterol levels were measured with a Roche/Hitachi 912 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Hepatic lipid content was determined as described previously (28).

RESULTS

The nuclear hormone receptors FXR and SHP are critical to the control of bile acid synthesis. To determine the effects of loss of SHP on lipid metabolism, SHP knockout ($SHP^{-/-}$) mice on a 129 strain background were created by generation of mice containing a floxed SHP exon 1 followed by crossing to mice containing a protamine CRE construct. Expression of SHP mRNA was undetectable in the livers or ileums of these mice (**Fig. 1**). The $SHP^{-/-}$ mice had elevated hepatic mRNA levels of the bile acid synthetic genes CYP7A1 and CYP8B1 similar to $FXR^{-/-}$ mice. Further, FXR mRNA levels were increased in the $SHP^{-/-}$ mice. As previously described, there was no decrease in hepatic FXR signal in the real time PCR assay in the $FXR^{-/-}$ mice due to the production of an aberrant transcript potentially encoding a truncated FXR protein (8). Fibroblast growth factor-15 (FGF-15) expression in the ileum has been demonstrated to be under the control of FXR and bile acid pool size (29, 30). FGF-15 expression was strongly increased in the ileum of the $SHP^{-/-}$ mice. These results are consistent with expansion of the bile acid pool size in $SHP^{-/-}$ (9, 10).

Although $FXR^{-/-}$ and $SHP^{-/-}$ mice had similar effects on CYP7A1 and CYP8B1, $FXR^{-/-}$ mice in a C57Bl/6 background had increased serum total cholesterol and TG levels as compared with control mice, while $SHP^{-/-}$ mice had no change in lipid levels as compared with control mice (**Fig. 2**). Expression of pregnane X receptor (PXR) regulated genes such as CYP3A11 and GSTA1 were increased in the $FXR^{-/-}$ mice as expected because bile acids can function as ligands for PXR (31), although this did not occur in the $SHP^{-/-}$ mice. Similarly, markers of hepatic inflammation including serum ALT and AST levels were elevated in the $FXR^{-/-}$ mice, but not in the $SHP^{-/-}$ mice, suggesting the loss of SHP was providing an offsetting beneficial activity on plasma lipids and hepatic inflammation.

To determine directly whether the loss of SHP could have beneficial effects on dyslipidemia, $SHP^{+/+}$, $SHP^{+/-}$, and $SHP^{-/-}$ mice were generated by interbreeding of $SHP^{+/-}$ mice. In order to induce dyslipidemia, the mice were challenged with a diet containing 2% cholesterol

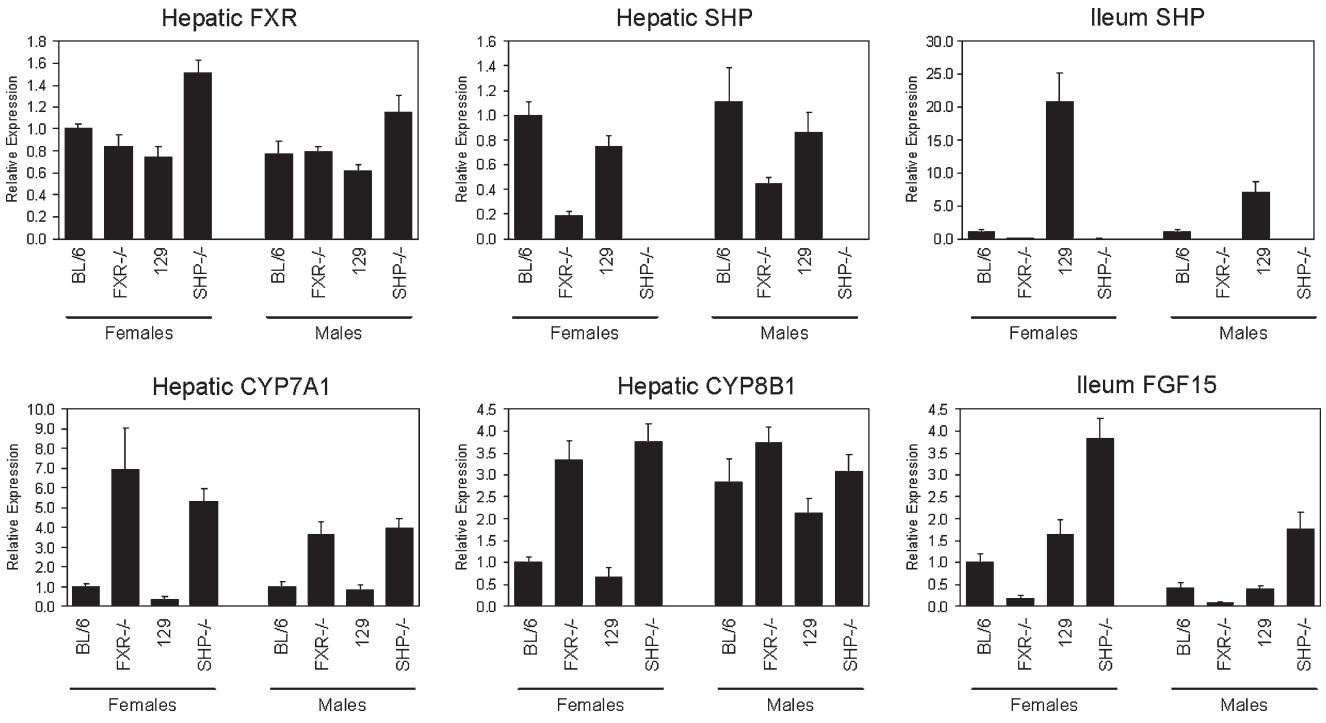

Fig. 1. Gene expression in C57Bl/6 wild-type (WT) mice, C57Bl/6 farnesoid X receptor $(FXR)^{-/-}$ mice, 129 WT mice, 129 small heterodimer partner $(SHP)^{-/-}$ mice. Total RNA was prepared from the livers and ileums of female and male mice between 8 and 10 weeks of age fed a standard chow diet. mRNA levels were quantified by real-time PCR, with expression levels normalized for GAPDH. The mean expression level in female C57Bl/6 mice was defined as 1.0 for each gene. Values shown are the mean ± SE (n = 6 per group).

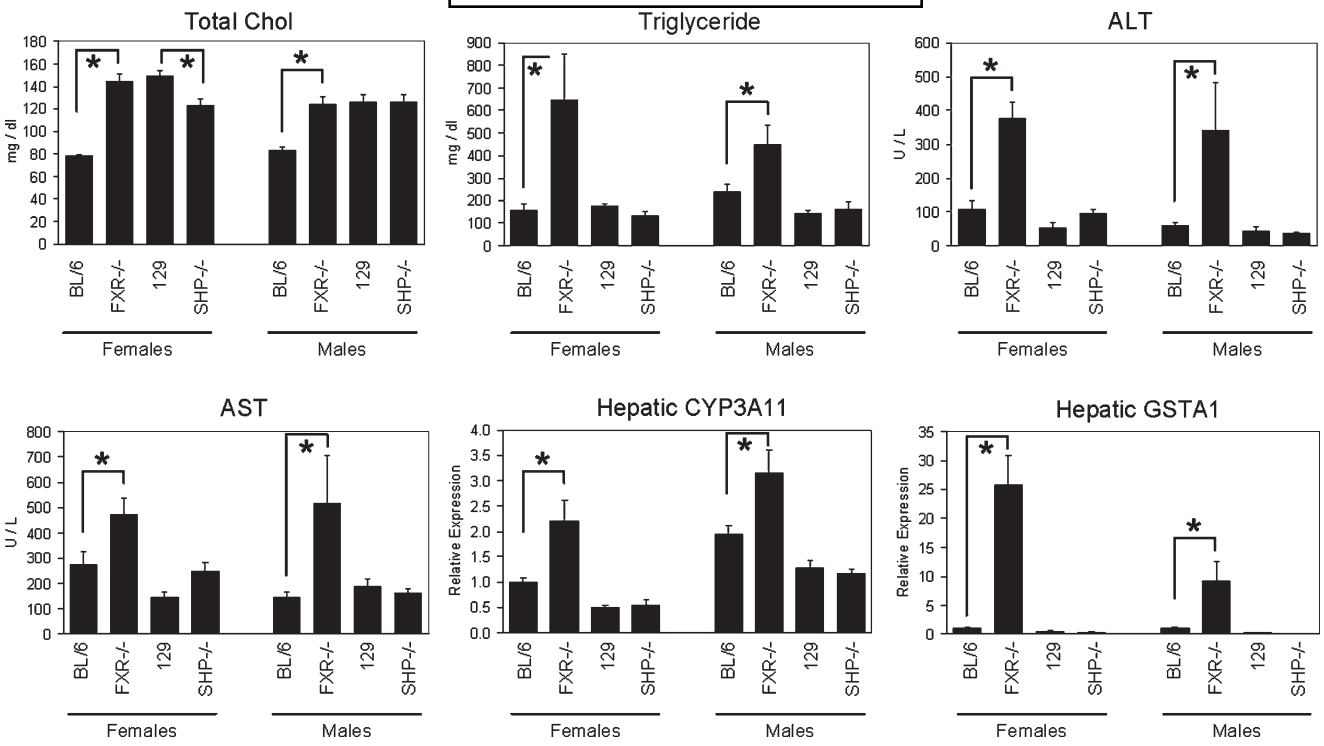

Fig. 2. Lipid levels and inflammatory markers in C57Bl/6 WT mice, C57Bl/6 FXR$^{-/-}$ mice, 129 WT mice, 129 SHP$^{-/-}$ mice. Total cholesterol, triglyceride (TG), ALT, and AST levels in serum obtained from female and male mice between 8 and 10 weeks of age fed a standard chow diet were quantified using a Hitachi 912 clinical chemistry analyzer. Hepatic mRNA levels were quantified by real-time PCR, with expression levels normalized for GAPDH. The mean expression level in female C57Bl/6 mice was defined as 1.0 for each gene. Values shown are the mean ± SE (n = 6 per group). * $P < 0.01$.

and 0.5% chol/CA for 5 weeks. The basal level of hepatic SHP mRNA was partially reduced in the SHP$^{+/-}$ mice (**Fig. 3A**). In the SHP $^{+/+}$ and SHP$^{+/-}$ mice, the chol/CA diet increased SHP mRNA levels to a similar extent. Repression of CYP7A1 and CYP8B1 mRNA by chol/CA occurred to a similar magnitude in the SHP$^{+/+}$ and SHP$^{+/-}$ mice, but was completely absent in the SHP$^{-/-}$ mice. The serum levels of VLDL and LDL were strongly increased in the SHP$^{+/+}$ mice fed the chol/CA diet (Fig. 3B and **Table 1**). This was accompanied by a decrease in the HDL cholesterol levels in the SHP$^{+/+}$ mice on the chol/CA diet. Compared with their WT littermates, the SHP$^{-/-}$ mice were almost completely resistant to the chol/CA diet effects on VLDL, LDL, or HDL cholesterol levels. The SHP$^{+/-}$ appeared to show an intermediate effect, with a partial loss of SHP being sufficient to confer significant protection against diet-induced elevations in VLDL cholesterol but providing less protection against diet-induced elevations in LDL cholesterol. Similar protective effects were seen in male and female SHP$^{-/-}$, and this protection was maintained when the mice were fed the chol/CA diet for 10 weeks (Table 1).

Diets containing CA are known to induce hepatic inflammation. In agreement with this, the expression of several inflammatory marker genes including VCAM, ICAM-1, and TNFα was increased in the livers of the SHP$^{+/+}$ mice (Fig. 3C). The basal level of expression of these genes was not altered in the SHP$^{-/-}$ mice, and there was no induction of these genes in the SHP$^{-/-}$ mice by the chol/CA diet. In the SHP$^{+/-}$ mice, the chol/CA diet induced VCAM expression to the same magnitude as seen in the SHP$^{+/+}$ mice. In contrast, the induction of ICAM-1 and TNFα was partially reduced in the SHP$^{+/-}$ mice. These results suggest that various inflammatory genes may have differing sensitivity to the loss of SHP. Further, since the regulation of CYP7A1 and CYP8B1 was similar in the SHP $^{+/+}$ and SHP$^{+/-}$ mice, the effects observed in the SHP$^{+/-}$ mice on VLDL cholesterol or ICAM-1 or TNFα expression suggest these effects may be mediated partly via pathways other than regulation of bile acid synthesis.

To study the effect of the loss of SHP in a more physiological model of dyslipidemia not dependent upon the presence of CA in the diet, mice were fed an iodine-deficient diet supplemented with propylthiouracil in order to induce hypothyroidism (32). Hypothyroid WT mice had an increase in total cholesterol primarily due to a large increase in LDL cholesterol accompanied by a small increase in HDL cholesterol (**Fig. 4**). VLDL levels were not increased in hypothyroid mice (not shown). A similar degree of hypothyroidism was produced in the SHP$^{-/-}$ as in the WT mice. However, the levels of total, LDL, and HDL cholesterol were only slightly elevated in SHP$^{-/-}$ mice.

To characterize further if the loss of SHP could protect aginst dyslipidemia, SHP$^{-/-}$ mice were crossed with LDLR$^{-/-}$ to generate LDLR$^{-/-}$SHP$^{-/-}$ mice (**Fig. 5A**). Dyslipidemia was induced in these mice by feeding a Western diet without any CA. When the LDLR$^{-/-}$ mice were

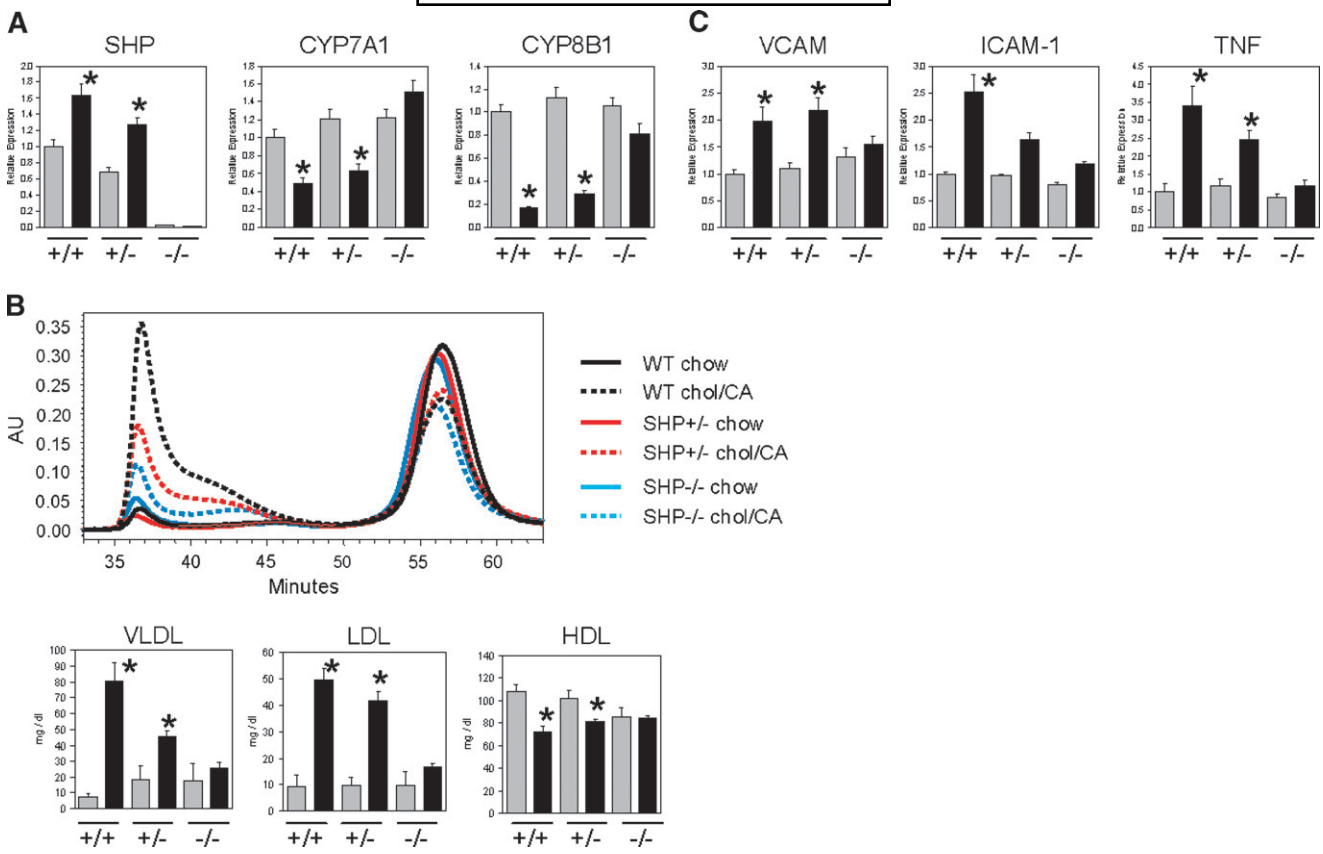

Fig. 3. $SHP^{-/-}$ mice are protected from diet-induced hypercholesterolemia. $SHP^{+/-}$ mice were interbred to generate $SHP^{+/+}$, $SHP^{+/-}$, and $SHP^{-/-}$ mice. At 8 to 10 weeks of age, the mice were either maintained on a chow diet (gray bars) or switched to a diet supplemented with 2% cholesterol and 0.5% cholic acid (CA) (black bars). After 5 weeks of feeding, serum lipid levels and mRNA expression was determined. A: Hepatic SHP, CYP7A1, and CYP8B1 expression were quantified by real-time PCR with normalization for GAPDH expression. The expression level in $SHP^{+/+}$ mice fed a chow diet was defined as 1.0. All values are the mean ± SE (n = 12 to 23 mice per group). B: Serum total cholesterol levels were determined using a Hitachi 912 clinical chemistry analyzer. Fast protein liquid chromatography (FPLC) was performed to determine the cholesterol distribution in VLDL, LDL, and HDL. FPLC profiles are for representative animals from each group. Bar graphs are the group mean ± SE. C: Hepatic mRNA levels for vascular cell adhesion molecule (VCAM), intracellular adhesion molecule-1 (ICAM-1), and tumor necrosis factor α (TNF) were determined by real-time PCR. * $P < 0.01$ for diet effect.

fed a Western diet for 7 days, serum TG, VLDL cholesterol, and LDL cholesterol were greatly increased (Fig. 5B). In contrast, the $LDLR^{-/-}SHP^{-/-}$ mice consuming a Western diet showed no increase in TG or VLDL cholesterol levels, and had a greatly reduced elevation in LDL cholesterol levels. The Western diet had no effect on HDL cholesterol levels in the $LDLR^{-/-}$ mice, while HDL cholesterol levels were increased by the Western diet in the $LDLR^{-/-}SHP^{-/-}$

TABLE 1. Lipoprotein analysis of $SHP^{+/+}$ and $SHP^{-/-}$ mice

	VLDL		LDL		HDL	
	Chow[a]	Chol/CA	Chow	Chol/CA	Chow	Chol/CA
5 weeks						
$SHP^{+/+}$ Male	4 ± 1[b]	87 ± 9	5 ± 1	71 ± 6	127 ± 2	96 ± 5
$SHP^{-/-}$ Male	5 ± 1	24 ± 6	5 ± 1	24 ± 4	112 ± 5	85 ± 7
$SHP^{+/+}$ Female	21 ± 8	118 ± 11	23 ± 9	44 ± 3	91 ± 16	73 ± 8
$SHP^{-/-}$ Female	33 ± 14	51 ± 11	21 ± 7	20 ± 2	70 ± 13	58 ± 12
10 weeks						
$SHP^{+/+}$ Male	10 ± 3	125 ± 25	21 ± 15	61 ± 13	114 ± 17	63 ± 24
$SHP^{-/-}$ Male	13 ± 5	25 ± 3	9 ± 3	24 ± 4	85 ± 13	84 ± 9
$SHP^{+/+}$ Female	9 ± 1	130 ± 11	8 ± 1	46 ± 7	100 ± 7	66 ± 13
$SHP^{-/-}$ Female	69 ± 24	68 ± 20	6 ± 1	13 ± 3	56 ± 22	76 ± 18

[a] At 8 to 10 weeks of age, $SHP^{+/+}$ mice and $SHP^{-/-}$ mice were either maintained on a chow diet or switched to a diet supplemented with 2% cholesterol and 0.5% cholic acid (CA) for 5 or 10 weeks.
[b] All values are the mean cholesterol concentration (mg/dl) ± SE. n = 6 to 12 mice per group.

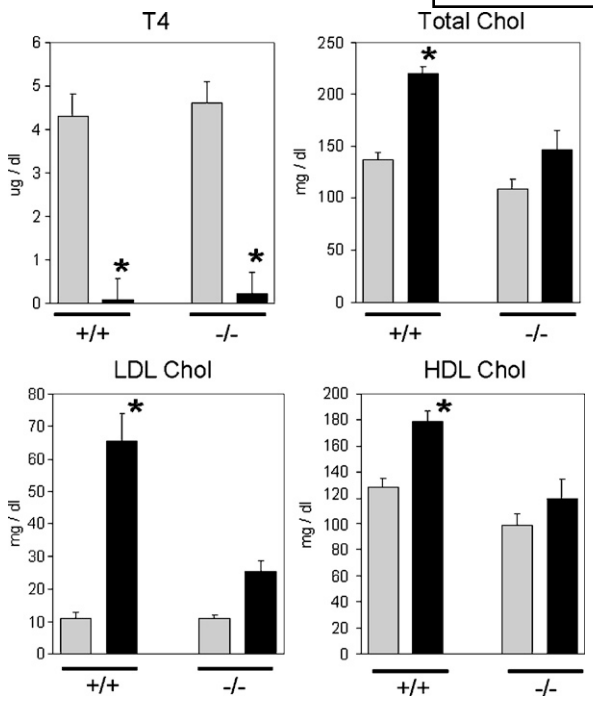

Fig. 4. Hypothyroid SHP$^{-/-}$ mice are protected from increased LDL cholesterol levels. SHP$^{+/+}$ and SHP$^{-/-}$ mice at 8 to 10 weeks of age were maintained on a standard chow diet (gray bars) or switched to an iodine-deficient diet supplemented with propylthiouracil (black bars) for three weeks. Serum T4, total cholesterol, LDL cholesterol, and HDL cholesterol levels were determined using a Hitachi clinical chemistry analyzer. *$P < 0.01$ for diet effect (n = 4 males and 4 females per group). Values are the mean ± SE.

mice. The Western diet also resulted in increased hepatic expression of hepatic inflammatory marker genes including VCAM, ICAM-1, and TNFα in the LDLR$^{-/-}$ mice (Fig. 5C). Again the loss of SHP expression in the LDLR$^{-/-}$SHP$^{-/-}$ mice resulted in a near complete block in the induction of these genes.

In contrast to the reduced serum cholesterol levels seen in the LDLR$^{-/-}$SHP$^{-/-}$ mice, hepatic total cholesterol, nonesterified cholesterol, and TG contents were equally increased by the Western diet in the LDLR$^{-/-}$ and LDLR$^{-/-}$SHP$^{-/-}$ mice (Fig. 5D). Similarly, the expression of 20 genes involved in cholesterol biosynthesis (see supplementary Table I) were down-regulated to the same magnitude by the Western diet in the LDLR$^{-/-}$ and LDLR$^{-/-}$SHP$^{-/-}$ mice (Fig. 5E). Finally, although cholesterol content of the ileum could not be measured directly due to diet remaining within the tissue sample, repression of a set of 20 cholesterol metabolism genes was used to determine cholesterol content of the ileum. For all genes, dietary repression was either the same or greater in the LDLR$^{-/-}$SHP$^{-/-}$ mice as compared with the LDLR$^{-/-}$ mice, suggesting that cholesterol levels in the enterocytes of the LDLR$^{-/-}$SHP$^{-/-}$ mice were at least the same or greater than the LDLR$^{-/-}$ mice. Together these results suggest that similar levels of cholesterol were absorbed and delivered to the liver in the LDLR$^{-/-}$ and LDLR$^{-/-}$ SHP$^{-/-}$ mice consuming the Western diet. A potential explanation for decreased serum cholesterol levels in the context of increased hepatic cholesterol levels is for increased cholesterol elimination. In support of this model, there was significantly higher levels of expression of CYP7A1 in the liver of the LDLR$^{-/-}$SHP$^{-/-}$ compared with the LDLR$^{-/-}$ mice on the Western diet (Fig. 5F). The increased CYP7A1 expression would be expected to increase the bile-acid pool size, resulting in the elevated expression of the FXR target genes IBABP, organic solute transporter α, and FGF-15 in the ileums of the LDLR$^{-/-}$ SHP$^{-/-}$ mice (Fig. 5F). Although CYP7A1 expression further increased when the LDLR$^{-/-}$SHP$^{-/-}$ mice were fed a Western diet, in the ileum only FGF15 expression further increased under these conditions.

The above results suggest that the loss of SHP expression specifically within the hepatocyte is responsible for the protection against dyslipidemia. To confirm this, mice containing the floxed SHP allele (SHP$^{flox/flox}$) were crossed with mice expressing Cre under the control of the albumin promoter to generate mice selectively deficient in SHP in the hepatocyte (SHP$^{hep/hep}$). SHP expression was undetectable in the livers of the SHP$^{hep/hep}$ mice (**Fig. 6A**), but normal levels of SHP were expressed in the ileum of the SHP$^{hep/hep}$ mice. When mice were fed a chol/CA diet, the SHP$^{-/-}$ and SHP$^{hep/hep}$ mice maintained significantly higher levels of CYP7A1 and CYP8B1 expression than did the WT or SHP$^{flox/flox}$ mice. Analysis of serum lipids revealed an identical degree of protection in the SHP$^{-/-}$ and SHP$^{hep/hep}$ mice fed a chol/CA diet (Fig. 6B). Finally, the SHP$^{hep/hep}$ mice were also resistant to hypothyroidism-induced increases in LDL cholesterol (Fig. 6C). In these mice, neither the SHP$^{-/-}$ nor SHP$^{hep/hep}$ mice showed any reduction of the HDL cholesterol increase produced by the hypothyroid diet as compared with WT or SHP$^{flox/flox}$ mice, suggesting this effect is variable. In both models, the magnitude of protection was nearly identical in the SHP$^{-/-}$ and SHP$^{hep/hep}$ mice, indicating it was loss of SHP within hepatocytes that was protective for elevations in serum cholesterol, particularly in regard to LDL cholesterol elevations.

DISCUSSION

Hypercholesterolemia is a well-known risk factor for the development of atherosclerosis. Recent clinical trials have demonstrated the aggressive lowering of LDL cholesterol levels not only stops atherosclerotic lesion progression but may actually regress lesion size (33). Presently the two major methods for lowering LDL cholesterol levels are statins to inhibit cholesterol synthesis and ezetimibe to inhibit cholesterol absorption. Yet many treated patients fail to achieve target LDL cholesterol levels (34).

Here we demonstrate that loss of SHP significantly reduces LDL cholesterol levels in three distinct models of dyslipidemia in 129 strain or 129 mixed strain mice. First, SHP$^{-/-}$ mice were protected from increased plasma total cholesterol levels when fed a cholesterol plus CA diet as seen previously (23). Importantly, the loss of SHP blocked the diet-induced increases in VLDL and LDL cholesterol

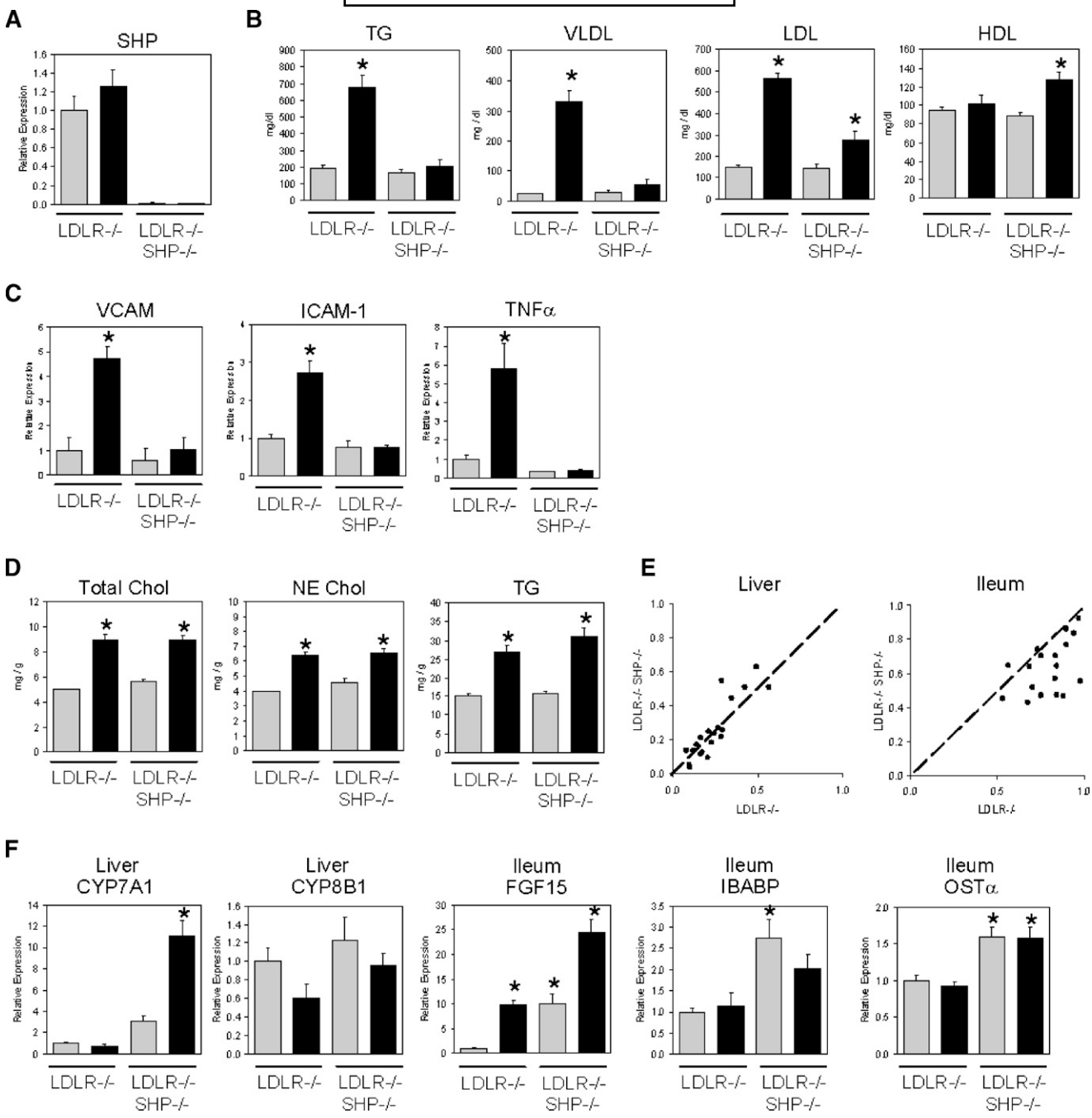

Fig. 5. Low density lipoprotein receptor (LDLR)$^{-/-}$SHP$^{-/-}$ mice are protected from Western diet dyslipidemia. LDLR$^{-/-}$ mice were crossed with SHP$^{-/-}$ mice to generate LDLR$^{+/-}$SHP$^{+/-}$ mice, which were then interbred to generate LDLR$^{-/-}$SHP$^{+/+}$ and LDLR$^{-/-}$SHP$^{-/-}$ mice. At 8 to 10 weeks of age, LDLR$^{-/-}$ and LDLR$^{-/-}$SHP$^{-/-}$ male mice (n = 5 per group) were either maintained on a chow diet (gray bars) or placed on a Western diet for 7 days (black bars). A: Hepatic SHP mRNA was quantified by real-time PCR, with values normalized for GAPDH expression. Expression in LDLR$^{-/-}$ mice fed a chow diet was defined as 1.0. B: Serum total cholesterol and TG levels were determined using a Hitachi 912 clinical chemistry analyzer. VLDL, LDL, and HDL cholesterol levels (mg/dl) were determined by FPLC. * $P < 0.01$ for diet effect. C: Hepatic VCAM, ICAM-1, and TNFα expression was determined by real-time PCR. * $P < 0.01$ for diet effect. D: Hepatic total cholesterol, nonesterified cholesterol, and TG content (mg/g) was determined as previously described (28). * $P < 0.01$ for diet effect. E: Expression of a panel of 20 cholesterol regulated genes was determined by GeneChip as described in supplementary Table I. The fold repression by the Western diet in the LDLR$^{-/-}$SHP$^{-/-}$ mice is plotted against the fold repression by the Western diet in the LDLR$^{-/-}$ mice. The dotted line denotes equivalent repression in the LDLR$^{-/-}$ and LDLR$^{-/-}$SHP$^{-/-}$ mice. F: Expression of CYP7A1 and CYP8B1 in the liver and expression of FGF15, ileal bile acid binding protein (IBABP), and OSTα in the ileum were determined by real-time PCR. * $P < 0.01$ expression level as compared with LDLR$^{-/-}$ mice on chow diet. Values are the mean ± SE.

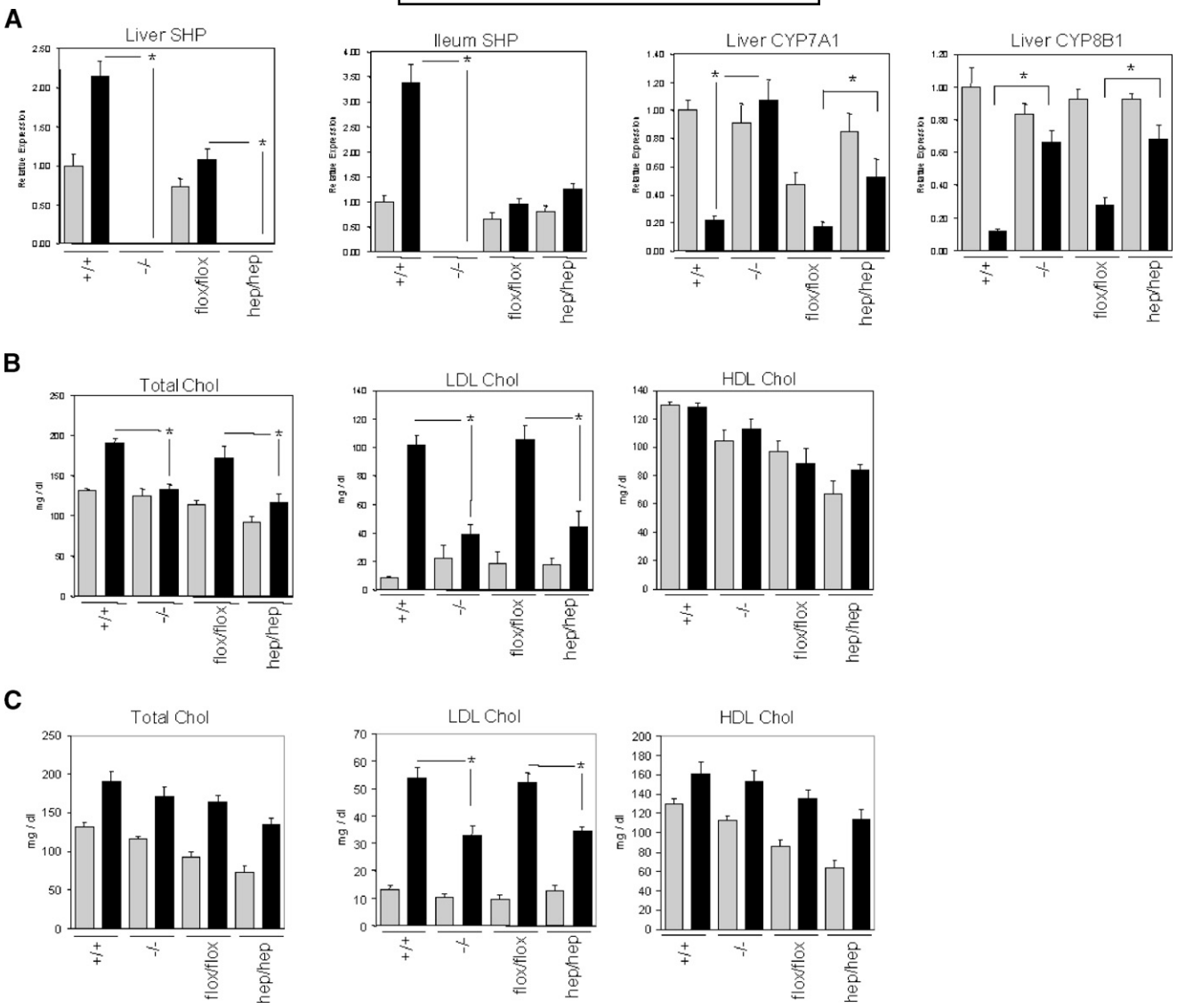

Fig. 6. Selective loss of SHP expression in hepatocytes protects against diet-induced dyslipidemia. Mice containing the floxed SHP exon 1 ($SHP^{flox/flox}$) were crossed with albumin Cre transgenic mice to create mice with the SHP gene selectively inactivated in hepatocytes ($SHP^{hep/hep}$). A: $SHP^{+/+}$, $SHP^{-/-}$, $SHP^{flox/flox}$, and $SHP^{hep/hep}$ mice at 8 to 10 weeks of age were either maintained on a chow diet (gray bars) or switched to a diet supplemented with 2% cholesterol and 0.5% CA (black bars) for 5 weeks. Gene expression in the liver and ileum was quantified by real-time PCR. All values were normalized for GAPDH expression, with expression in $SHP^{+/+}$ mice on a chow diet defined as 1.0 for each gene. * $P < 0.01$ (n = 12 for each group). B: Serum total cholesterol, LDL cholesterol, and HDL cholesterol were determined using a Hitachi 912 clinical chemistry analyzer. * $P < 0.01$ (n = 12 for each group). C: $SHP^{+/+}$, $SHP^{-/-}$, $SHP^{flox/flox}$, and $SHP^{hep/hep}$ mice at 8 to 10 weeks of age were either maintained on a chow diet (gray bars) or switched to a low iodine diet supplemented with propylthiouracil (black bars) for 3 weeks. Serum total cholesterol, LDL cholesterol, and HDL cholesterol were determined using a Hitachi 912 clinical chemistry analyzer. * $P < 0.01$. Values are the mean ± SE.

levels (Fig. 3). This diet also decreased HDL cholesterol levels in WT mice but had no effect on HDL cholesterol levels in the $SHP^{-/-}$. While the effects of FXR on lipids have been demonstrated to differ between male and female mice (22), a similar sustained protection of $SHP^{-/-}$ males and females was observed at all time points examined.

Because the cholesterol plus CA diet dyslipidemia does not well reflect human dyslipidemia, the effects of the loss of SHP were evaluated in two additional models in which LDL cholesterol is elevated via changes in LDLR expression. Hypothyroidism increases LDL cholesterol levels in humans and in mice, mediated in part by the ability of thyroid hormone to regulate transcription of the LDLR gene (32). $SHP^{-/-}$ were highly resistant to the large increase in LDL cholesterol that occurs in hypothyroid mice (Fig. 4).

Second, the $SHP^{-/-}$ mice were intercrossed with $LDLR^{-/-}$ mice to generate mixed 129-C57Bl/6 background $LDLR^{-/-}SHP^{-/-}$ mice. When fed a Western diet, the $LDLR^{-/-}SHP^{-/-}$ mice were almost completely resistant to diet-mediated increases in TGs, VLDL cholesterol, or LDL cholesterol but had increased HDL cholesterol as

compared with the LDLR$^{-/-}$ mice (Fig. 5). The mechanistic basis for these effects may reside in part in the regulation of CYP7A1 expression. In the LDLR$^{-/-}$ mice, the Western diet slightly repressed CYP7A1 expression, while in the LDLR$^{-/-}$SHP$^{-/-}$ mice, the Western diet strongly induced CYP7A1 expression. Feeding the Western diet will increase hepatic cholesterol levels and also hepatic oxysterol levels. Oxysterols are endogenous ligands for liver X receptor (LXR) that induce expression of CYP7A1 (35, 36). However, the increased production of bile acids will also activate FXR, induce SHP, and repress CYP7A1 expression. Additionally, increased bile acids will also induce FGF-15 expression in the intestine, which can signal to reduce CYP7A1 expression in the liver. In the absence of SHP, both of these inhibitory pathways for CYP7A1 expression are eliminated (30), while the LXR activation pathway for CYP7A1 expression remains intact. Thus the LDLR$^{-/-}$SHP$^{-/-}$ mice had increased basal expression of CYP7A1, and feeding the Western diet actually further induced CYP7A1 expression. Human genetic evidence and numerous animal experimental models have suggested that an increase in CYP7A1 expression would also lower LDL cholesterol levels. Bile acid sequestrants induce CYP7A1 expression, reduce LDL cholesterol levels, and reduce the incidence of coronary heart disease but are seldom used due to undesirable side effects including hypertriglyceridemia (37). This would not appear to be an issue for inhibition of SHP because the SHP$^{-/-}$ mice were also protected from diet-induced hypertriglyceridemia.

Overexpression of CYP7A1 blocks the increase in hepatic lipids that occurs when LDLR$^{-/-}$ mice are fed a Western diet (14). The LDLR$^{-/-}$SHP$^{-/-}$ mice did not have reduced hepatic lipid levels nor altered patterns of cholesterol regulated gene expression. Thus upregulation of CYP7A1 expression is unlikely to provide the sole mechanism for the observed protective lipid effects. The loss of SHP also reduced markers of hepatic inflammation in either SHP$^{-/-}$ mice fed a cholesterol plus CA diet or in LDLR$^{-/-}$SHP$^{-/-}$ mice fed a Western diet. The precise mechanism(s) responsible for these diet-induced inflammatory effects are unknown, although both the cholesterol and CA components can produce hepatic inflammation (38). Monocytic differentiation of HL-60 leukemia cells increases SHP expression in part through c-Jun activation of the SHP promoter (39). Further, SHP is a coactivator for nuclear factor κB, and is essential for nuclear factor κB activation by palmitoyl lysophosphatidylcholine, a major component of oxidized LDL (40). This inhibition of the proinflammatory effects of lipid accumulation in the livers of SHP$^{-/-}$ may also have contributed to the beneficial effects on plasma lipids.

Because SHP has been demonstrated to repress the activity of many other nuclear hormone receptors in vitro, it could be postulated that many of the beneficial effects of SHP$^{-/-}$ may in fact be mediated by increased activity of other nuclear hormone receptors. SHP represses the activity of the human LXRα (41), and LXRα regulates plasma lipid levels and has anti-inflammatory effects in mice (42). No differences in T0901317-mediated gene induction were observed between SHP$^{-/-}$ and WT mice for several LXR target genes in either the liver or the ileum (data not shown). Similarly, SHP also represses estrogen receptor α activity (43), and estrogen receptor α induces SHP expression (26) suggesting a putative negative feedback pathway. Again though no changes were observed in induction of several estrogen receptor α target genes by ethynylestradiol in livers of ovariectomized female SHP$^{-/-}$ mice compared with WT mice (data not shown). Finally, SHP has also been shown to be able to repress PXR activity (44). However no change in expression of PXR target genes was observed in SHP$^{-/-}$ mice (Fig. 2), and no difference in induction of CYP3A11 by the PXR agonist pregnenolone 16α-carbonitrile (PCN) is evident in SHP$^{-/-}$ mice (data not shown). Extensive characterization of 12-week-old SHP$^{-/-}$ mice has failed to identify the numerous pathologies that might be predicted if SHP altered the constitutive activity of multiple nuclear receptors in vivo (data not shown). The number of nuclear receptors regulated in vivo by SHP may thus be fewer than predicted by in vitro studies. However, one nuclear receptor that may be activated in the SHP$^{-/-}$ mice is FXR due to the expansion of the bile acid pool size (as evidenced by the increase in IBABP, OSTα, and FGF-15 expression in the ileum, Fig. 5). FXR activation reduces TG levels (45) and may have a protective activity in inflammation (46). A major concern though of FXR activation has been the potential negative consequences of repression of CYP7A1 expression, which has led to the search for FXR regulators with gene selectivity (47). In certain respects, the effects observed in the SHP$^{-/-}$ mice may predict the effects that could be obtained with gene-selective FXR agonists that were inactive for induction of SHP expression. Delineation of the role of FXR activation in the beneficial effects observed in SHP$^{-/-}$ mice will require analysis of FXR$^{-/-}$SHP$^{-/-}$ mice.

No crystal structure is known for SHP, and no ligands for SHP have been reported. Development of a SHP antagonist may thus prove to be difficult. Here though we have demonstrated that the selective inactivation of SHP within hepatocytes has the same beneficial activities as the complete loss of SHP, opening up alternative methods for the pharmacological inhibition of SHP, such as antisense oligonucleotides. For example, antisense oligonucleotides which reduce PCSK9 expression in the liver increase LDLR levels and decrease LDL cholesterol (48). Whether a similar approach will work for SHP is not yet known. Distinct from PCSK9 inhibition, the protection against dyslipidemia conferred by the loss of SHP appears to be independent of LDLR expression. SHP inhibitors can thus be predicted to be additive with the LDL cholesterol lowering effects observed with statins or PCSK9 inhibitors and may have utility in the treatment of dyslipidemia.

We thank Christine Huard and Robert Martinez for performing the microarray analysis, Kim Milarski and Roger Askew for assistance with generation of the knockout mice, the Wyeth Genetically Modified Animal Program for maintaining mutant strains, and Erin Vogelsong and Cathy Felegi-Rudolph for assistance with animal studies.

REFERENCES

1. Seol, W., H. S. Choi, and D. D. Moore. 1996. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science.* **272:** 1336–1339.
2. Bavner, A., S. Sanyal, J. A. Gustafsson, and E. Treuter. 2005. Transcriptional corepression by SHP: molecular mechanisms and physiological consequences. *Trends Endocrinol. Metab.* **16:** 478–488.
3. Wang, H., J. Chen, K. Hollister, L. C. Sowers, and B. M. Forman. 1999. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell.* **3:** 543–553.
4. Chiang, J. Y., R. Kimmel, C. Weinberger, and D. Stroup. 2000. Farnesoid X receptor responds to bile acids and represses cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription. *J. Biol. Chem.* **275:** 10918–10924.
5. Lu, T. T., M. Makishima, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Auwerx, and D. J. Mangelsdorf. 2000. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell.* **6:** 507–515.
6. Goodwin, B., S. A. Jones, R. R. Price, M. A. Watson, D. D. McKee, L. B. Moore, C. Galardi, J. G. Wilson, M. C. Lewis, M. E. Roth, et al. 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol. Cell.* **6:** 517–526.
7. Kok, T., C. V. Hulzebos, H. Wolters, R. Havinga, L. B. Agellon, F. Stellaard, B. Shan, M. Schwarz, and F. Kuipers. 2003. Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. *J. Biol. Chem.* **278:** 41930–41937.
8. Sinal, C. J., M. Tohkin, M. Miyata, J. M. Ward, G. Lambert, and F. Gonzalez. 2000. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell.* **102:** 731–744.
9. Kerr, T. A., S. Saeki, M. Schneider, K. Schaefer, S. Berdy, T. Redder, B. Shan, D. W. Russell, and M. Schwarz. 2002. Loss of nuclear receptor SHP impairs but does not eliminate negative feedback regulation of bile acid synthesis. *Dev. Cell.* **2:** 713–720.
10. Wang, L., Y. K. Lee, D. Bundman, Y. Han, S. Thevananther, C. S. Kim, S. S. Chua, P. Wei, R. A. Heyman, M. Karin, et al. 2002. Redundant pathways for negative feedback regulation of bile acid production. *Dev. Cell.* **2:** 721–731.
11. Wang, J., D. J. Freeman, S. M. Grundy, D. M. Levine, R. Guerra, and J. C. Cohen. 1998. Linkage between cholesterol 7alpha-hydroxylase and high plasma low-density lipoprotein cholesterol concentrations. *J. Clin. Invest.* **101:** 1283–1291.
12. Pullinger, C. R., C. Eng, G. Salen, S. Shefer, A. K. Batta, S. K. Erickson, A. Verhagen, C. R. Rivera, S. J. Mulvihill, M. J. Malloy, et al. 2002. Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J. Clin. Invest.* **110:** 109–117.
13. Rudel, L., C. Deckelman, M. Wilson, M. Scobey, and R. Anderson. 1994. Dietary cholesterol and downregulation of cholesterol 7 alpha-hydroxylase and cholesterol absorption in African green monkeys. *J. Clin. Invest.* **93:** 2463–2472.
14. Ratliff, E. P., A. Gutierrez, and R. A. Davis. 2006. Transgenic expression of CYP7A1 in LDL receptor-deficient mice blocks diet-induced hypercholesterolemia. *J. Lipid Res.* **47:** 1513–1520.
15. Miyake, J. H., X. T. Duong-Polk, J. M. Taylor, E. Z. Du, L. W. Castellani, A. J. Lusis, and R. A. Davis. 2002. Transgenic expression of cholesterol-7-alpha-hydroxylase prevents atherosclerosis in C57BL/6J mice. *Arterioscler. Thromb. Vasc. Biol.* **22:** 121–126.
16. Fiorucci, S., G. Rizzo, A. Donini, E. Distrutti, and L. Santucci. 2007. Targeting farnesoid X receptor for liver and metabolic disorders. *Trends Mol. Med.* **13:** 298–309.
17. Claudel, T., E. Sturm, H. Duez, I. P. Torra, A. Sirvent, V. Kosykh, J. C. Fruchart, J. Dallongeville, D. W. Hum, F. Kuipers, et al. 2002. Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. *J. Clin. Invest.* **109:** 961–971.
18. Boulias, K., N. Katrakili, K. Bamberg, P. Underhill, A. Greenfield, and I. Talianidis. 2005. Regulation of hepatic metabolic pathways by the orphan nuclear receptor SHP. *EMBO J.* **24:** 2624–2633.
19. Lambert, G., M. J. Amar, G. Guo, H. B. Brewer, F. J. Gonzalez, and C. J. Sinal. 2003. The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. *J. Biol. Chem.* **278:** 2563–2570.
20. Kim, I., S. H. Ahn, T. Inagaki, M. Choi, S. Ito, G. L. Guo, S. A. Kliewer, and F. J. Gonzalez. 2007. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. *J. Lipid Res.* **48:** 2664–2672.
21. Hanniman, E. A., G. Lambert, T. C. McCarthy, and C. J. Sinal. 2005. Loss of functional farnesoid X-receptor increases atherosclerotic lesions in apolipoprotein E-deficient mice. *J. Lipid Res.* **46:** 2595–2604.
22. Zhang, Y., X. Wang, C. Vales, F. Y. Lee, H. Lee, A. J. Lusis, and P. A. Edwards. 2006. FXR deficiency causes reduced atherosclerosis in LDLR$^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* **26:** 2316–2321.
23. Wang, L., Y. Han, C. S. Kim, Y. K. Lee, and D. D. Moore. 2003. Resistance of SHP-null mice to bile acid-induced liver damage. *J. Biol. Chem.* **278:** 44475–44481.
24. Huang, J., J. Iqbal, P. K. Saha, J. Liu, L. Chan, M. M. Hussain, D. D. Moore, and L. Wang. 2007. Molecular characterization of the role of orphan receptor small heterodimer partner in development of fatty liver. *Hepatology.* **46:** 147–157.
25. Dietschy, J. M., S. D. Turley, and D. K. Spady. 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* **34:** 1637–1659.
26. Lai, K., D. C. Harnish, and M. J. Evans. 2003. Estrogen receptor alpha regulates expression of the orphan receptor small heterodimer partner. *J. Biol. Chem.* **278:** 36418–36429.
27. Jelinsky, S. A., H. A. Harris, E. L. Brown, K. Flanagan, X. Zhang, C. Tunkey, K. Lai, M. V. Lane, D. K. Simcoe, and M. J. Evans. 2003. Global transcription profiling of estrogen activity: Estrogen receptor a regulates gene expression in the kidney. *Endocrinology.* **144:** 701–710.
28. Quinet, E. M., D. A. Savio, A. R. Halpern, L. Chen, G. U. Schuster, J. A. Gustafsson, M. D. Basso, and P. Nambi. 2006. Liver X receptor (LXR)-beta regulation in LXRalpha-deficient mice: implications for therapeutic targeting. *Mol. Pharmacol.* **70:** 1340–1349.
29. Holt, J. A., G. Luo, A. N. Billin, J. Bisi, Y. Y. McNeill, K. F. Kozarsky, M. Donahee, D. Y. Wang, T. A. Mansfield, S. A. Kliewer, et al. 2005. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev.* **17:** 1581–1591.
30. Inagaki, T., M. Choi, A. Moschetta, L. Peng, C. L. Cummins, J. G. McDonald, G. Luo, S. A. Jones, B. Goodwin, J. A. Richardson, et al. 2005. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* **2:** 217–225.
31. Schuetz, E. G., S. Strom, K. Yasuda, V. Lecureur, M. Assem, C. Brimer, J. Lamba, R. B. Kim, V. Ramachandran, B. J. Komoroski, et al. 2001. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. *J. Biol. Chem.* **276:** 39411–39418.
32. Shin, D. J., and T. F. Osborne. 2003. Thyroid hormone regulation and cholesterol metabolism are connected through sterol regulatory element-binding protein-2 (SREBP-2). *J. Biol. Chem.* **278:** 34114–34118.
33. Nissen, S. E., S. J. Nicholls, I. Sipahi, P. Libby, J. S. Raichlen, C. M. Ballantyne, J. Davignon, R. Erbel, J. C. Fruchart, J. C. Tardif, et al. 2006. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *JAMA.* **295:** 1556–1565.
34. Parris, E. S., D. B. Lawrence, L. A. Mohn, and L. B. Long. 2005. Adherence to statin therapy and LDL cholesterol goal attainment by patients with diabetes and dyslipidemia. *Diabetes Care.* **28:** 595–599.
35. Janowski, B. A., M. J. Grogan, S. A. Jones, G. B. Wisely, S. A. Kliewer, E. J. Corey, and D. J. Mangelsdorf. 1999. Structural requirements of ligands for the oxysterol liver X receptors LXRα and LXRβ. *Proc. Natl. Acad. Sci. USA.* **96:** 266–271.
36. Lehmann, J. M., S. A. Kliewer, L. B. Moore, T. A. Smith-Oliver, B. B. Oliver, J. L. Su, S. S. Sundseth, D. A. Winegar, D. E. Blanchard, T. A. Spencer, et al. 1997. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **272:** 3137–3140.
37. Lipid Research Clinics Program. 1984. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. *JAMA.* **251:** 351–364.
38. Vergnes, L., J. Phan, M. Strauss, S. Tafuri, and K. Reue. 2003. Cholesterol and cholate components of an atherogenic diet induce distinct stages of hepatic inflammatory gene expression. *J. Biol. Chem.* **278:** 42774–42784.
39. Choi, Y. H., M. J. Park, K. W. Kim, H. C. Lee, and J. Cheong. 2004. The orphan nuclear receptor SHP is involved in monocytic differentiation, and its expression is increased by c-Jun. *J. Leukoc. Biol.* **76:** 1082–1088.
40. Kim, Y. S., C. Y. Han, S. W. Kim, J. H. Kim, S. K. Lee, D. J. Jung, S. Y. Park, H. Kang, H. S. Choi, J. W. Lee, et al. 2001. The orphan nuclear

receptor small heterodimer partner as a novel coregulator of nuclear factor-kappa b in oxidized low density lipoprotein-treated macrophage cell line RAW 264.7. *J. Biol. Chem.* **276:** 33736–33740.
41. Brendel, C., K. Schoonjans, O. A. Botrugno, E. Treuter, and J. Auwerx. 2002. The small heterodimer partner interacts with the liver X receptor alpha and represses its transcriptional activity. *Mol. Endocrinol.* **16:** 2065–2076.
42. Joseph, S. B., A. Castrillo, B. A. Laffitte, D. J. Mangelsdorf, and P. Tontonoz. 2003. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat. Med.* **9:** 213–219.
43. Seol, W., B. Hanstein, M. Brown, and D. D. Moore. 1998. Inhibition of estrogen receptor action by the orphan receptor SHP (short heterodimer partner). *Mol. Endocrinol.* **12:** 1551–1557.
44. Ourlin, J. C., F. Lasserre, T. Pineau, J. M. Fabre, A. Sa-Cunha, P. Maurel, M. J. Vilarem, and J. M. Pascussi. 2003. The small heterodimer partner interacts with the pregnane X receptor and represses its transcriptional activity. *Mol. Endocrinol.* **17:** 1693–1703.
45. Kast, H. R., C. M. Nguyen, C. J. Sinal, S. A. Jones, B. A. Laffitte, K. Reue, F. J. Gonzalez, T. M. Willson, and P. A. Edwards. 2001. Farnesoid X-activated receptor induces apolipoprotein C–II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. *Mol. Endocrinol.* **15:** 1720–1728.
46. Li, Y. T., K. E. Swales, G. J. Thomas, T. D. Warner, and D. Bishop-Bailey. 2007. Farnesoid X receptor ligands inhibit vascular smooth muscle cell inflammation and migration. *Arterioscler. Thromb. Vasc. Biol.* **27:** 2606–2611.
47. Dussault, I., R. Beard, M. Lin, K. Hollister, J. Chen, J. H. Xiao, R. Chandraratna, and B. M. Forman. 2003. Identification of gene-selective modulators of the bile acid receptor FXR. *J. Biol. Chem.* **278:** 7027–7033.
48. Graham, M. J., K. M. Lemonidis, C. P. Whipple, A. Subramaniam, B. P. Monia, S. T. Crooke, and R. M. Crooke. 2007. Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. *J. Lipid Res.* **48:** 763–767.