Hepatic overexpression of sterol carrier protein-2 inhibits VLDL production and reciprocally enhances biliary lipid secretion

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Abstract **We examined in vivo a role for sterol carrier protein-2 (SCP-2) in the regulation of lipid secretion across the hepatic sinusoidal and canalicular membranes. Recombinant adenovirus *Ad.rSCP2* was used to overexpress SCP-2 in livers of mice. We determined plasma, hepatic, and biliary lipid concentrations; hepatic fatty acid (FA) and cholesterol synthesis; hepatic and biliary phosphatidylcholine (PC) molecular species; and VLDL triglyceride production. In *Ad.rSCP2* mice, there was marked inhibition of hepatic fatty acids and cholesterol synthesis to <62% of control mice. Hepatic triglyceride contents were decreased, while cholesterol and phospholipids concentrations were elevated in *Ad.rSCP2* mice. Hepatic VLDL triglyceride production fell in *Ad.rSCP2* mice to 39% of control values. As expected, biliary cholesterol, phospholipids, bile acids outputs, and biliary PC hydrophobic index were significantly increased in *Ad.rSCP2* mice. These studies indicate that SCP-2 overexpression in the liver markedly inhibits lipid synthesis as well as VLDL production, and alters hepatic lipid contents. In contrast, SCP-2 increased biliary lipid secretion and the proportion of hydrophobic PC molecular species in bile. These effects suggest a key regulatory role for SCP-2 in hepatic lipid metabolism and the existence of a reciprocal relationship between the fluxes of lipids across the sinusoidal and canalicular membranes.**—Amigo, L., S. Zanlungo, J. F. Miquel, J. M. Glick, H. Hyogo, D. E. Cohen, A. Rigotti, and F. Nervi. **Hepatic overexpression of sterol carrier protein-2 inhibits VLDL production and reciprocally enhances biliary lipid secretion. *J. Lipid Res.* 2003.** 44: **399–407.**

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Hepatic fatty acid (FA), triglyceride, and cholesterol metabolism are highly regulated processes determined by the concerted feedback regulation of genes governing their synthesis, lipoprotein uptake and production, and biliary lipid secretion (1–7). Regulation of FA and cholesterol synthesis are highly interrelated processes and share common transcription regulatory factors (8, 9). Even though the physiological role(s) for sterol carrier protein-2 (SCP-2) gene products remain elusive, several studies suggest a number of important transport and catalytic functions of these proteins in the regulation of lipid metabolism (10–14).

SCP-2 gene products not only bind cholesterol, FA, FA-acyl-CoA, and phospholipids, but also enhance trafficking of these lipids within cells (15–18). Experiments in cultured cell systems transfected with cDNAs encoding for the SCP-2/sterol carrier protein X (SCP-X) products demonstrated the role of these proteins in microsomal membrane utilization of FA for phospholipids, triglyceride, and cholesterol ester synthesis (19–22). Overexpression of SCP-2 in rat hepatoma cells enhanced intracellular cholesterol cycling, increased plasma membrane cholesterol content, and inhibited cholesterol esterification and HDL production (23). In addition, studies in mice with disruption of the *SCP-2/SCP-X* gene have shown a failure in the oxidation of 2-methyl-branched FA and the side chain of cholesterol. Furthermore, liver triglyceride and cholesterol ester concentrations significantly decreased in these SCP-2-knockout mice, suggesting that these animals have altered phospholipid, fatty acid, and triglyceride metabolism (24, 25). We have recently shown that transient hepatic overexpression of SCP-2 in mice altered plasma LDL cholesterol (LDL-C) and HDL-C concentrations, and decreased hepatic apolipoprotein B (apoB), apoE, and apoAI, and LDL receptor (LDLR) expression (26). These

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Abbreviations: apo, apolipoprotein; bw, body weight; FA-acyl-CoA, long chain fatty acids-acyl CoA; SCP-2, sterol carrier protein-2; SCP-X, sterol carrier protein-X.

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various changes were associated with a simultaneous enhancement of the enterohepatic circulation of cholesterol and bile acids and intestinal cholesterol absorption. These data, together with the studies of SCP-2 knockout mice, strongly support the concept that SCP-2 has a coordinate regulatory role in hepatic lipid metabolism by modulating expression of various genes involved in cholesterol, bile acid, FA, and triglyceride metabolism.

Since SCP-2 proteins are located in peroxisomes as well as in endoplasmic reticulum and cytosol (22, 27–29), we speculate here that the SCP-2 gene could have important regulatory functions on VLDL production. Specifically, we postulate that hepatic SCP-2 gene products inhibit VLDL production by preferentially channeling phospholipids and sterols to the canalicular pole rather than to the sinusoidal domain of the hepatocyte. The reciprocal relationship between plasma and biliary lipid secretion has been previously reported (3, 30). To further examine whether SCP-2 indeed plays an interrelated physiological role in lipoprotein and biliary lipid metabolism in vivo, we determined the effect of adenovirus-mediated SCP-2 gene transfer into the mouse liver on FA synthesis and VLDL production, as well as biliary cholesterol and phospholipids secretion and composition.

EXPERIMENTAL PROCEDURES

Animals and diet

Adult male C57BL/6 mice over 8 weeks of age were used in all experiments as previously described (26). Briefly, they had free access to commercial rodent diet Prolab RMH 3000 (PMI Nutritional International Inc., Brentwood, MO). Animals were subjected to experimental protocols approved by the Research Advisory Committee of our institution. All experiments were carried out during the dark phase of reversal diurnal cycle between 8 AM to 1 PM (dark phase, 8 AM to 8 PM).

Recombinant adenoviruses preparation and administration

The recombinant adenovirus *Ad.rSCP2* was generated by homologous recombination in 293 cells as described previously (26, 31, 32). Briefly, the adenoviral backbone used for the construction of the vector-containing rat scp2 cDNA under control of the CMV enhancer/promoter was derived from a replication-deficient first-generation type-5 adenovirus with deletions of E1 and E3 genes. The control adenovirus *Ad.E1Δ* contained the same E1 and E3 deletions without the transgene expression cassette. Large-scale production of recombinant adenoviruses was performed after purification from infected 293 cells (33). Viruses were administered intravenously as previously described (26). Animals were studied 7 days after adenoviral infection during the dark phase of a reversed diurnal cycle. Western blotting of liver homogenates for SCP2 was performed using a rabbit polyclonal anti-rat SCP2 serum from each sample as previously described (26, 34, 35).

Bile and blood sampling

Bile was collected through a common bile duct fistula for 30 to 60 min in preweighed tubes and stored at −20°C. Blood was removed by puncture of the inferior vena cava with a heparinized syringe. Plasma was immediately separated by centrifugation at 10,000 rpm × 10 min at 4°C and stored at −20°C.

Plasma lipoprotein separation, lipid analyses, and liver histology

Plasma lipoprotein separation was performed by Superose 6-fast protein liquid chromotography gel filtration of fresh plasma specimens (36). For other determinations, liver, bile, and plasma samples were frozen at −20°C until processing. Total plasma and lipoprotein cholesterol, and triglyceride concentrations were measured by enzymatic kits (Sigma Chemicals Co., St. Louis, MO). Hepatic triglycerides were extracted, solubilized, and measured as previously described (37). Hepatic and biliary cholesterol, biliary phospholipids, and bile acids were determined by routine methods (30, 38, 39). Molecular species of phosphatidylcholine (i.e., *sn*-1 and *sn*-2 fatty acyl compositions) were determined as previously described (40, 41). Hydrophobic index is a concentration-weighted average of HPLC-determined hydrophobicities of individual phosphatidylcholines present in a mixture and was determined according to Hay et al. (41, 42). Conventional liver biopsies were performed in 4 *Ad.E1Δ* and *5 Ad.rSCP2* mice. Specimens were stained with hematoxilin-eosin and were blindly analyzed by a pathologist.

Hepatic fatty acid and cholesterol synthesis in vivo

After a 2-h fasting period, rates of hepatic fatty acid and cholesterol synthesis were measured at the mid-dark phase of the diurnal cycle (10 AM). Each mouse received 50 mCi [^{3}H] water (Amersham Pharmacia Biotech, Piscataway, NJ) by intraperitoneal injection as previously described (43, 44). One hour after radiolabel injection, animals were anesthetized and ~0.5 ml of blood was obtained from the inferior vena cava for determination of water-specific activity in plasma. After liver removal, tissue specimens were saponified and digitonin-precipitable sterols were isolated (43, 44). For determination of fatty acid synthesis, liver homogenates were extracted twice with 10 ml of petroleum ether after acidification with 1 ml 1 N HCl (43). Results were expressed as mmol of [^{3}H]water incorporated into fatty acids, or digitonin-precipitable sterol nmol/h/g liver weight.

Hepatic triglyceride production

To measure hepatic VLDL triglyceride production, we used the well-characterized method of Triton WR-1339 (tyloxapol) (Sigma Chemicals Co.) injection to block peripheral removal of newly secreted VLDL (45–47). Food was removed from the cages at 8:30 PM the day prior to the experiment at the beginning of the light phase of the diurnal cycle, when animals normally eat a scarce amount of food. (Rodents usually ingest the major proportion of their daily food during the dark phase of the diurnal cycle.) An aliquot of blood was drawn from a tail vein the next day for determination of basal triglyceride concentration in plasma. Triton WR-1339 was intravenously injected at a dose of 35 mg/kg body weight in 50 μl of saline solution. Three hours later, blood was drawn from the inferior vena cava. We chose this dose of Triton WR-1339 and the 3 h postinjection sampling period because preliminary experiments demonstrated that higher doses, or longer time periods after Triton WR-1339 injection, were deleterious for animals infected with the SCP-2 recombinant adenovirus. All animals participating in this series of experiments tolerated well the administration of Triton WR1339 and looked healthy during the period of anesthesia and bleeding. An aliquot of plasma post-Triton WR1339 injection was stored for total triglyceride determination, while plasma lipoproteins were immediately isolated for VLDL triglyceride measurements. For the calculation of VLDL triglyceride production, we assumed that intestinal contribution to the plasma VLDL pool was minimal. Plasma volume was calculated assuming a value of 0.071 ml/g body weight (45).

Statistics

Data are presented as mean ± SE. The two-tailed unpaired Student's *t*-test was used to compare the sets of data. Statistically significant differences were considered at $P < 0.05$.

RESULTS

To evaluate the relevance of SCP-2 in hepatic lipid metabolism, we studied C57BL/6 mice that transiently overexpressed SCP-2 in the liver by adenovirus-mediated gene transfer. Controls included noninfected saline-injected animals or mice infected with a control adenovirus that lacks a cDNA transgene (*Ad.E1Δ*). Hepatic SCP-2 expression was increased by 8-fold in *Ad.rSCP2*-infected mice 7 days postinfection (26). Mice looked healthy and body weight remained within the normal range in SCP-2-overexpressing mice. Similar mild elevations of alanine aminotransferase and aspartate aminotransferase were observed in the serum of both groups of infected mice. The acute phenotypic changes induced by *Ad.rSCP2* on plasma lipid concentration, biliary lipid secretion and hepatic cholesterol, phospholipids, and triglyceride concentrations are shown in **Table 1**. Liver weight significantly increased in *Ad.rSCP2*-infected mice compared with control saline-injected mice and *Ad.E1Δ*-infected mice. In both adenovirus-infected groups of mice, liver histology showed minor changes with scarce parenchymal cell ballooning, scattered necrotic hepatocytes around the periportal areas, and absence of significant inflammation. No differences were found between *Ad.E1Δ* and *Ad.rSCP2* groups of animals. When compared with control *Ad.E1Δ*-infected mice, total plasma cholesterol and triglyceride levels were unchanged in *Ad.rSCP2* animals, whereas biliary lipid outputs were significantly increased. An unexpected finding was the remarkable 4-fold decrease of hepatic triglyceride concentration and the significant increase in total hepatic cholesterol and phospholipids concentrations found in *Ad.rSCP2* mice. Taken together, these results confirmed that hepatic SCP-2 expression has a significant role in the regulation of hepatic cholesterol, phospholipids, and triglyceride metabolism, as well as biliary lipid secretion.

As previously reported (26), plasma lipoprotein cholesterol distribution was altered in *Ad.rSCP2* mice compared with the *Ad.E1Δ* mice: plasma LDL-C concentration increased by 100%, whereas HDL-C levels decreased by 25%. VLDL-C was not changed (results not shown). Since we previously found a significant decrease in hepatic apoB and apoE expression in *Ad.rSCP2*-infected mice (26), we hypothesized that lipid availability for VLDL assembly and VLDL secretion by the liver was impaired. We therefore studied hepatic FA and cholesterol synthesis, as well as VLDL production in vivo. As shown in **Table 2**, SCP-2 overexpression decreased in vivo hepatic synthesis of cholesterol and free fatty acid (FFA) by 40% and 56%, respectively. Furthermore, hepatic VLDL triglyceride secretion was markedly reduced by 63% in *Ad.rSCP2*-infected mice (**Fig. 1**). As shown in **Fig. 2**, post-Triton WR1339 plasma VLDL-C concentration was significantly decreased, which was consistent with the inhibition of hepatic VLDL production in *Ad.rSCP2*-infected mice compared with control virus-infected animals. The increase in hepatic cholesterol concentration was consistent with conserved feedback inhibition of hepatic cholesterol and fatty acid synthesis and hepatic LDLR expression (26) found in *Ad.rSCP2*-infected mice compared with control virus-infected animals.

On the other hand, biliary bile acid, phospholipids, and cholesterol outputs were significantly increased as a consequence of hepatic SCP-2 overexpression (Table 1). Proportionally, the increased lipid output was more pronounced in biliary cholesterol secretion, a finding probably related in part to the increased biliary bile acid output and the augmented intestinal cholesterol absorption (26). Since biliary cholesterol output is tightly coupled to phospholipids output and hepatic FA metabolism was markedly modified in *Ad.rSCP2*-infected mice, we

TABLE 1. Acute phenotypic differences among sterol carrier protein-2 recombinant adenoviral infection, control adenoviral infection and noninfected saline-injected mice

Parameter	Saline	Ad.E1Δ	Ad.rSCP2
Body weight (g)	22 ± 0.5	22 ± 0.6	21 ± 0.5
Liver weight (g)	1.1 ± 0.03	1.0 ± 0.03	1.3 ± 0.04
Total plasma lipid concentration (mg/dl)			
Cholesterol	87 ± 2.0	80 ± 2.1	90 ± 4.2
Triglycerides	18 ± 3.2	29 ± 3.4	25 ± 4.0
Biliary lipid secretion (nmol/min ×100 g bw)			
Bile acids	248 ± 31	232 ± 29	387 ± 44[a]
Phospholipids	50 ± 5	46 ± 3	78 ± 3[a]
Cholesterol	5.5 ± 0.5	5.1 ± 0.7	11 ± 0.4[a]
Hepatic lipid content (mg/g liver wt)			
Total cholesterol	2.0 ± 0.3	2.4 ± 0.2	3.9 ± 0.6[a]
Cholesterol esters	0.32 ± 0.3	0.39 ± 0.1	0.51 ± 0.2
Triglycerides	22.4 ± 5.7	13.1 ± 5.5	3.1 ± 1.0[a]
Phospholipids (organic P)	1.0 ± 0.06	1.0 ± 0.04	1.4 ± 0.06[a]

Determinations were performed 7 days after IV administration of adenoviruses or saline. Values represent the mean ± SE. There were 6–8 fasted mice in each group. All experiments were performed during the mid-dark phase of the reversed diurnal cycle.

[a] Significantly different at $P < 0.01$ compared with control groups.

TABLE 2. Effect of SCP-2 recombinant adenoviral infection on hepatic cholesterol and fatty acid synthesis, triglyceride secretion, and lipid content in mice

Group	Hepatic Lipid Synthesis	
	Cholesterol	Fatty Acids
	μmol/h/100 g bw	
A. Saline	1.7 ± 0.04	15.1 ± 2.9
B. Ad.E1Δ	3.4 ± 0.16	22.3 ± 1.6
C. Ad.rSCP2	1.8 ± 0.34[a]	9.7 ± 2.3[a]

All values are expressed as mean ± SE. Experiments (4–6 fasted animals in each group) were performed during the mid-dark phase of the diurnal cycle.

[a] Significantly different at $P < 0.001$ compared with group B.

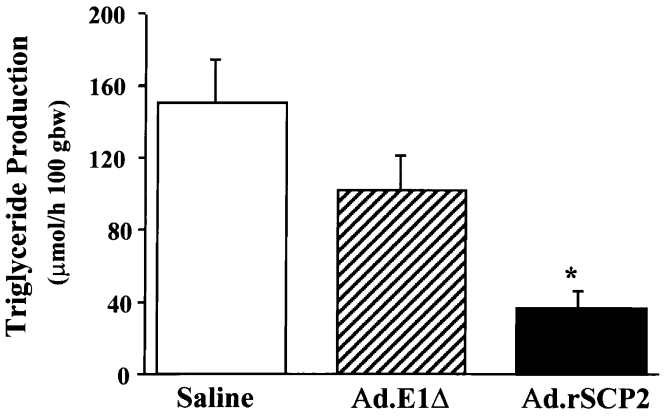

Fig. 1. Effect of Triton TW1339 administration on VLDL triglyceride production in mice with adenovirus-mediated sterol carrier protein-2 (SCP-2) gene transfer. Food was removed from the cages at 8:30 PM at the beginning of the light phase of the diurnal cycle (reversed dark phase from 8 AM to 8 PM, the physiological period of food ingestion) the day prior to the experiment. An aliquot of blood was drawn from a tail vein the next day for determination of basal triglyceride concentration in plasma. Triton WR-1339 was intravenously injected at a dose of 35 mg/kg bw. Three hours later, blood was drawn from the inferior vena cava. An aliquot of plasma post-Triton WR1339 injection was stored for total triglyceride determination, while plasma lipoproteins were immediately isolated for VLDL triglyceride measurement. For the calculation of VLDL triglyceride production, we assumed that intestinal contribution to the plasma VLDL pool was minimal. Plasma volume was calculated assuming a value of 0.071 ml/g bw.

tested the hypothesis that the composition of biliary phosphatidylcholines, the principal form of phospholipids in bile, might have changed to favor biliary cholesterol solubilization and transport. As shown in **Table 3**, SCP2 overexpression led to significant changes in the proportions of hepatic and biliary phosphatidylcholine molecule species. There was a 100% increase in the hydrophobic index of biliary phosphatidylcholines in *Ad.rSCP2* relative to *Ad.E1Δ* mice (**Fig. 3**). These differences were not associated with changes in the hydrophobic index of hepatic phosphatidylcholines.

DISCUSSION

These studies provide the first description in the live animal of the multiple and pleotrophic alterations in hepatic cholesterol and triglyceride metabolism, biliary lipid secretion and composition, and lipoprotein metabolism that result from adenovirus-mediated overexpression of SCP-2 gene products in the mouse liver. The marked acute changes in hepatic VLDL production, accompanied by major changes in plasma lipoprotein cholesterol concentration, biliary lipid output, and phosphatidylcholine composition, supported the concept that SCP-2 might play an important role in the control of hepatic FA and cholesterol trafficking, influencing through these mechanisms the expression of other genes responsible for the package and secretion of lipids as nascent lipoproteins. The major metabolic changes induced by overexpression of the SCP-2 gene, including the present and prior (26) series of experiments, are outlined in **Fig. 4**. The central effects were initiated in the liver and determined major changes in serum lipoprotein cholesterol concentrations, hepatic lipid concentration, association with marked inhibition of fatty acid and relative decrease in cholesterol synthesis, inhibition of VLDL production, enhanced cholesterol absorption, and increase in biliary bile acid, phospholipids, and cholesterol secretion rates. In contrast, but consistent with present observations, disruption of the SCP2 gene in mice determined a phenotype characterized by a decrease of bile acid secretion rate and pool size, decrease hepatic cholesterol content, and intestinal absorption (48). SCP-2-knockout mice also presented significantly decreased liver triglyceride and cholesterol ester

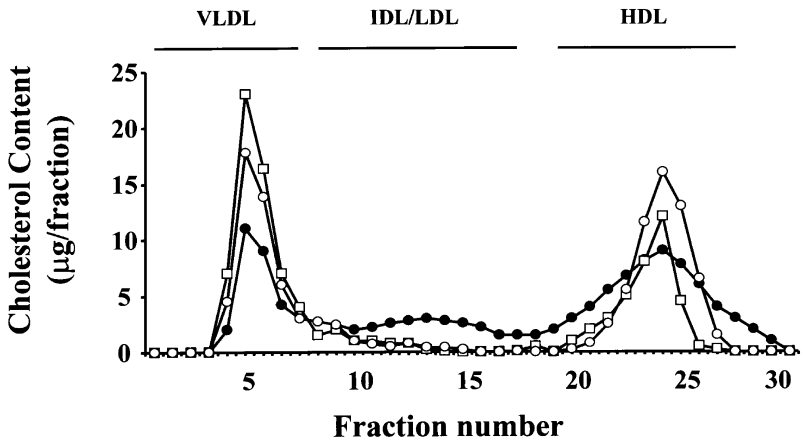

Fig. 2. Effect of Triton TW1339 administration on plasma lipoprotein cholesterol distribution in mice with adenovirus-mediated SCP-2 gene transfer. Mice were treated as described in Fig. 1. Cholesterol was measured in lipoprotein fractions. Curves correspond to one representative lipoprotein cholesterol profile of pooled plasma obtained from two mice in each group. More than 85% of post-Triton TW1339 plasma triglycerides were present in VLDL in the three groups of mice. Square, control saline-injected; open circle, *Ad.E1Δ*-infected mice; closed circle, *Ad.rSCP2*-infected mice.

TABLE 3. Effect of SCP-2 recombinant adenoviral infection on molecular species of phosphatidylcholine in mice liver and bile

	A. Saline	B. Ad.E1Δ	C. Ad. rSCP2
Liver			
Phosphatidylcholine species			
16:1-20:4	1.5 ± 0.6	2.1 ± 0.5	2.2 ± 0.3
16:1-18:2	2.7 ± 0.2	2.1 ± 0.6	1.6 ± 0.2[a]
16:0-22:6	9.7 ± 1.8	10.9 ± 2.1	12.4 ± 0.2
16:0-20:4	10.1 ± 1.6	10.7 ± 0.8	14.4 ± 4.6
16:0-18:2	43.8 ± 3.0	41.4 ± 3.0	34.8 ± 3.8[a]
16:0-18:1 + 18:0-20:4	22.7 ± 1.1	24.7 ± 2.8	28.4 ± 1.5[b]
18:0-18:2	8.1 ± 1.8	7.6 ± 1.5	5.2 ± 0.1[b]
18:0-18:1	1.3 ± 0.4	0.4 ± 0.3	0.9 ± 1.0
Bile			
Phosphatidylcholine species			
16:1-20:4	3.97 ± 0.50	3.90 ± 0.41	2.88 ± 0.27[a]
16:1-18:2	2.78 ± 0.47	2.98 ± 0.21	2.90 ± 0.54
16:0-22:6	5.08 ± 0.58	4.40 ± 0.54	5.37 ± 1.80
16:0-20:4	8.04 ± 0.48	7.55 ± 1.17	9.65 ± 2.71
16:0-18:2	62.60 ± 2.14	61.50 ± 2.48	54.15 ± 5.34[a]
16:0-18:1 + 18:0-20:4	14.30 ± 2.42	16.30 ± 1.50	20.90 ± 2.67[a]
18:0-18:2	3.10 ± 0.26	3.37 ± 0.44	4.10 ± 0.68[a]
18:0-18:1	0.12 ± 0.03	0.05 ± 0.02	0.07 ± 0.02[a]

Values represent the mean ± SE.
[a] $P < 0.05$ group C compared with groups A and B.
[b] $P < 0.05$ group C compared with group A.

concentrations, suggesting that these animals also have altered phospholipids, fatty acid, and triglyceride metabolism (24, 25). It is important to emphasize that the present studies of acute SCP2 gene overexpression in the liver may not reciprocally parallel the experimental findings of the SCP-2-knockout mice. In this model, the SCP2 gene is permanently deleted from all tissues; therefore, a number of adaptative mechanisms should have developed in the live animal, particularly in lipid cell trafficking. Presumably, one example of this adaptative mechanism was the increased expression of liver fatty acid binding protein found in SCP-2 knockout mice; this protein probably replaced some of the functions of SCP2 (48). The inhibition of hepatic VLDL triglyceride production was expected since hepatic apoB and apoE expressions were markedly reduced in *Ad.rSCP2* mice (26). The expressions of both apoB (5–7) and apoE (49, 50) are critical for VLDL synthesis and secretion. It is known that SCP-2 interacts directly with free FA and acyl-CoA FA (11, 14) and thus, it is conceivable that hepatic FAs in SCP-2-overexpressing mice were diverted from triglyceride synthesis to other metabolic pathways, including newly synthesized biliary phosphatidylcholine, decreasing VLDL production. Reduced FA synthesis determined by SCP-2 overexpression might have also contributed to the lower hepatic VLDL triacylglycerol production observed in *Ad.rSCP2*-infected mice. Hepatic SCP-2 overexpression also decreased nonfasting plasma HDL-C concentration and increased LDL-C levels. These changes were correlated with decreased expression of hepatic apo AI and LDLR as previously reported (26).

The relative inhibition of hepatic cholesterogenesis found in the *Ad.rSCP2* mice was correlated with increased concentration of hepatic cholesterol and the expected feedback regulation in hepatic LDLR expression, suggesting that the feedback regulation of the genes that control hepatic cholesterol metabolism was operating normally in the *Ad.rSCP2*-infected mice. Interestingly, hepatic FA synthesis and VLDL triglyceride production were also reduced in SCP-2-overexpressing mice. The complex coordinate regulation of hepatic FA and cholesterol metabolism was found after SCP-2 overexpression, suggesting the potential involvement of the sterol regulatory element binding proteins (SREBPs) in mediating the biological activity of SCP-2. These membrane-bound transcription factors regulate the expression levels of HMG-CoA reductase and other enzymes involved in the cholesterol and FA biosynthetic pathways, and the expression of the LDLR (51). In fact, genetic manipulation of some SREBPs in mice has shown marked effects in FFA metabolism (52, 53). Further studies are required to evaluate whether the decreased cholesterol and FA synthesis observed in the liver of SCP-2-overexpressing mice was indeed mediated by decreased transcriptional regulatory activity of SREBPs.

It is important to note that inflammatory mediators released in response to viral infection may induce disturbances in lipid metabolism (54). Nevertheless, several lines of evidence suggest that the observed effects on lipid metabolism in this study were not nonspecific effects of vi-

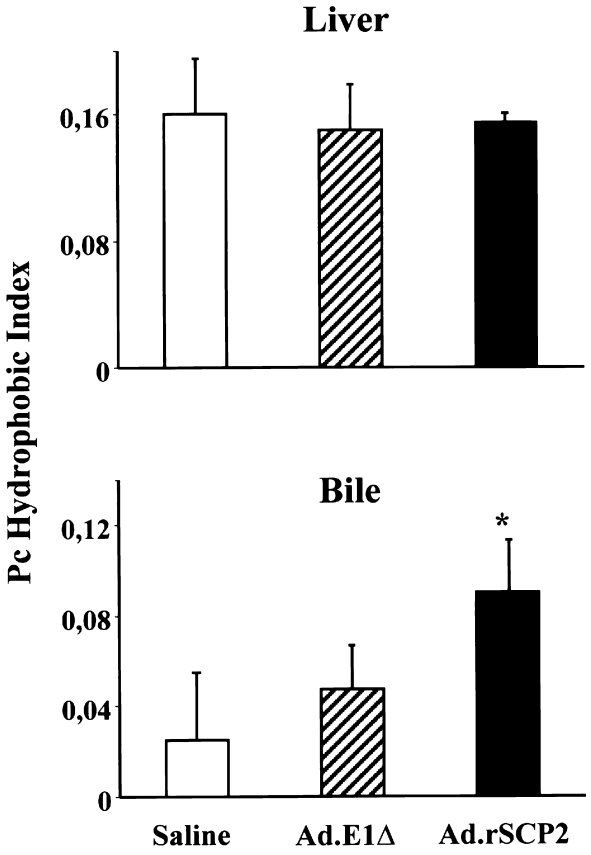

Fig. 3. Hepatic and biliary phosphatidylcholine (Pc) hydrophobic index. Liver and bile specimens were obtained as described in Table 1. There were four mice in each group. Values represent the mean ± SE. *Significantly different at $P < 0.01$ compared with control groups.

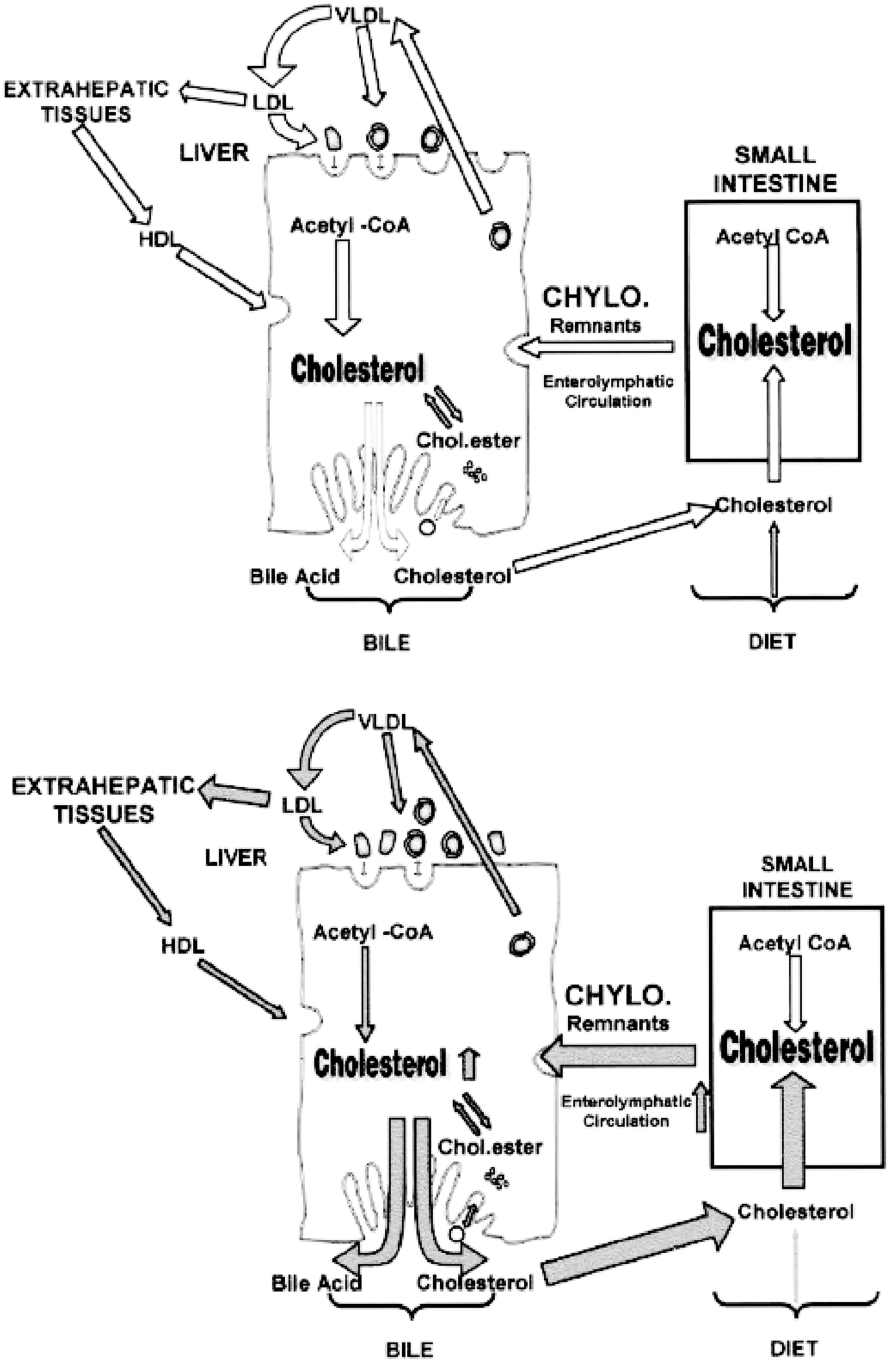

Fig. 4. Enterohepatic cholesterol metabolism in the mouse with adenovirus-mediated hepatic overexpression of SCP-2 gene. This figure summarizes the effects of the acute hepatic overexpression of SCP-2 gene on the enterohepatic flux of cholesterol through the plasma as lipoproteins and through the intestine after secretion into bile. The normal situation is represented in the upper panel and the effects of SCP-2 gene overexpression in the lower one. The thickness of the lines showing cholesterol fluxes represents semiquantitative values. The five more significant changes of cholesterol metabolism found in the Ad.rSCP2 mice are remarked in gray arrows as follows: *1*) VLDL production is reduced with presumably a lower formation of LDL in the Ad.rSCP2 mice. *2*) Plasma LDL cholesterol concentration increases due to a lower expression of hepatic LDL receptor (LDLR) (24). *3*) Plasma HDL concentration decreases, presumably because of a marked inhibition of hepatic AI synthesis (26) in the *Ad.rSCP2* mice. *4*) Hepatic cholesterogenesis is inhibited as expected, because of the enhanced cholesterol concentration and the diminution of sinusoidal LDLR expression in the experimental animal. *5*) The cholesterol and bile acid fluxes are markedly increased, a phenomenon that is associated with enhanced intestinal cholesterol absorption.

ral infection. First, the deleterious effects of viral infection were similar in both infected groups of animals, as judged by the scarce necrosis found in the livers and the mild elevations of serum transaminases. Moreover, high-resolution immunohistochemistry of thin liver sections of *Ad.rSCP2* mice showing subcellular distribution of SCP2 in round organelles highly suggestive of peroxisomes (26) also showed normal appearance of other organelles, including mitochondria. Second, bile flow and lipid secretion, both complex energy-dependent functions of hepatocytes, were maintained within the normal range. Bile flow and biliary lipid secretion require the functional and subcellular integrity of sinusoidal receptors and transporters, energy production, lysosomal processing, intracellular protein, and vesicular-mediated transport and canalicular excretion through a number of putative solute carriers (55, 56). In addition, administration of control *Ad.E1Δ* viruses increased hepatic cholesterogenesis, an effect that was reversed by the increase hepatic cholesterol content in the *Ad.rSCP2* mice, probably as the consequence of enhanced intestinal cholesterol absorption found in these animals (26). Similarly, total serum cholesterol concentration was unchanged in SCP2-overexpressed mice, a result consistent with previous studies showing that recombinant adenoviral infection, per se, does not change total plasma cholesterol levels (57).

We hypothesize that the triggering event involved in the multiple effects of hepatic SCP-2 overexpression on lipid metabolism in mice was the increased FA and cholesterol cycling and intracellular cholesterol and phosphatidylcholine redistribution. This possibility is consistent with the results of previous studies in SCP-2-transfected cultured rat hepatoma cells (23) and in the whole animal (26). It was apparent that the velocity of the bidirectional flux of cholesterol and phosphatidylcholine between endoplasmic reticulum and plasma membranes was critically dependent upon SCP-2 expression levels. Consequently, SCP-2 transfection might specifically determine free cholesterol and phosphatidylcholine accumulation in the plasma membrane of hepatocytes, with a simultaneous reduction in cholesterol esterification, triglyceride synthesis, and VLDL and HDL production. These latter changes are correlated with decreased apoB, apoAI, and apoE expression (26), VLDL production, and reduced plasma HDL-C levels.

Increasing evidence has now accumulated supporting the concept that SCP-2 plays a physiological role in hepatic cholesterol, phospholipids, and lipoprotein metabolism, as well as in biliary lipid secretion. We can postulate that the effect of SCP-2 overexpression on biliary phospholipids and cholesterol output may be related to vectorial enrichment of biliary lipids in some specific canalicular plasma membrane domains associated with a reciprocal effect of lipids through the sinusoidal membrane. Specific canalicular membrane domains could represent the immediate source of cholesterol to be recruited for bile secretion, particularly those enriched with the *ABCG5-ABCG8* transporters responsible for canalicular cholesterol secretion (58) and the MDR2 transporter responsible for canalicular membrane phosphatidylcholine flipping and biliary lipid vesicle secretion (2). Consistent with this possibility, SCP-2 can regulate cholesterol distribution between different kinetic domains of the plasma membrane (59). Furthermore, SCP-2 transfection in rat hepatoma cells determined cholesterol accumulation in the plasma membrane (23). On the other hand, previous studies have shown that cholesterol hypersecretion was correlated with a significant increase in the concentration of cholesterol in the canalicular membrane (39). Cholesterol-rich plasma membrane regions may exist in liver plasma membranes and correspond to detergent-resistant domains, such as caveolae and lipid rafts, which seem to participate in cellular cholesterol efflux in nonhepatic cells (60). Whether the increase of biliary lipid secretion found in the *Ad.rSCP2*-infected animal is associated with overexpression of the canalicular ATP binding cassette transporters remains to be elucidated.

Our results in *Ad.rSCP2*-infected mice are consistent with the hypothesis of the existence of a common metabolically active pool of cholesterol and phospholipids for VLDL production and biliary secretion, as previously postulated (3, 30). SCP-2 preferentially channels cholesterol and phospholipids into bile and reciprocally decreases the availability of hepatic lipids for VLDL assembly and secretion, which in this case is paralleled by a marked inhibition of hepatic expression of apoB and apoE, two key apolipoproteins that regulate VLDL production. Under these circumstances, cholesterol and phosphatidylcholine, which are the lipid components of nascent VLDL surface monolayer (5–7), will accumulate and eventually be delivered to the apical pole of the hepatocyte and into bile. Our data demonstrate that hepatic overexpression of SCP-2 favored biliary secretion of more hydrophobic phosphatidylcholine molecular species. These phosphatidylcholine species pack more tightly with cholesterol (61), and this could provide a mechanism by which SCP-2 increases biliary cholesterol secretion. Recent studies have shown that more hydrophilic phosphatidylcholine species promote rapid cholesterol crystallization in bile, whereas more hydrophobic species retard cholesterol crystallization (62). Both the detailed mechanisms by which SCP-2 overexpression directly influences hepatocellular trafficking of biliary phosphatidylcholines and the relevance of this finding for biliary cholesterol precipitation and gallstone formation remain to be established.

In summary, these studies support two important concepts. First, that hepatic SCP-2-mediated lipid trafficking represents a major physiological regulatory mechanism for cholesterol, FA and lipoprotein metabolism, and biliary lipid secretion. Second, these results strongly support the concept that SCP-2 preferentially channels lipids to the apical pole of the hepatocyte, while simultaneously decreasing sinusoidal lipoprotein secretion. Therefore, SCP-2 expression levels in the liver may serve an important physiological role in lipid homeostasis. Pharmacological manipulation of the SCP-2 gene could modulate hepatic overproduction of VLDL and biliary lipid secretion. If so, SCP2 may be a novel target for drug development

aimed to treat dyslipidemias, atherosclerosis, and gallstone disease.

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