Sterol Carrier Protein 2 Gene Transfer Changes Lipid Metabolism and Enterohepatic Sterol Circulation in Mice

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Background & Aims: Sterol carrier protein 2 (SCP-2) enhances sterol cycling and facilitates cholesterol translocation between intracellular organelles and plasma membrane in cultured cells, including hepatocytes. We examined the role of SCP-2 in hepatic cholesterol and lipid trafficking through the sinusoidal and canalicular secretory pathways of the liver in vivo. Methods: Recombinant adenovirus–mediated SCP-2 gene transfer was used to obtain hepatic overexpression of SCP-2 in C57BL/6 mice. Results: SCP-2 overexpression in the mouse liver resulted in an 8-fold increase of SCP-2 protein levels and determined various effects on lipid metabolism. It decreased high-density lipoprotein cholesterol and increased low-density lipoprotein (LDL) cholesterol concentrations. The expressions of hepatic LDL receptor, apolipoprotein (apo) A-I, apoB, and apoE were decreased. SCP-2 overexpression also increased hepatic cholesterol concentration, associated with decreased cholesterol neosynthesis. Increased biliary cholesterol and bile acid secretion, bile acid pool size, and intestinal cholesterol absorption were also observed. Conclusions: These results indicate that modulation of SCP-2 expression in the liver determines important modifications on lipoprotein metabolism, hepatic cholesterol synthesis and storage, biliary lipid secretion, bile acid metabolism, and intestinal cholesterol absorption.

The liver plays a central role in the maintenance of cholesterol homeostasis in vivo.1–3 Hepatocytes obtain cholesterol primarily through its biosynthesis from acetate in the endoplasmic reticulum and peroxisomes,4,5 and its uptake from apolipoprotein (apo) B–containing lipoproteins by low-density lipoprotein (LDL) receptor family protein–mediated endocytosis1,2 or from high-density lipoprotein (HDL) by selective cholesterol uptake mediated by the scavenger receptor class B type I (SR-BI).6,7 In addition, hepatocytes are relevant for cholesterol disposal by incorporating cholesterol and triglycerides into very-low-density lipoproteins, which, after the delivery of endogenously synthesized lipids to peripheral tissues, are converted into LDL.8 Liver cells also produce a significant fraction of apoA-I, which is secreted into the plasma complexed with phospholipids in HDL particles.8 Finally, the liver represents a unique organ involved in sterol elimination through the bile as cholesterol and newly synthesized bile acids.1,4,9 Given the complex function of the liver in cholesterol metabolism, sterol trafficking within the hepatocytes must be particularly intricate as well as exquisitely regulated.

Under steady-state conditions, the plasma membrane and the endocytic apparatus contain approximately 80%–90% of the total cell cholesterol.10 This cholesterol gradient between plasma membrane and intracellular compartments implies the existence of a complex, efficient, and well-controlled system for sterol trafficking among cell compartments through different intracellular cholesterol transport mechanisms, including vesicles and a variety of carrier proteins.10,11 Based on several in vitro experiments, it has been postulated that sterol carrier protein 2 (SCP-2) and sterol carrier protein X (SCP-X), which originate from different transcripts of the same gene,12 may have an important role in intracellular cholesterol trafficking and metabolism.13 Purified SCP-2 stimulated interorganellar cholesterol transfer14,15 as well as the conversion of free cholesterol into cholesteryl esters,16,17 steroid hormones,18 and 7α-hydroxycholesterol.19,20 Similarly, SCP-X increased the transfer of sterols among intracellular membranes in vitro.21 In addi-
tion, SCP-X had thiolitic activity on the side chain of cholesterol, suggesting its relevance in bile acid biosynthesis.\(^{22}\)

We have shown that SCP-2 appears to be critical for rapid transport of newly synthesized cholesterol from the endoplasmic reticulum to the plasma membrane in cultured human fibroblasts.\(^{23}\) More recently, Baum et al.\(^{24}\) reported that overexpression of SCP-2 in rat hepatoma cells enhanced intracellular cholesterol cycling, increased plasma membrane cholesterol content, and decreased cholesterol esterification and HDL production. Furthermore, we found that SCP-2 expression was important to control secretion of hepatic newly synthesized cholesterol into bile in the rat, suggesting a role of SCP-2 function in biliary cholesterol secretion.\(^{25}\) Consistent with this latter proposal, Fuchs et al.\(^{26}\) showed that hepatic SCP-2 expression levels correlated with biliary cholesterol hypersecretion in mice with genetic predisposition to cholesterol gallstone disease.\(^{26}\) Similarly, hepatic SCP-2 levels were also elevated in patients with cholesterol gallstones.\(^{27}\) These various in vivo studies strongly support the hypothesis that SCP-2 constitutively participates in hepatocellular cholesterol trafficking and suggest that hepatic SCP-2 expression can influence overall cholesterol metabolism under physiologic and pathophysiologic conditions.

To further examine whether SCP-2 indeed plays a major physiologic role in whole-body cholesterol homoeostasis in vivo, we determined whether adenovirus-mediated transfer of SCP-2 into the mouse liver altered lipoprotein and hepatic cholesterol metabolism and biliary lipid secretion. We show that transient hepatic overexpression of SCP-2 in mice decreased HDL cholesterol levels, whereas it increased plasma LDL cholesterol concentrations. These changes were associated with decreased hepatic apoA-I, apoB, apoE, and LDL receptor expression. In addition, SCP-2 overexpression enhanced enterohepatic circulation of cholesterol and bile acids, bile acid pool size, and intestinal cholesterol absorption. These results show that hepatic SCP-2 expression can regulate lipoprotein metabolism, hepatic sterol synthesis and content, biliary lipid secretion, and enterohepatic circulation of cholesterol and bile acids.

**Materials and Methods**

**Animals and Diet**

Adult male C57BL/6 mice over 8 weeks of age were used in all experiments. Mice had free access to commercial rodent diet Prolab RMH 3000 (PMI Nutritional International Inc., Brentwood, MO), except where otherwise specified. This diet contained small amounts of cholesterol (0.02%, wt/wt).

The animals were housed at 25°C in a well-ventilated room with controlled reverse light cycling (middark was set at 10 AM and midnight at 10 PM). All experiments were carried out during the dark phase of the diurnal cycle.

**Recombinant Adenoviruses Preparation and Administration**

The recombinant adenovirus Ad.rSCP2 was generated by homologous recombination in 293 cells, essentially as described previously.\(^{28,29}\) The adenoviral backbone used for the construction of the vector containing rat SCP-2 complementary DNA (cDNA) under control of the cytomegalovirus enhancer/promoter was derived from a replication-deficient first-generation type 5 adenovirus with deletions of E1 and E3 genes. The control adenovirus, AdE1\(\Delta\), contained the same E1 and E3 deletions without the transgene expression cassette. Large-scale production of recombinant adenoviruses was performed from infected 293 cells, as described previously.\(^{20}\)

For administration of viruses, mice were anesthetized by intraperitoneal injection of 45 mg/kg body wt sodium pentobarbital (Abbott Laboratories, North Chicago, IL). A jugular vein was exposed, and \(1 \times 10^{11}\) particles (in 0.1 mL of isotonic saline buffer) of control or recombinant adenoviruses were injected intravenously. An additional control group received 0.1 mL of saline buffer only. In preliminary experiments, we observed that the effects of adenovirus-mediated transfer of SCP-2 cDNA on cholesterol metabolism peaked 5–8 days after infection and began to decline within 10 days. Therefore, we performed all subsequent experiments 7 days after adenoviral infections.

**Bile and Blood Sampling**

After 12 hours of fasting, mice were anesthetized as described above. The cyst duct was ligated, and a common bile duct fistula was performed using a PE10 polyethylene catheter (Clay-Adams, New York, NY). Hepatic bile specimens were collected for 30–60 minutes in preweighed tubes, and constant body temperature was maintained under a heating lamp. At the end of the experiments, blood was removed from the inferior vena cava. Plasma was immediately separated by centrifugation at 10,000 rpm for 10 minutes at 4°C. In some experiments, consecutive 30-minute bile samples were obtained under depletion of the bile acid pool or under intravenous infusion of sodium taurocholate (1 mmol/min).

**cDNA Probes**

cDNA probes for apoB, cholesterol-7alpha-hydroxylase, and oxysterol-7alpha-hydroxylase were kindly provided by Dr. Roger Davis (San Diego State University, San Diego, CA). cDNA probes for apoA-I and apoE were obtained as follows. First-strand cDNA was prepared by random priming and reverse transcription from liver (for the apoA-I probe) and small bowel (for the apoE probe) total RNA and used as a template in polymerase chain reaction (PCR) reactions. The PCR primer pair for the apo A-I probe was (5’)-AGTTGGTACCTCCTGGAAAACTGGGACA-3’ and (3’)-AGTTGGTACCTCCTGGAAAACTGGGACA-3’.
5'-CGGAAACCTTACGGCTGGCCTTTG-3'. The primer pair for the apo E probe was: (5') 5'-CCTGAAACCGCTGTTGATTAC-3' and (3') 5'-CCTAGCTGGTCATGATGTTGC-3'. PCR products were subcloned into pGEM-T (Promega, Madison, WI), sequenced, and purified on agarose gel before radiolabeling.

**Quantitative Immunoblotting Analysis**

For SCP-2 immunoblotting, liver homogenates were prepared as previously described. Each sample (30 μg) was subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using a rabbit polyclonal anti-rat SCP-2 serum. An antialbumin antibody was used for protein loading control. For lipoprotein receptor expression analysis, total membrane extracts (post-nuclear 100,000 g membrane pellets) from mouse liver were prepared. For SR-BI immunoblotting, membranes (40–100 μg of protein/sample) were size-fractionated on 8% SDS-PAGE and immobiloblotted with a rabbit polyclonal antipeptide antibody against murine SR-BI provided by Dr. Monty Krieger (Massachusetts Institute of Technology, Cambridge, MA). For LDL receptor Western blotting, membrane extracts (100 μg of protein/sample) were separated on 8% SDS-PAGE under nonreducing conditions and immunoblotted with a rabbit polyclonal anti-bovine LDL receptor antibody provided by Dr. Helen Hobbs and Dr. Joachim Herz (Southwestern Medical Center, Dallas, TX). Anti-ε-coatomer protein (anti-ε-COP) antibody obtained from Dr. Monty Krieger was used for membrane protein loading control. Antibody binding to protein samples was visualized using the enhanced chemiluminescence procedure. Densitometric analysis was performed with a Macintosh Color One scanner (Cupertino, CA) and NIH Image software.

**Hepatic Immunohistochemistry**

Liver slices (2–4 mm thick) were fixed by immersion in Bouin’s solution, dehydrated, embedded in Paraplast (Monoject Scientific, St. Louis, MO), sectioned at 7-μm thickness in a rotatory microtome, mounted on glass slides, and stored until processing. Immunostaining was performed according to the peroxidase/antiperoxidase method with some modifications previously described. After inhibition of endogenous pseudoperoxidase activity with 3% (vol/vol) hydrogen peroxide in absolute methanol, tissue sections were incubated with the anti–SCP-2 antibody (1:1000–1:2000) overnight at 22°C, followed by the secondary antibody (1:20), the peroxidase/antiperoxidase complex (1:150), and color development with 3,3'-diaminobenzidine-hydrogen peroxide. Sections were counterstained with hematoxylin, dehydrated, cleared with xylene, and coverslipped. For high-resolution morphologic analysis, a modification of a pre-embedding ultrastructural immunohistochemistry protocol was performed as previously described. All tissue sections were visualized and photographed with a Nikon Optiphot microscope with a Nikon Microflex UFX IIA filter (Nikon, Tokyo, Japan).

**Blot Hybridization of RNA**

Total RNA was prepared from mouse liver using the acid guanidinium thiocyanate–phenol–chloroform method. Aliquots of 15 μg of total RNA were size-fractionated on a 1% (wt/vol) agarose-formaldehyde gel and transferred to nylon membranes. Filters were hybridized with 32P-labeled probes (1 × 10^6 cpm/mL) for 2 hours at 65°C using Rapid-hyb buffer (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were then washed with 0.1% (wt/vol) SDS/2X standard saline citrate for 10 minutes at room temperature, followed by another wash with the same solution for 10 minutes at 65°C, and finally autoradiographed with Kodak film at −80°C. Densitometric analysis was performed as described above. Results were normalized to the signal of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messenger RNA (mRNA). For cholesterol 7α-hydroxylase expression analysis, poly(A)T-enriched RNA was prepared from 200 μg of total liver RNA using the PolyAT Tract kit (Promega, Madison, WI) and subjected to blot hybridization, as described for the other cDNA probes.

**Quantitation of Hepatic Cholesterol Synthesis In Vivo**

After a 2-hour fasting period, the rate of cholesterol synthesis was measured at the middark phase of the diurnal cycle (10 AM). Each mouse received 50 mCi [3H]water (Amersham Pharmacia Biotech, Piscataway, NJ) by intraperitoneal injection as previously described. One hour after radiolabel injection, animals were anesthetized and approximately 0.5 mL of blood was obtained for determination of water-specific activity in plasma. After liver removal, tissue specimens were saponified, and digitonin-precipitable sterols were isolated as previously described. Results were expressed as micromoles of [3H]water incorporated into digitonin-precipitable sterols per hour per gram of liver weight.

**Measurement of Bile Acid Pool Size and Fecal Excretion**

Bile acid pool size was quantified as the total mass of bile acids extracted from the small intestine, liver, and gall-bladder as previously described. Briefly, organs were minced and extracted at 60°C for 4 hours in ethanol containing [24-14C]taurocholic acid (New England Nuclear, Boston, MA) as internal standard. After filtration, aliquots of the extracts were dried in a vial and the recovery of radiolabeled taurocholic acid was determined by scintillation counting. Recoveries were always >91%. Bile acid pool size was expressed as micromoles of bile acids/100 g body wt. To determine daily fecal bile acid excretion, stools were collected from each animal housed individually during a 24-hour period from the sixth to the seventh day after intravenous administration of adenoviral preparations or saline controls. Stools were extracted with [14C]cholic acid (New England Nuclear, Boston, MA) added as internal standard, and bile acids were quantified and corrected for recovery.
Measurement of Intestinal Cholesterol Absorption

Intestinal cholesterol absorption was measured by the dual-isotope ratio method. Briefly, mice were individually housed in wire cages, fasted for 4 hours, and at noon were given an intragastric bolus of 100 μL of corn oil containing 1 mCi [14C]cholesterol (New England Nuclear) and 2 mCi [3H]sitostanol (American Radiolabeled Chemicals, St. Louis, MO). Feces was collected for 24 hours, dried overnight at 40°C, and extracted with chloroform-methanol (2:1 vol/vol). After phase separation, a fraction of the cholesterin phase was transferred into scintillation vials and dried under a hood. Radioactivity of each specimen was measured and corrected by the channel ratio method using external standards. Intestinal cholesterol absorption was calculated as the percent of cholesterol absorbed per day by using the formula: % Absorption = [1 - (Fecal [14C/3H])/(Administered [14C/3H])] × 100.

Plasma Lipoprotein Separation and Plasma, Hepatic, and Biliary Lipid Analyses

Plasma lipoprotein separation was performed by Superose 6–fast protein liquid chromatography gel filtration of fresh plasma specimens. For other determinations, liver, bile, and plasma samples were frozen at 20°C until processing. Total plasma and lipoprotein cholesterol concentrations were measured using enzymatic kits (Sigma Chemical Co., St. Louis, MO). Hepatic and biliary cholesterol, biliary phospholipids, and bile acids were determined by routine methods. Bile acid pool composition analysis was kindly performed by Dr. Stephen Turley (Southwestern Medical Center, Dallas, TX). The bile acid–independent bile flow fraction was calculated by linear regression analysis of bile flow as a function of bile acid output according to the equation y = a + bx, where a represents the bile acid–independent bile flow at the interception of the y-axis.

Statistics

Data are presented as means ± SE. The 2-tailed, unpaired Student t test was used to compare the sets of data. Statistically significant differences were considered at a P value of <0.05.

Results

To evaluate the relevance of SCP-2 in hepatic lipid metabolism in vivo, we studied C57BL/6 mice that transiently overexpressed SCP-2 in the liver by adenovirus-mediated gene transfer (Ad.rSCP2). Controls included noninfected saline-injected animals or mice infected with a control adenovirus that lack a cDNA transgene (Ad.E1Δ). Hepatic SCP-2 expression was increased 8-fold in Ad.rSCP2–infected mice as evaluated by immunoblotting of liver homogenates 7 days after infection (Figure 1). As expected, hepatic SCP-X expression remained unchanged in Ad.rSCP2-infected mice.

Immunostaining of liver tissue readily revealed SCP-2 protein overexpression in a large number of hepatocytes from animals infected with Ad.rSCP2 (Figures 2A and C) compared with tissue from noninfected mice (not shown) or control Ad.E1Δ–infected mice (Figure 2B and D). In Ad.rSCP2-infected mice, overexpression of SCP-2 protein was heterogeneous, ranging from faint to heavy immunostaining. SCP-2–positive hepatocytes were heavily stained with a subcellular pattern, suggesting a granular distribution over the cytoplasm (Figure 2C). When high-resolution immunohistochemistry was used to examine thin liver sections, the subcellular distribution of SCP-2 was evidenced in round liver sections, the subcellular distribution of SCP-2 was evidenced in round liver segments, the subcellular distribution of SCP-2 was evidenced in the cytoplasm (Figure 2C).

Compared with those in control Ad.E1Δ–infected mice, plasma cholesterol levels were unchanged in Ad.rSCP2 animals (Table 1). These results were consistent with previous studies showing that recombinant adeno-viral infection per se does not change total plasma cholesterol levels. However, plasma lipoprotein cholesterol distribution was altered in Ad.rSCP2 mice compared with that in Ad.E1Δ mice (Figure 3); the plasma LDL cholesterol concentration increased by 100%, and HDL cholesterol levels decreased by 25%. Very-low-density lipoprotein cholesterol was not changed. Together, these results indicate that hepatic SCP-2 expression can regulate lipoprotein cholesterol metabolism in vivo.

Because the modifications observed in plasma lipoprotein cholesterol distribution in Ad.rSCP2-overexpressing mice might have been caused by changes in hepatic
lipoprotein synthesis and/or receptor-mediated lipoprotein cholesterol clearance, we evaluated hepatic expression of key apolipoproteins and cell surface receptors involved in lipoprotein metabolism in vivo. The expressions of apoA-I, apoB, and apoE were analyzed by Northern blot and normalized to GADPH expression (Figure 4). Liver mRNA levels of apoA-I and apoE, 2 major apolipoprotein constituents of HDL in rodents, and

Table 1. Effect of SCP-2 Recombinant Adenoviral Infection on Body and Liver Weight and Serum Lipid Concentrations in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Liver wt (g)</th>
<th>Total plasma cholesterol (mg/dL)</th>
<th>Total plasma triglyceride (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Saline (n = 17)</td>
<td>21 ± 0.6</td>
<td>0.92 ± 0.02</td>
<td>88 ± 2.7</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>B. Ad.E1Δ (n = 15)</td>
<td>22 ± 0.6</td>
<td>0.94 ± 0.03</td>
<td>87 ± 3.9</td>
<td>104 ± 6</td>
</tr>
<tr>
<td>C. Ad.rSCP2 (n = 12)</td>
<td>21 ± 0.3</td>
<td>1.48 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98 ± 5.1</td>
<td>84 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NOTE. Determinations were made 7 days after intravenous administration of saline or adenoviruses. Values represent means ± SE. The number of mice in each group is shown in parenthesis.

<sup>a</sup>Plasma triglyceride values were determined in 5–6 mice in each group.

<sup>b</sup>Significant difference (P < 0.001) compared with groups A and B.
apoB, the unique protein component of LDL, were decreased in Ad.rSCP2-infected mice by 50%, 80%, and 40%, respectively. Liver expression of LDL receptor and HDL receptor SR-BI were evaluated by immunoblotting in hepatic membrane extracts (Figure 5). Compared with control mice, hepatic LDL receptor expression decreased by 40%, whereas SR-BI levels remained unchanged. These findings showed that SCP-2 can influence hepatic apolipoprotein and LDL receptor expression.

As shown in Table 2, SCP-2 overexpression increased hepatic-free cholesterol concentration 1.6-fold and decreased in vivo hepatic synthesis of cholesterol by 40%. The increase in cholesterol content was consistent with feedback inhibition of hepatic cholesterol synthesis and LDL receptor expression (Figure 5) found in Ad.rSCP2-infected mice compared with control virus-infected animals. Although no change was observed in absolute hepatic cholesteryl ester concentration, the relative levels of esterified cholesterol and total cholesterol were significantly reduced in Ad.rSCP-infected mice. These results indicate that SCP-2 expression regulates intrahepatic cholesterol metabolism.

Next, we studied the effects of hepatic SCP-2 overexpression on bile flow and biliary lipid concentrations (Table 3). Total bile flow increased by 73% in Ad.rSCP2-infected mice. This increased bile flow was most likely caused by an approximately 3-fold increase in the bile acid–independent bile flow in Ad.rSCP2 mice compared with Ad.E1Δ-infected mice. Biliary lipid concentrations remained within normal range, with the exception of biliary cholesterol levels, which were slightly but significantly increased by 20% in Ad.rSCP2 mice. Biliary bile acid and phospholipid outputs increased by approximately 50%–60%, whereas biliary cholesterol output doubled in Ad.rSCP2 mice compared with Ad.E1Δ animals (Table 4). Cholesterol–bile acid and cholesterol–phospholipid molar ratios were significantly increased in hepatic bile from Ad.rSCP2 mice by 34% and 25%, respectively, indicating that hepatic SCP-2 gene overexpression had a more important effect on biliary cholesterol secretion, which is independent of the observed effect on biliary bile acid secretion.

Consistent with the increase in biliary bile acid output, the bile acid pool size was significantly expanded by approximately 15% in Ad.rSCP2-infected mice, whereas bile acid pool composition remained unchanged (Table 5). The daily fecal bile acid excretion rate, which is a good indicator of hepatic bile synthesis, was also similar in the different experimental groups, a finding that was consistent with unchanged cholesterol 7α-hydroxylase expression, the most relevant rate-limiting enzyme in
bile acid synthesis (Figure 6, left panel). However, mRNA levels for the oxysterol-7α-hydroxylase were decreased by 50% in Ad.rSCP2 mice (Figure 6, right panel), suggesting selective down-regulation of the alternative pathway for hepatic bile acid synthesis. Given the measured basal biliary bile acid outputs and assuming that the intrinsic motility of the biliary tree remained unchanged, it can be calculated that the bile acid pool recirculated 5.5 times/day in the Ad.rSCP2 mice compared with 4 times/day in control noninfected and Ad.E1Δ-infected mice, suggesting that the turnover of the bile acid pool was increased in the Ad.rSCP2 mice. This increased enterohepatic circulation of bile acids could have had an effect on intestinal cholesterol absorption. In fact, dietary cholesterol absorption was significantly increased from 63% in the saline-injected and Ad.E1Δ-infected animals to 71% in Ad.rSCP2 mice. Taken together, these studies indicate that hepatic overexpression of SCP-2 results in increased bile acid pool size and turnover and, subsequently, enhanced intestinal cholesterol absorption.

**Discussion**

These studies show that adenovirus-mediated overexpression of SCP-2 in the mouse liver was associated with marked changes in lipoprotein metabolism, hepatic cholesterol synthesis and content, biliary lipid output, and enterohepatic circulation of cholesterol and bile acids. Hepatic SCP-2 overexpression decreased HDL cholesterol concentration and increased LDL cholesterol levels, which were correlated with lowered expression of hepatic apoA-I and LDL receptor. Multiple effects on hepatic cholesterol and bile acid metabolism were found: (1) unesterified cholesterol accumulation and cholesterol synthesis inhibition; (2) increased bile flow and biliary lipid secretion; and (3) increased bile acid pool size associated with normal cholesterol 7α-hydroxylase expression and fecal bile acid excretion. In addition, adenovirus-mediated SCP-2 gene transfer resulted in increased intestinal cholesterol absorption.

SCP-2 protein is mainly localized to peroxisomes. As a consequence, the mechanism by which peroxisomal SCP-2 overexpression can regulate extraperoxisomal cholesterol trafficking in the liver remains to be elucidated. However, a number of studies have suggested that at least in hepatocytes, a significant proportion of SCP-2 is also present in the cytosol. These observations have been supported recently by studies in both mice that are

<table>
<thead>
<tr>
<th>Fold Increase</th>
<th>-</th>
<th>1.0</th>
<th>0.6^A</th>
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</table>

*Figure 5.* Hepatic LDL receptor and SR-BI expression in SCP-2–overexpressing mice. Total membrane extracts were prepared, size fractionated by SDS-PAGE, immunoblotted with anti–SR-BI and LDL receptor antibodies, and subjected to densitometric analysis. Anti-γ-COP antibody was used for membrane protein loading control. The fold change in receptor expression of Ad.rSCP-2 mice compared with control Ad.E1Δ mice is shown after correction for γ-COP signal. ^A^ *P* < 0.05.

Table 2. Effect of SCP-2 Recombinant Adenoviral Infection on Hepatic Cholesterol Synthesis and Content in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic cholesterol synthesis (µmol·h⁻¹·100 g body wt⁻¹)</th>
<th>Cholesterol (mg/g liver)</th>
<th>Cholesteryl esters (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Saline</td>
<td>1.63 ± 0.09</td>
<td>2.0 ± 0.3</td>
<td>0.32 ± 0.3</td>
</tr>
<tr>
<td>B. Ad.E1Δ</td>
<td>3.14 ± 0.19</td>
<td>2.4 ± 0.1</td>
<td>0.39 ± 0.1</td>
</tr>
<tr>
<td>C. Ad.rSCP2</td>
<td>1.91 ± 0.33^a</td>
<td>3.9 ± 0.6^b</td>
<td>0.52 ± 0.2</td>
</tr>
</tbody>
</table>

*NOTE.* All values are expressed as means ± SE. Experiments (4–6 animals in each group) were performed during the middark phase of the diurnal cycle. ^a^Significant difference (*P* < 0.001) compared with group B. ^b^Significant difference (*P* < 0.001) compared with groups A and B.
Table 3. Effect of SCP-2 Recombinant Adenoviral Infection on Bile Flow and Biliary Lipid Concentrations in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Bile flow (μL · min⁻¹ · 100 g body wt⁻¹)</th>
<th>Hepatic bile lipid concentration (mmol/L)</th>
<th>Gallbladder cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Bile acid independent</td>
<td>Bile acids</td>
</tr>
<tr>
<td>A. Saline</td>
<td>8.0 ± 0.4</td>
<td>6.0 ± 0.8</td>
<td>28.5 ± 2.1</td>
</tr>
<tr>
<td>B. Ad.E1Δ</td>
<td>7.4 ± 0.3</td>
<td>3.8 ± 0.8</td>
<td>31.3 ± 2.2</td>
</tr>
<tr>
<td>C. Ad.rSCP2</td>
<td>12.8 ± 0.7a</td>
<td>10.7 ± 0.7a</td>
<td>28.2 ± 2.7</td>
</tr>
</tbody>
</table>

Note. Values represent means ± SE. Experiments were performed during the dark phase of the diurnal cycle. Measurements were performed in 11 mice in each experimental group (except for gallbladder cholesterol concentration (5–6 mice in each group). Some animals were depleted of bile salts or intravenously infused with 1 mmol sodium taurocholate. Linear regression analysis of bile flow as a function of bile acid secretion rates were significantly correlated (r = 0.93, P < 0.001).

*P < 0.001, †P < 0.05; group C compared with groups A and B.

terol-sensitive genes, such as apoA-I and apoE. In fact, apoA-I and E gene expression have been correlated with changes in cholesterol content in putative regulatory sterol pools.46,47 Because apoA-I synthesis is the main determinant of HDL production,8 it is likely that the decreased plasma HDL cholesterol levels observed in SCP-2–overexpressing mice were caused by decreased hepatic HDL synthesis and secretion. Hepatic SCP-2 overexpression also had a major impact on hepatic cholesterol metabolism. The relative inhibition of hepatic cholesterologenesis found in the Ad.rSCP2 mice was correlated with increased concentration of hepatic cholesterol and the expected feedback regulation in hepatic LDL receptor expression. The mechanism by which SCP-2 down-regulates apolipoprotein expression and cholesterol synthesis is not known. It might be related to changes in the cholesterol distribution within the cell, presumably in the endoplasmic reticulum, where cholesterol sensing occurs. Although our measurements of LDL receptor and SR-BI protein levels, as well as message levels for apoproteins, can explain the physiologic basis for changes in plasma lipoproteins in the SCP-2–overexpressing model, it is important to note that they are indirect data and not direct measurements. Further studies are needed to establish the molecular basis and physiologic significance of the observed changes.

Although increased hepatic SCP-2 expression stimulated secretion of the 3 major biliary lipids, it had a more important effect on biliary cholesterol secretion. These findings are consistent with the decreased transport of

Table 4. Effect of SCP-2 Gene Transfer on Biliary Lipid Secretion and Cholesterol Molar Ratio in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Biliary lipid output (nmol · min⁻¹ · 100 g body wt⁻¹)</th>
<th>Cholesterol molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile acids</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>A. Saline</td>
<td>235 ± 29</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>B. Ad.E1Δ</td>
<td>235 ± 25</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>C. Ad.rSCP2</td>
<td>360 ± 38a</td>
<td>74 ± 4a</td>
</tr>
</tbody>
</table>

Note. All values are means ± SE. Each experimental group had 9–11 animals.

*P < 0.01, group C compared with groups A and B.
newly synthesized cholesterol from liver to bile observed in SCP-2 antisense oligonucleotide–treated rats. They are also consistent with the correlation between increased SCP-2 expression and biliary cholesterol hypersecretion in mice genetically predisposed to cholesterol gallstone formation, as well as in patients with cholelithiasis. The major driving forces of biliary cholesterol secretion are the rate of bile acid secretion, the hydrophobicity of bile acid pool, and the availability of free cholesterol in the metabolically active pool of the hepatocyte. In the present study, we showed that 2 potential factors responsible for the enhanced biliary cholesterol output in Ad.r-SCP2 mice were the increased biliary bile acid output and the potentially augmented hepatic cholesterol availability as a consequence of increased intestinal cholesterol absorption. Because the bile acid pool composition remained unchanged, increased biliary cholesterol secretion was not attributable to a higher proportion of hydrophobic bile salt species.

We can postulate that the effect of SCP-2 overexpression on biliary cholesterol output might be explained by vectorial enrichment of cholesterol in some specific canalicular plasma membrane domains, which are the immediate source of cholesterol to be recruited for bile secretion. SCP-2 transfection in rat hepatoma cells determined cholesterol accumulation in the plasma membrane. Furthermore, SCP-2 can regulate cholesterol distribution between different kinetic domains of the plasma membrane. Cholesterol-rich plasma membrane regions may exist in liver plasma membranes and correspond to detergent-resistant domains.

<table>
<thead>
<tr>
<th>Cholesterol group</th>
<th>Bile acid pool size (µmol/100 g body wt)</th>
<th>Bile acid pool composition (%)</th>
<th>Fecal bile acid excretion (µmol · day⁻¹ · 100 g body wt⁻¹)</th>
<th>Intestinal absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Saline</td>
<td>81 ± 3.2</td>
<td>43 ± 4</td>
<td>19.0 ± 0.5</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>B. Ad.E1Δ</td>
<td>82 ± 2.8</td>
<td>47 ± 3</td>
<td>18.3 ± 0.6</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>C. Ad.rSCP2</td>
<td>94 ± 4.3ᵃ</td>
<td>39 ± 2</td>
<td>18.2 ± 0.3</td>
<td>71 ± 2ᵃ</td>
</tr>
</tbody>
</table>

NOTE. Values are means ± SE. Bile acid pool size was determined in 11 mice in each group, bile acid pool composition in 4–6 animals/group, and fecal bile acid outputs in 6 animals/group. Cholesterol absorption was measured in 4–8 mice/group. ᵃP < 0.02.

**Figure 6.** Hepatic expression of cholesterol 7α-hydroxylase and oxysterol 7α-hydroxylase in Ad.rSCP2-infected mice. Hepatic RNA was prepared, electrophoresed, and transferred to nylon membranes. Hydroxylase gene expression was evaluated by RNA blot hybridization with ³²P-labeled cDNA probes and densitometric analysis. The fold change in mRNA expression of Ad.rSCP2 mice compared with AdE1Δ-infected mice is shown after normalization against GAPDH mRNA. ᵇP < 0.05.
 mains, such as caveolae and lipid rafts that seem to participate in cellular cholesterol efflux in nonhepatic cells. In fact, caveolin-1 and -2, 2 major protein markers of caveolae, are highly expressed in hepatocytes and copurify with Triton X-100–resistant domains prepared from hepatocellular membranes. Another consequence of an SCP-2–dependent cholesterol enrichment in some specific plasma membrane domains may have important functional implications for the activities of a variety of plasma membrane enzymes and transporters. One of these potential implications might be related to the increase in the bile acid–independent bile flow found in Ad.rSCP2 mice. Increased cholesterol concentration in the canalicular membrane could have stimulated the activities of apical transporters (i.e., multidrug resistance–associated protein) that regulate secretion of key bile constituents (i.e., glutathione) and control bile flow.

Previous studies show that cholesterol from HDL is preferentially secreted into bile compared with cholesterol from other lipoproteins. However, the results of the present study suggest that SR-BI may not be involved in the enhanced secretion of biliary cholesterol in the SCP-2–overexpressing mice because hepatic SR-BI protein levels do not change in these animals. Therefore, in this model the decreased levels of plasma HDL cholesterol seem to be a consequence of decreased hepatic HDL synthesis, rather than increased clearance of HDL by the liver. More likely, the origin of the increase of biliary cholesterol output was related to the enhanced intestinal cholesterol absorption in the Ad.rSCP2 mice. ApoE-rich chylomicron remnants and its sinusoidal membrane receptor mediate this pathway of dietary cholesterol delivery to the liver and bile. A major finding in the SCP-2–overexpressing mice was a greater than normal bile acid pool size, which was associated with an increased enterohepatic circulation of bile salts and correlated with enhanced absorption of dietary cholesterol in the small intestine. Interestingly, fecal bile acid excretion remained unchanged, suggesting a normal rate of bile acid synthesis. This latter finding was consistent with a normal hepatic expression of the cholesterol 7α-hydroxylase gene. The increase in bile acid pool size and enhanced biliary secretion of bile acids associated with a normal rate of synthesis may be related to an increase in the turnover of the bile acid pool. Another plausible explanation for these results is that the bile acid pool size increased because of a transitory increase in bile acid synthesis that might have taken place before we decided to measure bile acid kinetics. A potential weakness of this study was a lack of a temporal vision of the events that took place between the moment of the infection and the time chosen for performing the protocols (7 days).

Unexpectedly, the expression of oxysterol-7α-hydroxylase, an early enzyme of the alternative bile acid synthesis pathway that is coordinately regulated with sterol 27-hydroxylase but not with cholesterol 7α-hydroxylase in the mouse liver, was markedly reduced in Ad.rSCP2 mice. The functional significance of this finding is not apparent, but it suggests that expression of this gene might be under coordinate control together with other genes sensitive to SCP-2–dependent intracellular cholesterol channeling, such as those genes encoding apoA-I, apoB, and apoE.

In contrast to the present study, Seedorf et al. and Kannereberg et al. have not reported a major abnormal phenotype for cholesterol or lipoprotein metabolism in mice with disruption of the SCP-2/SCP-X gene. These investigators found only a failure in the oxidation of 2-methyl-branched fatty acids and the side chain of cholesterol. The peroxosomal 58-kilodalton SPC-X or SCP-2/thiolase normally performs these functions. We speculate that this apparent discrepancy between the SCP-2/SCP-X knockout mouse model and the present study could be caused by compensatory mechanisms activated in SCP-2/SCP-X knockout mice. These alternative mechanisms are normally able to transport cholesterol among intracellular organelles and might be up-regulated as a consequence of the SCP-2 deficiency in the gene-manipulated mouse model. We have previously reported that the transfer of newly synthesized cholesterol from the endoplasmic reticulum to the plasma membrane in normal human fibroblasts and from the liver into the bile in the rat was a rapid SCP-2–dependent transport process. However, when similar studies were performed in fibroblasts obtained from patients with Zellweger disease that lack peroxisomes and do not express SCP-2, cholesterol trafficking to the plasma membrane was delayed, but not completely abolished. In fact, it was switched to a vesicle-mediated intracellular cholesterol transport pathway that was sufficient to maintain normal cell viability. In summary, these studies support the concept that hepatic SCP-2 lipid–mediated trafficking represents a major physiologic regulatory mechanism for hepatic cholesterol metabolism, lipoprotein metabolism, biliary cholesterol secretion, and enterohepatic circulation of cholesterol and bile salts. The more striking new contribution of these series of experiments is the finding that hepatic SCP-2 overexpression determines a cascade of sterol-regulatory mechanisms, which led to increased
hepatic cholesterol concentration, a decrease in the rate of tissue cholesterologenesis, and enhanced cholesterol absorption. It is possible that SCP-2 expression levels in the liver play an important role in pathophysiologic processes related to abnormal hepatic cholesterol metabolism such as atherosclerosis and cholesterol gallstone disease. Further studies would be required to elucidate the complex interaction among different hepatocellular sterol carrier proteins, the enterohepatic circulation of sterol molecules, and whole body cholesterol homeostasis.

References

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Received May 5, 2000. Accepted July 26, 2000.

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Supported by a donation of Elliot Marcus, Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, grants 1971092 and 1970056). The authors thank Dr. J. Chianale and V. Vollrath for providing the apoE cDNA probe; Drs. Helen Hobbs, Joachim Herz, and Monty Krieger for providing antibodies; Dr. Roger Davis for supplying cDNA probes; and Dr. Stephen Turley for measuring bile acid pool composition.