Altered hepatic cholesterol metabolism compensates for disruption of phosphatidylcholine transfer protein in mice

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Wu, Michele K., and David E. Cohen. Altered hepatic cholesterol metabolism compensates for disruption of phosphatidylcholine transfer protein (PC-TP) in mice. Am J Physiol Gastrointest Liver Physiol 289: G456–G461, 2005. First published April 21, 2005; doi:10.1152/ajpgi.00107.2005.—Phosphatidylcholine transfer protein (PC-TP) is a member of the steroidogenic acute regulatory transfer protein-related domain superfamily and is enriched in liver. To explore a role for PC-TP in hepatic cholesterol metabolism, Pctp−/− and wild-type C57BL/6J mice were fed a standard Chow diet or a high-fat, high-cholesterol lithogenic diet. In Chow-fed Pctp−/− mice, acyl CoA:cholesterol acyltransferase (Acat) activity was markedly increased, 3-hydroxy-3-methylglutaryl-CoA reductase activity was unchanged, and cholesterol 7α-hydroxylase activity was reduced. Consistent with increased Acat activity, esterified cholesterol concentrations in livers of Pctp−/− mice were increased, whereas unesterified cholesterol concentrations were reduced. Hepatic phospholipid concentrations were also decreased in the absence of PC-TP and consequently, unesterified cholesterol-to-phospholipid ratios in liver remained unchanged. The lithogenic diet downregulated 3-hydroxy-3-methylglutaryl-CoA reductase in wild-type and Pctp−/− mice, whereas Acat was increased only in wild-type mice. In response to the lithogenic diet, a greater reduction in cholesterol 7α-hydroxylase activity in Pctp−/− mice could be attributed to increased size and hydrophobicity of the bile salt pool. Despite higher hepatic phospholipid concentrations, the unesterified cholesterol-to-phospholipid ratio increased. The lack of Acat upregulation suggests that, in the setting of the dietary challenge, the capacity for esterification to defend against hepatic accumulation of unesterified cholesterol was exceeded in the absence of PC-TP expression. We speculate that regulation of cholesterol homeostasis is a physiological function of PC-TP in liver, which can be overcome with a cholesterol-rich lithogenic diet.

Hepatic cholesterol homeostasis is achieved by a balance of uptake, biosynthesis, storage, catabolism, and export (9). Most cholesterol secreted from the liver enters bile in the form of biliary vesicles (9) or plasma as nascent pre-β-HDL and VLDL particles (3, 27, 37). Hepatocellular secretion of phosphatidylcholines is critical to the formation of each of these particles (9, 27). In the liver, coordinate regulation of cholesterol synthesis and catabolism occurs due to feedback and feedforward mechanisms, principally involving nuclear hormone receptors (7, 8) and sterol regulatory element-binding proteins (11).

Phosphatidylcholine transfer protein (PC-TP) is a member of a recently described superfamily of proteins that contains steroidogenic acute regulatory transfer protein-related domains (21, 23, 25, 28). It is robustly expressed in liver and binds with absolute specificity to phosphatidylcholines (33). Although the absence of PC-TP expression in mice does not disrupt the secretion of biliary phosphatidylcholines or cholesterol in chow-fed mice (29), we recently showed that the response of biliary lipid secretion to a dietary cholesterol challenge is impaired in Pctp−/− mice (35). In addition, experiments in cultured Chinese hamster ovary cells (1) and in mouse peritoneal macrophages (2) suggest a role for PC-TP in apolipoprotein A-I-mediated phosphatidylcholine efflux during pre-β-HDL formation.

Considering its putative roles in the response of biliary lipid secretion to dietary cholesterol and in HDL metabolism, we utilized Pctp−/− and wild-type control mice to examine the influence of PC-TP expression on hepatic cholesterol homeostasis. This study shows that the absence of PC-TP in C57BL/6J mice resulted in compensatory changes in the activities of key enzymes of hepatic cholesterol homeostasis, which apparently functioned to prevent the accumulation of excess unesterified cholesterol within the liver.

MATERIALS AND METHODS

Materials

[4-14C]cholesterol (50 mCi/mmol) and oleoyl-[1,14C]CoA (55 mCi/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). 3α-Hydroxy-[3,4-14C]methylglutaryl-CoA (57 mCi/mmol) and DL-[3H]mevalonolactone (58 Ci/mmol) were obtained from Perkin Elmer Life Sciences (Torrance, CA). Cholesteryl-[1α,2α,3H]oleate (24 Ci/mmol) was from Amersham Biosciences (Piscataway, NJ). Aluminum and glass silica gel plates were purchased from EM Science (Gibbstown, NJ). β-Nicotinamide-adenine dinucleotide phosphate and α-β-glucose-6-phosphate were obtained from Calbiochem (La Jolla, CA). All other chemicals and general reagents were purchased from Sigma (St. Louis, MO).

Animals

Male 6-wk-old to 8-wk-old Pctp−/− and wild-type littermate control mice on a C57BL/6J genetic background (35) were fed either a Chow diet containing 4% fat and <0.02% cholesterol (LabDiet 5001, PMI Nutrition International, Brentwood, MO) or a lithogenic diet containing 15% fat, 1.25% cholesterol, and 0.5% sodium cholate (Harlan Teklad, Madison, WI) for 7 days. At 6:00 AM on the day of an experiment, mice were fasted 3 h and then anesthetized with intraperitoneal injections of 87 mg/kg body wt ketamine (Fort Dodge Animal Health, Fort Dodge, IA) and 13 mg/kg body wt xylazine (Lloyd Laboratories, Shenandoah, IA). Livers were excised, rinsed with 0.15 M NaCl to remove blood, weighed, snap frozen in liquid nitrogen, and stored at −80°C. In separate experiments, the liver,

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gallbladder, and small intestine were harvested as a single block of tissue and stored at −80°C. These procedures were conducted with the approval of the Institutional Animal Care and Use Committee.

**Analytical Techniques**

*Hepatic lipid concentrations.* Hepatic lipid concentrations were measured after organic extraction. Concentrations of total and unesterified cholesterol were measured enzymatically, and esterified cholesterol was calculated as the difference between total and unesterified cholesterol concentrations (30). We determined hepatic phospholipid concentrations using an inorganic phosphorus procedure (30).

*Hepatic enzyme activities.* Microsomal membranes were purified from 0.5 g of liver by differential ultracentrifugation (24). Hepatic activities of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase (EC 1.1.1.34), acyl-CoA:cholesterol acyltransferase (Acat) (EC 2.3.1.26), and cholesterol 7α-hydroxylase (Cyp7A1) (EC 1.14.13.17) were determined by thin-layer chromatography as previously described (12).

*Bile salt pool size and composition.* Liver, gallbladder, and intestines were homogenized, and lipids were extracted in 50 ml of ethanol at 60°C overnight together with glycocholate added as an internal standard (12). Ethanolic extracts (10 ml) were then dried under nitrogen and resuspended in 1 ml of methanol. Bile salt concentrations and compositions were determined by HPLC (12). Bile salt pool size was calculated and expressed as micromoles of bile salts per 20 g body wt. The content of individual bile salt molecular species in the bile salt pool was calculated for each mouse as the product of mole fraction of the bile salt times the bile salt pool size. The hydrophobic index of bile salts, which represents a concentration-weighted average hydrophobicity of a mixture of bile salts, was calculated according to Heuman (10).

**Statistical Analysis**

Data are expressed as means ± SE. Student’s t-test assuming equal variance was employed to determine statistical significance between experimental groups. Differences were considered significant for two-tailed P < 0.05.

**RESULTS**

Figure 1 shows the hepatic concentrations of total as well as unesterified and esterified cholesterol in Pctp−/− and wild-type littermate control mice. In chow-fed Pctp−/− and wild-type mice (Fig. 1A), hepatic total cholesterol concentrations were similar. However, concentrations of unesterified cholesterol were lower and esterified cholesterol concentrations were similar. However, concentrations of unesterified cholesterol were lower and esterified cholesterol concentrations were similar. After mice were fed the lithogenic diet, the pool size of cholesterol decreased in both genotypes; however, in Pctp−/− mice, they were 1.6-fold lower than in littermate controls.

Hepatic homeostasis in the liver depends on a substantial degree on the activities of HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis (11), Acat, which catalyzes the formation of cholesteryl esters (5), and Cyp7A1, the rate-limiting enzyme in the classical pathway for catabolism of cholesterol to bile salts (8). As shown in Fig. 2A, the hepatic activity of HMG-CoA reductase was not affected by the absence of PC-TP. Dietary cholesterol downregulated HMG-CoA reductase in both genotypes, but there was no effect on PC-TP expression. In contrast, Acat activity in Pctp−/− mice was 8.4-fold higher than in wild-type controls when fed chow (Fig. 2B). Whereas the lithogenic cholesterol increased Acat activity in wild-type mice, no changes were observed in Pctp−/− mice. Cyp7A1 activity in livers of chow-fed Pctp−/− mice was 1.8-fold lower than in wild-type mice. When fed the lithogenic diet, activities decreased in both genotypes; however, in Pctp−/− mice, they were 1.6-fold lower than in littermate controls.

Bile salt pool size and composition are key regulators of Cyp7A1 activity. Figure 3A demonstrates that bile salt pool sizes in chow-fed C57BL/6J Pctp−/− and wild-type mice were similar. After mice were fed the lithogenic diet, the pool size increased 1.8-fold in Pctp−/− mice but did not change in wild-type mice. Figure 3B displays hydrophobic indexes of the bile salt pools. In chow-fed mice, the hydrophobic index in Pctp−/− mice was only slightly higher than in wild-type mice. However, after the dietary cholesterol challenge, the hydrophobicity of the bile salt pool increased to a greater degree in Pctp−/− mice.

![Fig. 1. Influence of phosphatidylcholine transfer protein (PC-TP) on hepatic cholesterol concentrations: Hepatic cholesterol (total, unesterified, and esterified) concentrations ([cholesterol]) were determined for wild-type (solid bars) and Pctp−/− (open bars) C57BL/6J mice fed either a chow (A; wild type: n = 10; Pctp−/−: n = 11) or a lithogenic (B; wild type: n = 8; Pctp−/−: n = 10) diet for 7 days. Note that the scale of the vertical axis in B differs from A by 10-fold. Error bars represent SE. *P < 0.05, wild-type vs. Pctp−/− mice. †P < 0.05, chow-fed vs. lithogenic diet-fed mice.](http://ajpgi.physiology.org/)

**Fig. 1. Influence of phosphatidylcholine transfer protein (PC-TP) on hepatic cholesterol concentrations: Hepatic cholesterol (total, unesterified, and esterified) concentrations ([cholesterol]) were determined for wild-type (solid bars) and Pctp−/− (open bars) C57BL/6J mice fed either a chow (A; wild type: n = 10; Pctp−/−: n = 11) or a lithogenic (B; wild type: n = 8; Pctp−/−: n = 10) diet for 7 days. Note that the scale of the vertical axis in B differs from A by 10-fold. Error bars represent SE. *P < 0.05, wild-type vs. Pctp−/− mice. †P < 0.05, chow-fed vs. lithogenic diet-fed mice.**
The compositions of individual bile salt molecular species in bile salt pools of \( Pctp^{-/-} \) and wild-type mice are presented in Fig. 4. Contents of tauromuricholates were lower in \( Pctp^{-/-} \) mice fed the chow diet (Fig. 4A). However, the bile salt pool of chow-fed \( Pctp^{-/-} \) and wild-type mice contained similar amounts of tauroursodeoxycholate, taurocholate, taurochenodeoxycholate, and taurodeoxycholate. When mice were fed the lithogenic diet, tauromuricholate contents decreased to similar levels, tauroursodeoxycholate remained unchanged, and taurocholate increased to similar levels (Fig. 4B). Amounts of taurochenodeoxycholate increased in both genotypes but to greater extents in \( Pctp^{-/-} \) mice. Whereas levels of taurodeoxycholate did not change in the wild-type mice, they increased substantially in \( Pctp^{-/-} \) mice.

Unesterified cholesterol in cells resides only in phospholipid bilayers, and most (~90%) is contained in plasma membranes (14, 15). Figure 5 shows the influence of PC-TP on hepatic phospholipid concentrations. In chow-fed \( Pctp^{-/-} \) C57BL/6J mice, hepatic phospholipid concentrations were reduced by 1.4-fold compared with wild-type littermate control mice. Whereas phospholipid concentrations in wild-type mice did not change in response to the dietary challenge, they were 1.6-fold higher in \( Pctp^{-/-} \) mice (Fig. 5A). Figure 5B displays the unesterified cholesterol-to-phospholipid ratios in mouse livers. In \( Pctp^{-/-} \) mice fed chow, unesterified cholesterol-to-phospho-

![Fig. 2. Influence of PC-TP on activities of key regulating enzymes in livers of C57BL/6J mice. Activities of HMG-CoA reductase (A), ACAT (B), and Cyp7A1 (C) were determined for wild-type (solid bars) and \( Pctp^{-/-} \) (open bars) mice (\( n = 5 \) /group) fed either chow or lithogenic diet for 7 days. Error bars represent SE. * \( P < 0.05 \), wild-type vs. \( Pctp^{-/-} \) mice. † \( P < 0.05 \), chow-fed vs. lithogenic diet-fed mice.](http://ajpgi.physiology.org/)

![Fig. 3. Influence of PC-TP expression on the bile salt pool and hydrophobicity. Bile salt pool size (A) was determined, and bile salt hydrophobic index (B) was calculated for wild-type (solid bars) and \( Pctp^{-/-} \) (open bars) C57BL/6J mice (\( n = 5 \) /group) fed either chow or lithogenic diet for 7 days. Error bars represent SE. * \( P < 0.05 \), wild-type vs. \( Pctp^{-/-} \) mice. † \( P < 0.05 \), chow-fed vs. lithogenic diet-fed mice.](http://ajpgi.physiology.org/)

![Fig. 4. Influence of PC-TP on bile salt composition of the bile salt pool. Individual bile salt species were quantified for wild-type (solid bars) and \( Pctp^{-/-} \) (open bars) C57BL/6J mice (\( n = 5 \) /group) fed either chow (A) or a lithogenic diet (B) for 7 days. TMC, tauromuricholate; TUDC, tauroursodeoxycholate; TC, taurocholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate. Error bars represent SE. * \( P < 0.05 \), wild-type vs. \( Pctp^{-/-} \) mice. † \( P < 0.05 \), chow-fed vs. lithogenic diet-fed mice.](http://ajpgi.physiology.org/)
DISCUSSION

This study was designed to assess the role of PC-TP in hepatic cholesterol homeostasis. The main findings were that the absence of PC-TP in chow-fed mice was associated with substantial compensatory changes in hepatic activities of key enzymes that control cholesterol homeostasis but no changes in unesterified cholesterol-to-phospholipid ratio in the liver. When mice were fed the lithogenic diet, the absence of PC-TP was associated with a larger increase in unesterified cholesterol relative to phospholipid concentration within the liver.

We have recently demonstrated that, in chow-fed Pctp<sup>−/−</sup>-C57BL/6J mice, secretion rates of phospholipid into bile were increased 1.3-fold compared with wild-type mice in the absence of changes in biliary secretion of cholesterol or bile salts (35). Although a limitation of the present study is that we have not measured VLDL production, which represents a source of lipid export from liver (20), we have demonstrated that steady-state plasma phospholipid and cholesterol concentrations were not influenced by PC-TP expression in chow-fed mice (34). This suggests that the increase in biliary secretion accounted for the 1.4-fold reduction in hepatic phospholipid concentrations in Pctp<sup>−/−</sup> compared with wild-type mice. Although we cannot exclude that the absence of PC-TP expression was associated with reduced phospholipid synthesis by the liver, recent studies in our laboratory (1) showed that PC-TP expression does not influence rates of cellular phosphatidylcholine synthesis in tissue culture.

Increased membrane concentrations of unesterified cholesterol within cells may be associated with cytotoxicity (26). In chow-fed Pctp<sup>−/−</sup> mice, decreased hepatic phospholipid concentrations were accompanied by decreased concentrations of unesterified cholesterol and increased esterified cholesterol, without changes in total cholesterol concentrations. As a result, the unesterified cholesterol-to-phospholipid ratio was unchanged within the liver. This shift in the cholesterol distribution could be attributed to marked upregulation in the rate of cholesterol esterification in Pctp<sup>−/−</sup> compared with wild-type mice, which was associated with a decrease in the catabolic rate of cholesterol (activity of Cyp7A1) but no change in the rate of synthesis (HMG-CoA reductase activity).

Acat activity is regulated largely by cholesterol availability (14, 16) rather than cholesterol-mediated transcriptional up-regulation of Acat1 or Acat2 (6). Accordingly, Northern blot analysis did not reveal upregulation of Acat mRNA transcripts in Pctp<sup>−/−</sup> mice (data not shown). Apparently due to the compensatory increases in Acat activity that occurred in the absence of PC-TP expression, we did not observe differences in unesterified cholesterol-to-phospholipid ratios in liver or in microsomes. However, it is noteworthy that important differences in the metabolically active pools of cholesterol that regulate Acat activity are not necessarily reflected by microsomal cholesterol contents. Large and colleagues (14) have shown that 87% of unesterified cholesterol resides in the plasma membrane in rat hepatoma cells and that Acat activity, when measured in intact cells and in vitro, is controlled by the cholesterol content of the plasma membrane. It is also possible that measurements of microsomal cholesterol content did not accurately reflect the microenvironment of Acat. This is because the microsomal fraction comprises appreciable propor-

![Figure 5](http://ajpgi.physiology.org/)
tions of other membrane fractions in addition to regions of the endoplasmic reticulum that contain the Acat activity (15).

In addition to being regulated by cholesterol, Acat activity is also controlled by phospholipids. Activity in vitro can be modulated by enrichment of microsomal membranes with different phospholipid classes (18). In addition, it is responsive to phosphatidylcholine fatty acyl composition, as evidenced by marked variability in activity when purified PC-TP is utilized to supplement microsomes with specific phosphatidylcholine molecular species (17). Interestingly, these changes occur in the absence of measurable changes in membrane fluidity (17), suggesting that direct interactions between Acat and neighboring phospholipids within a membrane are critical to the control of enzymatic activity. By reducing the transfer rate of cholesterol out of the plasma membrane, phospholipid added to cultured fibroblasts also reduces the flow of cholesterol to the endoplasmic reticulum (16). Therefore, by several distinct mechanisms, changes in the membrane microenvironment secondary to the absence of PC-TP may have led to Acat upregulation in livers of chow-fed Pctp−/− mice.

In the setting of a relative excess of hepatocellular cholesterol, a compensatory decrease in activity of HMG-CoA reductase and an increase in Cyp7A1 activity might have been expected due to feedback (11) and feedforward mechanisms (8, 38). Because Acat utilizes unesterified cholesterol derived from the same regulatory pool as HMG-CoA reductase (4), the upregulation of Acat activity most likely explained the absence of a decline in the activity of HMG-CoA reductase in Pctp−/− compared with wild-type mice. Acat-mediated consumption of unesterified cholesterol would be expected to reduce activation of the liver X receptor (22) and may account for the observed decrease of Cyp7A1 activity. Whereas Cyp7A1 is downregulated by increases in size and hydrophobicity of the bile salt pool via activation of the farnesoid X receptor (FXR) (8), bile salt pool size was not increased in chow-fed Pctp−/− mice and there was only a modestly reduced content of the hydrophilic bile salt tauromuricholate, which did not influence the overall hydrophobicity of the bile salt pool.

After the dietary challenge with the lithogenic diet, there was greater hepatic accumulation of phospholipid and cholesterol in Pctp−/− compared with wild-type mice. Considering that plasma cholesterol and phospholipid concentrations were each increased in the absence of PC-TP expression (34), hepatic lipid accumulation is most likely explained by the impairment in biliary secretion of both lipids in Pctp−/− mice fed the lithogenic diet (35). The lithogenic diet led to a similar decrease of HMG-CoA reductase in both Pctp−/− and wild-type mice, indicating that mechanisms for downregulation of hepatic cholesterol biosynthesis remained intact in the absence of PC-TP. Presumably due to the cholate content of the lithogenic diet (13), there was downregulation of Cyp7A1 in both wild-type and Pctp−/− mice due to FXR activation by the larger, more hydrophobic bile salt pool (8). The lower Cyp7A1 activity in Pctp−/− mice after they were fed the lithogenic diet could be explained by greater increases in both the size and hydrophobicity of the bile salt pool. Although we did not perform studies specifically directed toward localizing the major fraction of bile salts, gross and histological examination of livers (data not shown) did not reveal evidence of hepatocellular damage, such as would be expected if a substantial proportion of bile salts were retained in the liver. Considering that others have shown that gallbladder volume is increased in response to the lithogenic diet (32), it is most likely that the increase in bile salt mass in fasted Pctp−/− mice was accommodated by the gallbladder and that increased fluxes of more hydrophobic bile salts across the liver accounted for FXR activation (36).

The lithogenic diet upregulated Acat activity in wild-type controls but did not increase activity further in Pctp−/− mice. This presumably contributed to the greater rise in the unesterified cholesterol-to-phospholipid ratio that was observed in livers of Pctp−/− compared with wild-type mice. Apparently, Acat was maximally upregulated in chow-fed Pctp−/− mice; therefore, the limited capacity for esterification contributed to excess hepatic accumulation of unesterified cholesterol when Pctp−/− mice were challenged with the lithogenic diet.

To gain insight into whether expression level of PC-TP influenced the observed phenotype, we utilized FVB/NJ mice, which express PC-TP in liver at 20-fold lower levels than C57BL/6J mice (35). We chose this approach rather than the use of Pctp+/− C57BL/6J mice because, in a number of experiments, the magnitude of the differences observed between Pctp−/− and wild-type mice would not have permitted us to distinguish an intermediate phenotype associated with expression of half the complement of hepatic PC-TP. In contrast to PC-TP-deficient C57BL/6J mice, when fed chow or the lithogenic diet, Pctp−/− mice bred onto the FVB/NJ genetic background did not display appreciable changes in hepatic cholesterol metabolism. These findings strongly suggest that the absence of this phenotype was attributable to the much lower level of PC-TP in livers of FVB/NJ mice. Appreciating that strain-related differences in hepatobiliary lipid metabolism are well described in mice (13, 19, 31), we cannot exclude the possibility that genetic background per se contributed to the lack of influence of PC-TP expression on cholesterol homeostasis in livers of FVB/NJ mice. Therefore, definitive proof would require the use of Pctp−/− C57BL/6J mice to create a transgenic line of mice in which PC-TP is expressed at low levels in liver.

Together, these experiments support a role for PC-TP in the regulation of hepatic cholesterol homeostasis that depends on the expression level in the liver. Upregulation of Acat activity in the absence of coordinate regulation of HMG-CoA reductase and Cyp7A1 suggests that PC-TP expression may influence a key cellular pool of cholesterol. This idea is in keeping with our recent report that PC-TP in cultured mouse peritoneal macrophages defends against apoptosis induced by unesterified cholesterol via a mechanism that appears to involve the membrane properties of the endoplasmic reticulum (2). Detailed studies of the influence of PC-TP expression on compositions and physical-chemical characteristics of subcellular membrane fractions may help to elaborate mechanisms by which this specific phosphatidylcholine binding protein participates in the regulation of hepatic cholesterol homeostasis.

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