Targeted Deletion of Hepatic CTP:phosphocholine Cytidyltransferase α in Mice Decreases Plasma High Density and Very Low Density Lipoproteins*

Received for publication, April 12, 2004, and in revised form, August 23, 2004
Published, JBC Papers in Press, August 25, 2004, DOI 10.1074/jbc.M404027200

René L. Jacobs‡§, Cecilia Devlin%, Ira Tabas¶, and Dennis E. Vance‡‡

From the ‡Canadian Institutes of Health Research Group on the Molecular and Cell Biology of Lipids and Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada and the ¶Departments of Medicine, Anatomy and Cell Biology, and Physiology and Cellular Biophysics, Columbia University, New York, New York 10032

CTP:phosphocholine cytidyltransferase (CT) is the key regulatory enzyme in the CDP-choline pathway for the biosynthesis of phosphatidylcholine. Hepatic cells express both an α and a β2 isoform of CT and can also synthesize phosphatidylcholine via the sequential methylation of phosphatidylethanolamine catalyzed by phosphatidylethanolamine N-methyltransferase. To ascertain the functional importance of CTα, we created a mouse in which the hepatic CTα gene was specifically inactivated by the Cre/loxP procedure. In CTα knockout mice, hepatic CT activity (due to residual CTβ2 activity as well as activity in nonhepatic cells) was 15% of normal, whereas phosphatidylethanolamine N-methyltransferase activity was elevated 2-fold compared with controls. Lipid analyses of the liver indicated that female knockout mice had reduced phosphatidylcholine levels and accumulated triacylglycerols. The plasma phosphatidylcholine concentration was reduced in the CTα knockout (independent of gender), as were levels of very low density lipoproteins (cholesterol and apoA1) and very low density lipoproteins (triacylglycerols and apoB100). Experiments in which mice were injected with Triton WR1339 indicated that apoB secretion was decreased in hepatic-specific CTα knockout mice compared with controls. These results suggest an important role for hepatic CTα in regulating both hepatic and systemic lipid and lipoprotein metabolism.

Phosphatidylcholine (PC) is vital for the structural integrity of mammalian membranes and is the primary phospholipid in bile, lung surfactant, and plasma lipoproteins. In all nucleated mammalian cells, PC is synthesized from choline via the Kennedy (CDP-choline) pathway (1), and the flux through this pathway is regulated by the activity of CTP:phosphocholine cytidyltransferase (CT) (2–4). In the mouse, two genes encode CT, Pcyt1a and Pcyt1b (5). Hepatic cells have both a CTα isoform encoded by Pcyt1a and a CTβ2 isoform encoded by Pcyt1b. CTα is believed to be the predominant isoform in the liver (5). Liver cells are unique in that they can also synthesize PC via the sequential methylation of phosphatidylethanolamine catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) (6). The PEMT pathway accounts for ~30% of hepatic PC biosynthesis, whereas the enzymes of the Kennedy pathway produce the remaining 70% (7–9).

In recent years, several studies have described specific roles for the PEMT and CDP-choline pathways in PC biosynthesis and lipoprotein metabolism. Experiments on Pemt−/− mice suggested that PEMT has been retained during evolution to provide PC when dietary choline is deficient, such as during starvation (10–12). Indeed, when PEMT knockout mice were fed a choline-deficient diet, they developed severe liver pathology and died within 4 days (11), thus highlighting the requirement for PEMT activity when biosynthesis of PC through the CDP-choline pathway is insufficient. The PEMT-deficient mice appeared normal on a standard chow diet, suggesting that the CDP-choline pathway can compensate for the loss of PEMT activity when choline is available. A specific role for PEMT in VLDL secretion has been demonstrated in vivo and in experiments with hepatocytes (13, 14). In male Pemt−/− mice fed a high fat/high cholesterol diet, plasma TG and PC levels were reduced by 50 and 20%, respectively, compared with those in Pemt+/+ mice. In contrast, the plasma content of these lipids was the same in female Pemt−/− and Pemt+/− mice fed a high fat/high cholesterol diet (14). In hepatocytes from male Pemt−/−, compared with Pemt+/+ mice, apoB100 secretion was reduced by 60%, whereas TG secretion was reduced by 75% (13). This apparent reduction in VLDL secretion may explain why Pemt−/− mice fed the high fat/high cholesterol diet have a 5-fold higher level of hepatic TG than their Pemt+/− counterparts (13). The above experiments demonstrate that PEMT can be limiting in VLDL secretion and highlight a possible gender difference in the relationship between PC metabolism and lipoprotein homeostasis.

Studies investigating the importance of the CDP-choline pathway in lipoprotein metabolism have primarily consisted of feeding choline-deficient (CD) diets to animals (15–17), or applying CD medium to hepatocytes or other cells in culture (18–20). It has been well established that hepatic and plasma PC concentrations are reduced in rats fed a CD diet. This reduction in dietary choline results in decreased plasma VLDL-TG and VLDL-apoB levels and TG accumulation in the
liver (16). The presumption in these experiments was that the effects seen on lipid and lipoprotein metabolism were due to inhibition of PC biosynthesis via the CDP-choline pathway. Studies in hepatocytes initially seemed to agree with this conclusion, since VLDL secretion was impaired from rat hepatocytes incubated in medium deficient in methionine and choline and TG accumulated 6-fold in the cells (18). However, attributing these changes to impaired flux through the CDP-choline pathway was dubious for several reasons. First, supplementation of the medium with either choline or methionine returned cellular TG levels to normal and restored normal TG and apoB secretion (18). Second, the rate of PC synthesis from CDP-choline was not directly measured. However, these observations suggest that active PC biosynthesis from either PEMT or CDP-choline is required for normal VLDL secretion. Furthermore, Kulinski et al. (22) have shown that the rate of flux through the CDP-choline pathway is higher, not lower, than in choline-supplemented hepatocytes following incubation with [3H]choline. Moreover, Kuliniski et al. (22) have shown that the rate of flux through the CDP-choline pathway is higher, not lower, in CD than in choline-supplemented murine hepatocytes and that VLDL secretion from murine hepatocytes is not impaired by choline deficiency. In light of these experiments, choline deficiency appears not to be an appropriate model in which to investigate the specific role of the CDP-choline pathway in PC biosynthesis or in lipid and lipoprotein metabolism.

To gain further insight for the role of the CDP-choline pathway in hepatic lipoprotein metabolism, we have generated mice in which the CTα gene was selectively disrupted in the liver by the Cre-Lox system (23, 24). Our hypothesis predicted that CTα is vital for normal secretory of lipoproteins from liver. Consequently, we predicted that mice deficient in hepatic CTα would have decreased circulating lipoproteins and fat accumulation in the liver. The results describe an important role for the hepatic CDP-choline pathway, and particularly CTα, in regulating both hepatic and systemic lipid and lipoprotein metabolism.

**EXPERIMENTAL PROCEDURES**

**Materials—**Anti-human CTβ2 and anti-human CTα rabbit polyclonal antibodies were generous gifts from Dr. S. Jackowski (St. Jude Children’s Research Hospital, Memphis, TN). The sheep anti-human apolipoprotein B antibody was purchased from Roche Applied Science, and the rabbit anti-human apoA1 and goat anti-human apoE antibodies were gifts from Biodesigns (Kennebunk, ME). Both the donkey anti-rabbit and the goat anti-rabbit secondary antibodies conjugated to horseradish peroxidase were purchased from Pierce. The polyclonal antibody directed against the C-terminal dodecapeptide of rat PEMT2 was raised in rabbits (25). S-[methyl-3H]adenosylmethionine, [methyl-3H]choline, and [3S]Ptrimix (methionine/cysteine) were purchased from Amersham Biosciences. Triton WR1339 was purchased from Sigma. All other chemicals and reagents were from standard commercial sources.

**Generation and Identification of CTαmice and CTDαmice/Albumin-Cre Mice—**Homozygous CTDαmice mice, generated previously (23), were identified by PCR of tail DNA (isolated by the DNeasy Tissue Kit; Qiagen) using the following primers: CTS285 (5′-CTTTGCTTGGATCA-3′) and CTT3UL (5′-GAAGATGGCATTGGAACCTT-3′). A homozygous CTDαmice mouse was crossed with a mouse expressing the Cre recombinase gene driven by the hepatic-specific albumin promoter (albumin-Cre) kindly provided by Dr. Mark A. Magnuson (Vanderbilt University). The resulting pups, which were heterogeneous for both CTDαmice and albumin-Cre, were then mated with homozygous CTDαmice mice. Heterozygous mice for CTDαmice and heterozygous for albuminCre (i.e. mice having CTDα-deficient livers) were identified (Fig. 1A) by PCR screening of tail DNA from the pups.

**Mice—**All procedures were approved by University of Alberta’s Animal Welfare Committee and were in accordance with guidelines of the Canadian Council on Animal Care. Mice, housed 4–5 per cage, were exposed to a 12 h light/dark cycle beginning with light at 8:00 a.m. Adult male and female mice, 12–24 weeks old, were fed ad libitum a Chow diet from LabDiet (PICO laboratory Rodent Diet 20) and had free access to water. For all experiments, mice were fasted overnight (16 h) prior to sacrifice.

**Enzymatic Assays—**Tissues were homogenized in a glass/Teflon homogenizer in 2 ml of buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride) followed by sonication for 30 s. Protein concentration was determined using the Coomassie Plus protein protocol (Bio-Rad), with bovine serum albumin as a standard. Total CT activity was measured in homogenates (50 μg of protein) from livers and hearts by monitoring the conversion of [3H]phosphocholine into CDP-choline (26). For measurement of PEMT activity, 45 μg of protein of liver homogenates were incubated with phosphatidylmonomethylethanolamine and S-adenosyl[methyl-3H]methionine, and the incorporation of radiolabel into PC was measured as described previously (27).

**Immunoblotting of Hepatic CTα, PEMT, and CTβ2—**Liver homogenates (20–50 μg of protein) were boiled in buffer containing 1% SDS, and proteins were separated on a 10% SDS-polyacrylamide gel. Proteins were transferred to nylon membranes and probed with anti-CTα (dilution 1:1500), anti-PMT2 (1:5000), or anti-CTβ2 (1:1000) antibody. Immunoreactive bands were visualized by enhanced chemiluminescence according to the manufacturer’s instructions (Amersham Biosciences). Quantification of the immunoreactive bands was performed using Image Gauge version 3.0 software by Fujifilm.

**Analysis of ApoA1, ApoE, and ApoB48/l.100 Levels in Plasma—**Blood was collected from each mouse via cardiac puncture in the presence of trace amounts of 250 mM EDTA, and plasma was isolated by centrifugation. Plasma (2–4 μl) was resolved on a 10% (for apoA1 and apoE) or 5% (for apoB48/100) SDS-polyacrylamide gel. Proteins were transferred to nylon membranes and probed with anti-apoA1 (dilution 1:10,000), anti-apoE (dilution 1:2500), or anti-apoB (dilution 1:5000) antibody.

**Determination of the Mass of Cholesterol, Cholesteryl Ester, and TG in Liver and Plasma—**The amount of cholesterol, cholesteryl ester, and TG was determined in liver homogenates (0.5 mg of protein) or 50 μl of plasma. After digestion (2 h, 30 °C) of the phospholipids with phospholipase A2 and heparin necerin (20 μg of each) in reaction medium, and lipids were extracted. The mass of TG, cholesterol, and cholesteryl ester was determined by gas-liquid chromatography (28).

Plasma from individual animals was separated into lipoprotein fractions using high performance liquid chromatography with an American Biosciences Supersor 6 column attached to a Beckman Systems Gold or Nouveau Gold apparatus. In-line assays for total cholesterol (Sigma Diagnostics, Infinity cholesterol reagent) and TG (Sigma Triglyceride GPO Trinder kit) were performed as previously described (14).

**Determination of PE and PC Mass in Livers and Plasma—**Phosphatidylcholine was isolated by partition of the phospholipids with phospholipase A2 and heparin necerin (20 μg of each) in reaction medium, and phospholipids were separated and quantified by the HPLC method of Bergo et al. with minor modifications (30).

**Determination of the Mass of Cholesterol, PE, and Bile Acids in Plasma—**Plasma was collected directly from the gallbladders of anesthetized mice. Plasma was separated and quantified by the HPLC method of Torchia et al. (31) using 3α,12α-dihydroxy-23-nor-5β-cholanic acid as an internal standard. Assay kits were used to determine the mass of biliary cholesterol and PC (Sigma). Analysis of ApoB Secretion in Vivo—Mice were fasted overnight and injected with 100 μl of phosphate-buffered saline containing 5% Triton WR1339 (v/v) and 250 μCi of [3S]Ptrimix as previously described (14). After various times, the animals were sacrificed, blood was collected, and plasma was isolated. To 100 μl of plasma were added 450 μl of preincubation buffer (100 μM of buffer containing Triton X-100, 100 mM phosphate, pH 7.4), NaCl (0.75 mM), EDTA (25 mM), phenylmethylsulfonyl fluoride (5 mM) and Triton X-100 (5%, v/v), and 7.5 μl of anti-human apoB antibody. The mixture was incubated at 8 °C with gentle shaking. Next, 45 μg of protein A-Sepharose was added, and the sample was mixed and equilibrated prior to centrifugation for the sample for 2 min at 14,000 rpm in a microcentrifuge. The pellet was washed with 100 μl of sample buffer. Following centrifugation, samples were electrophoresed on 5% SDS-polyacrylamide gels, after which the gels were soaked in Amplify solution, dried, and exposed to film. Quantification of the bands was performed using Image Gauge version 3.0 software by Fujifilm.

**Histological Studies—**Livers were quickly extirpated and fixed in 10% formalin, and sections were stained by a standard protocol with hematoxylin and eosin. Some formalin-fixed samples were frozen in
liquid nitrogen before sectioning and were subsequently stained with Oil Red O.

**Measurement of Plasma Aminotransferase Activities**—Aspartate aminotransferase and alanine aminotransferase activities in plasma were measured using the INFINITY AST and ALT kits from ThermaTrace.

**Statistical Analysis**—Data are presented as means ± S.E. unless otherwise noted. There were 3–10 samples in each experimental group. Student’s unpaired t test was performed to compare means. A p value of <0.05 was interpreted as a significant difference.

---

**RESULTS**

Identification of the Liver-specific CTα Knockout Mice—Breeding of a homozygous CTα<sub>flox</sub> mouse with a mouse homozygous for CTα<sub>flox</sub> and heterozygous for Cre generated two possible genotypes: 1) homozygous CTα<sub>flox</sub> mice, which were used as controls in this study, and 2) homozygous CTα<sub>flox</sub> + heterozygous Cre mice, which lack hepatic CTα expression. DNA from tail clips were used to identify the genotype of the mice (Fig. 1A). In this figure, mice 1 and 2 were homozygous for CTα<sub>flox</sub> (right panel); however, only mouse 2 expressed Cre (left panel). Therefore, mouse 1 was a “control,” whereas mouse 2 was a liver-specific CTα “knockout.” Mouse 3 was homozygous for CTα<sub>wt</sub> (right panel) and did not have the Cre gene (left panel).

To determine whether disruption of the CTα gene resulted in decreased CTα protein expression, immunoblot analysis was performed on hepatic homogenates from control (CTα<sub>wt</sub>) and knockout (CTα<sub>flox</sub> + albuminCre) mice. As demonstrated in Fig. 1B, the presence of Cre reduced hepatic CTα expression by ~95%. The remaining CTα protein can be attributed to the presence of endothelial, Kupffer, and blood cells. Indeed, others have observed similar results in liver when using the albumin-Cre/LoxP technology for other hepatocyte-specific genes (32).

**Histological Characterization of Livers and Assay of Aminotransferase Activities**—The weights of knockout mice (21.6 ± 2.4 g) were comparable with those of their littermate controls (21.5 ± 3.6 g). Moreover, there was no difference in the weight of the liver as a percentage of body weight between genotypes (controls versus knockouts: 5.6 ± 1.2 versus 5.3 ± 0.7% body weight). Livers taken from female knockout mice appeared outwardly normal; however, histological examination with hematoxylin/eosin staining revealed many large vacuoles (Fig. 2B). These vacuoles were present throughout the hepatic acinus and showed no particular pattern. Livers from male knockout mice were also indistinguishable in outward appearance; however, unlike the female mice, male knockouts had only mild vaculization (Fig. 2D). Oil Red-O staining of liver slices confirmed the presence of neutral lipids in the vacuoles (data not shown).

Regardless of the degree of vaculization, the CTα-deficient livers appeared to have fewer hepatocytes that were larger in size compared with control livers. We therefore counted the number of cells present on the slide at × 63 magnification. The livers taken from the knockout mice had 40% fewer hepatocytes per viewing area (control versus knockout: 105–130 versus 60–80 cells/viewing area). To confirm these results, we also measured the amount of DNA present/g of hepatic tissue. The CTα-deficient livers contained 30% less DNA/g of tissue.

---

**Fig. 1.** Cre-mediated disruption of the CTα<sub>flox</sub> gene in liver. A, PCR assay of tail DNA for Cre (left panel) and CTα (right panel) from homozygous CTα<sub>flox</sub> (lane 1), homozygous CTα<sub>flox</sub> + Cre (lane 2), and wild type (lane 3) mice. A reaction with no DNA template (negative control) was performed with all assays (lane 4). B, immunoblots of liver homogenates obtained from control and knockout mice; a CTα-specific antibody was used at 1:1500 dilution. C, homogenates from livers and hearts were assayed for CT activity in the presence of phosphatidylcholine/oleate vesicles. Values are expressed as means ± S.E. for 3–8 mice. The asterisks signify differences versus control, p < 0.05.

**Fig. 2.** Histology of livers from control and knockout mice. Mice (12–24 weeks old) were fasted overnight and sacrificed, and livers were removed. Sections of liver were fixed in 10% formalin, sliced, and stained with hematoxylin/eosin. The hematoxylin/eosin sections are shown at a magnification of ×40. Livers from at least two mice for each condition were stained, and pictures are representative of all livers examined. The arrows indicate selected vacuoles.
Role of Hepatic CTα in Lipoprotein Metabolism

Concentration of PC, PE, cholesterol, cholesteryl ester, and TG in livers of hepatic CTα knockout and control mice

Lipids were extracted from livers (0.5 mg of protein) and separated by high pressure liquid chromatography (for phospholipids) or by gas chromatography (for neutral lipids). The results are expressed as means ± S.E. from 6–10 mice.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PC</th>
<th>PE</th>
<th>Cholesterol</th>
<th>Cholesteryl ester</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/mg</td>
<td>µg/mg</td>
<td></td>
<td></td>
<td>µg/g</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>66.0 ± 9.0</td>
<td>39.4 ± 6.7</td>
<td>6.61 ± 2.79</td>
<td>13.3 ± 1.8</td>
<td>126.8 ± 24.2</td>
</tr>
<tr>
<td>Knockout</td>
<td>48.0 ± 13.0</td>
<td>36.3 ± 7.7</td>
<td>6.85 ± 1.90</td>
<td>14.6 ± 2.7</td>
<td>220.7 ± 44.5</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>63.0 ± 12.1</td>
<td>31.7 ± 11.6</td>
<td>6.33 ± 0.89</td>
<td>11.7 ± 3.6</td>
<td>175.3 ± 38.6</td>
</tr>
<tr>
<td>Knockout</td>
<td>58.2 ± 13.5</td>
<td>26.0 ± 4.45</td>
<td>6.51 ± 1.61</td>
<td>11.8 ± 2.2</td>
<td>230.9 ± 31.3</td>
</tr>
</tbody>
</table>

* Differences versus control, p < 0.05.

pared with controls (0.78 ± 0.08 versus 1.17 ± 0.13 mg/g liver, p < 0.05). These results provide convincing evidence for the presence of fewer but larger hepatocytes in the knockout livers.

Because of the obvious differences between the livers of the CTα knock-out and control mice (Fig. 2, B and D) plasma aspartate aminotransferase and alanine aminotransferase activities were measured to assess the possibility of liver damage. The plasma activity of these enzymes was unaltered by disruption of the CTα gene in the liver, suggesting that the observed changes in plasma and hepatic lipids were due to loss of hepatic CTα activity rather than liver damage.

The Lipid Content of the Liver Is Altered in CTα Knockout Mice—The Kennedy (CDP-choline) pathway has been shown to produce ~70% of PC in the liver (7, 9). It was, therefore, expected that deletion of CTα would alter hepatic lipid concentrations. Table I shows data for hepatic PC, PE, cholesterol, cholesteryl esters, and TG. PC in the liver was decreased by 20% in female CTα knockout mice relative to controls. This decrease was not observed in male mice. Furthermore, hepatic TG was increased by 40% in the female knockout mice relative to control mice. Regardless of gender, no difference was observed in the levels of hepatic PE, cholesterol, or cholesteryl ester between control and knockout mice.

Expression of Hepatic PEMT and CTβ2 Is Induced in the Liver-specific CTα Knockout Mice—To ascertain how the livers of the CTα knockout mice respond to decreased CTα, we investigated the other major pathway by which the liver can synthesize PC, the PEMT pathway. In the CTα-deficient livers, PEMT activity was increased irrespective of gender by 90% (Fig. 3A). Similarly, hepatic PEMT protein levels were 2-fold higher in the knockout mice than in control mice. Hepatic CTβ2 protein was also increased ~2-fold in the CTα KO mouse (Fig. 3B), consistent with previous data with CTα-deficient mice (23). These results suggest that normally CTβ2 represents only ~5% of total hepatic CT activity. This observation is consistent with another study that reports only minimal hepatic expression of CTβ2 (5).

Plasma Lipids Are Decreased in the Liver-specific CTα Knockout Mice—The plasma concentration of PC, the major phospholipid in mammalian plasma (33), was 60% lower in male knockout mice than in their control counterparts (Fig. 4). Moreover, the amount of plasma PC in female knockout mice was 50% less than in female controls. Interestingly, the large change in plasma PC was mirrored by changes in plasma cholesterol concentrations. In male knockout mice, total plasma cholesterol was 40% of control levels (Fig. 5), and in female knockout mice the plasma cholesterol level was half of that in control mice.

Whereas the extent of the changes in plasma PC and cholesterol was similar in male and female mice, modulation of plasma TG levels by hepatic CTα deficiency was different between genders. The plasma TG content in male mice was only modestly (20%, p < 0.05) reduced in knockout mice compared with controls (Fig. 5). However, the amount of plasma TG was strikingly lower (45%, p < 0.05) in female knockout mice than in littermate controls. These data are consistent with the larger accumulation of TG in the livers of female knockout mice than in their male counterparts and suggest a greater defect in lipoprotein secretion from the CTα-deficient livers of female, compared with male, mice.

Plasma was separated into lipoprotein fractions by HPLC. The content of TG (Fig. 6, A and C) in the VLDL fraction from both male and female knockout mice was lower than in control mice, in agreement with the quantitative data shown in Fig. 5. However, the decrease in plasma VLDL-TG in the male knockout mice compared with the control mice was larger than was predicted from the quantitative data of Fig. 5. The amount of cholesterol (Fig. 6, B and D) in the HDL fractions of both male and female knockout mice was lower than in their control littermates. The cholesterol content of the VLDL and low den-
Role of Hepatic CTα in Lipoprotein Metabolism

FIG. 4. Plasma PC is decreased in the liver-specific CTα knockout mice. PC was extracted from 50 μl of plasma, separated from other lipids by HPLC, and quantified using phosphatidylcholine as an internal standard. Values are means ± S.E. for 6–10 mice for each condition. The asterisks signify differences versus control, p < 0.05.

FIG. 5. Plasma TG and cholesterol are decreased in the liver-specific CTα knockout mice. The amounts of total cholesterol (unesterified + esterified cholesterol) and TG were measured in 50 μl of plasma. After digestion (2 h, 30 °C) of the phospholipids with phospholipase C, tridecanoin (20 ng) was added as an internal standard, and lipids were extracted. The amounts of TG and total cholesterol were determined by gas-liquid chromatography. Values are expressed as means ± S.E. for 6–10 mice for each condition. The asterisks signify differences versus control, p < 0.05.

sity lipoprotein fractions of both male (Fig. 6D) and female (Fig. 6B) knockout mice appeared to be lower than in control mice. However, the cholesterol level of these fractions was close to the limit of detection of these experiments.

Bile Composition Is Unchanged in the Liver-specific CTα Knockout Mice—The concentration of PC in the gall bladders of male and female knockout mice (21.4 ± 3.2 nmol/liter) was similar to that in control mice (18.8 ± 3.5 nmol/liter). Cholesterol secretion into bile is closely associated with PC secretion. The amount of biliary cholesterol was also the same in the knockout (3.5 ± 0.8 nmol/liter) versus control (4.0 ± 1.6 nmol/liter) mice. Total bile acid concentration (control versus knockout: 213 ± 21.1 versus 246 ± 22.3 μmol/liter) and the levels of taurocholic acid (control versus knockout: 97.2 ± 7.4 versus 113.1 ± 9.9 μmol/liter) and taurocholic acid (control versus knockout: 115.4 ± 16.1 versus 134 ± 14.2 μmol/liter), the two major biliary acids in mice, were not significantly affected by deletion of the hepatic CTα gene.

Plasma ApoB100 and ApoA1 Are Decreased in the Liver-specific CTα Knockout Mice—Since the plasma levels of HDL and VLDL were decreased in the knockout mice, we next determined whether or not the amounts of the corresponding plasma apolipoproteins were altered. To this end, plasma samples were immunoblotted for apoB48, apoB100, apoA1, and apoE. The plasma level of apoAI was ~60% lower in both male and female knockout mice than in their gender controls (Fig. 7). These results are consistent with the observation that the knockout mice contained lower levels of HDL-cholesterol than did the control mice (Fig. 6). Unlike apoAI, the amount of plasma apoE was unaltered by a deficiency of hepatic CTα irrespective of gender (Fig. 7). This result is not surprising, since apoE is only found in a small fraction (15%) of HDL particles (34). ApoB48 and apoB100 are prominently found in VLDL in mice. The level of plasma apoB100, but not apoB48, was markedly lower in both male and female knockout mice than in their control littermates (Fig. 7).

To determine whether or not apoB secretion was decreased in the knockout mice, we performed in vivo labeling of proteins in the mice in the presence of Triton WR1339, a detergent that prevents the catabolism of TG in plasma VLDL by inhibition of lipoprotein lipase (35). Male mice were fasted overnight and then injected with 100 μl of 15% Triton WR1339 and 250 μCi of [35S]Promix. The animals were sacrificed at various times up to 3.5 h, and apoB100 and apoB48 were immunoprecipitated from plasma. The results from a time course are indicated in Fig. 8A, showing maximal incorporation for apoB100 at 2 h. For apoB48, the labeling continued to rise until 3.5 h. In subsequent experiments, the samples were taken at 1 h after injection of Triton WR1339 and radioactivity. As a means of controlling for injection volume, the labeling of plasma apoB was compared with the labeling of plasma albumin. Fig. 8B shows that the total amount of radioactivity was approximately equal in the plasma samples. The amount of labeled apoB100 recovered from the plasma was significantly lower in the liver-specific CTα knockout mice than in control mice (Fig. 8, B and C). Although the plasma apoB48 concentration was apparently unaltered by disruption of the CTα gene (Fig. 7), the amount of labeled apoB48 recovered in the plasma was significantly lower in the knockout mice than in the control mice (Fig. 8, B and C).

DISCUSSION

Choline was classified as an essential nutrient by the American Food and Nutrition Board in 1998 (61). A major phenotype of choline deficiency is lipid accumulation in the liver (16), suggesting that choline deficiency inhibited PC synthesis and consequently impaired lipoprotein secretion from the liver. Early studies in hepatocytes showed that an active synthesis of PC is required for VLDL secretion (18). However, these studies were unable to ascertain a direct role for either the CDP-choline or PEMT pathway in this process. To address the role of PEMT, our laboratory generated mice in which the Pemt gene was disrupted. Hepatocytes isolated from Pemt−/− mice had a defect in lipoprotein (VLDL) secretion. PEMT-deficient hepatocytes secreted 70% less apoB100 and 50% less TG and PC associated with VLDL (13) than did wild-type controls. In vivo, Pemt−/− mice (fed a high fat/high cholesterol diet) also had lower than normal HDL (PC and cholesterol) levels. However, VLDL secretion, under the same dietary conditions, appeared to be dependent on gender. Male, but not female, Pemt−/− mice exhibited a defect in the secretion of VLDL (TG and apoB100) (14). The reason for the gender difference is unclear, but the combined data from the Pemt−/− mice provided a convincing argument for a vital role of PEMT in lipoprotein metabolism.

We now report the generation of a liver-specific CTα knockout mouse. Since CT catalyzes the rate-limiting step in the Kennedy pathway for PC synthesis (3, 36), this model allows us, for the first time, to investigate directly the impact of inhibiting the hepatic CDP-choline pathway on lipid and lipoprotein metabolism. The results of this study demonstrate that CTα is required for normal secretion of VLDL (apoB, PC, and TG) in mice fed a normal chow diet. In addition, plasma HDL (PC, cholesterol, and apoAI) was 50% lower in the knock-
out mice than in the control mice. These results suggest that hepatic PC supply from CTα is vital for homeostasis of both plasma VLDL and HDL.

How Does Hepatic PC Biosynthesis Regulate Plasma HDL?—Plasma levels of HDL-cholesterol are inversely correlated with the development of cardiovascular disease (37, 38). HDL is thought to inhibit atherosclerosis by stimulating transport of cholesterol from peripheral cells to the liver, where the cholesterol is converted to bile acids and/or excreted (39). The importance of the hepatic uptake of cholesterol in reverse cholesterol transport has been demonstrated using transgenic and knockout models of the scavenger receptor class B type 1 that mediates the selective uptake of cholesteryl esters and probably other lipids from HDL (40). Overexpression of scavenger receptor B1 in the livers of mice is accompanied by a decreased level of plasma HDL-cholesterol (41). Furthermore, SR-B1 gene deletion in mice results in a 90% reduction in cholesterol uptake by the liver and a 2-fold increase in the plasma level of cholesterol (42, 43). In rats, the hepatic uptake of PC from HDL is also a quantitatively important process, since nearly 40% of biliary PC originates from HDL (44). These observations might explain why the amount of biliary PC is not altered in mice deficient in hepatic CTα. We suggest that our data indicate that hepatic CTα deficiency might result in an increased uptake of HDL-PC with a corresponding reduction in the level of plasma HDL as observed in our liver-specific CTα knockout mice.

Alternatively, or in addition, livers deficient in CTα might compensate for impaired PC biosynthesis by limiting the amount of PC and cholesterol available for HDL formation. The formation of HDL from apoAI requires the cellular efflux of both cholesterol and PC (45). A change in the rate of hepatic cholesterol efflux has been previously shown to alter circulating HDL levels (46, 47). Overexpression of hepatic ATP-binding cassette binding protein A1 (required for the efflux of cholesterol and PC to apoAI) results in a 2-fold increase in plasma levels of both PC and cholesterol in HDL (47). Whether or not decreased hepatic PC biosynthesis reduces cholesterol efflux remains to be determined. No change was observed in apoAI secretion or nascent HDL formation in cultured hepatocytes deficient in methionine and choline, suggesting that PC biosynthesis is not required for hepatic cholesterol efflux (18). However, these results should be interpreted carefully, since an increased, rather than a decreased, flux through the Kennedy

![Fractionation of plasma lipoproteins by high performance liquid chromatography](http://www.jbc.org/)

**FIG. 6.** Fractionation of plasma lipoproteins by high performance liquid chromatography. Plasma (25 μl) from female (A and B) and male (C and D) mice was separated into lipoprotein fractions on an Amersham Biosciences Superose 6 column with an in-line assay for either cholesterol (B and D) or triacylglycerol (A and C). Lipoproteins eluted in the following order: VLDL at 25 min, intermediate/low density lipoproteins (LDL) at 35 min, and HDL at 40–45 min. For both assays, 2–4 samples were measured in each group. Graphs are representative of all samples measured.
pathway has been observed in choline-deficient murine hepatocytes (22). Indeed, the generation of the PEMT knockout mice and the liver-specific CT\textsubscript{H9251}\textsuperscript{−/−} knockout mice allows a closer examination of the relationship between PC biosynthesis and cholesterol efflux to apoA1.

CT\textsubscript{H9251} and VLDL Secretion—The role of PC synthesis in VLDL secretion has been studied extensively in our laboratory (13, 14, 16–19, 21, 48, 49). When rats (16) and mice (22) are fed a CD diet, the level of plasma VLDL (apoB, PC, and TG) is reduced. Nevertheless, recent studies suggest that this reduction is not the result of either inhibition of the CDP-choline pathway or a defect in VLDL secretion from the liver (22). As mentioned above, studies in Pemt\textsuperscript{−/−} mice have strengthened the link between PE methylation and VLDL secretion. (We now provide evidence that hepatic CT\textsubscript{H9251} is required for normal VLDL secretion in mice.)

As mentioned above, studies in Pemt\textsuperscript{−/−} mice have strengthened the link between PE methylation and VLDL secretion. (We now provide evidence that hepatic CT\textsubscript{H9251} is required for normal VLDL secretion in mice.) Our data show that plasma TG, PC, and apoB100 are decreased in the knockout mice, compared with littermate controls, regardless of gender. It is interesting that the lower VLDL levels are independent of gender, since total hepatic PC is decreased only in female, not male, knockout mice. This observation further supports the idea that active PC biosynthesis is required for normal VLDL secretion. The PC level of Pemt\textsuperscript{−/−} hepatocytes is also normal, but secretion of apoB100 and TG is inhibited (13). Impairment of apoB100 and apoB48 secretion was seen in male CT\textsubscript{H9251}-deficient livers without a decrease in the level of PC in the liver. The decrease in apoB secretion may be partially explained by the reduction in the total number of hepatocytes in the CT\textsubscript{H9251}-deficient livers. However, preliminary results show that apoB100 secretion from hepatocytes isolated from knockout mice is reduced compared with that from control hepatocytes.\textsuperscript{2} It should be noted that induction of expression of PEMT and CTβ2, while probably important in maintaining hepatic PC levels, was insufficient to normalize hepatic VLDL secretion.

Other Roles for CT\textsubscript{H9251}—It is now clear that PC production via the CDP-choline pathway is vital for lipoprotein homeostasis in mice. However, regulation of CT activity has also been implicated in many other biological processes, including neurite growth (50), neural tube closure (51), and fetal development (52). It has also been suggested that dysregulation of hepatic PC biosynthesis disrupts the distribution of long chain polyunsaturated fatty acids in liver and plasma (53). Furthermore, induction of PC biosynthesis is an essential step during cell division (54–56). In this process, CT\textsubscript{H9251} gene expression is stimulated by Sp1 during the S phase of the cell cycle (57). Consequently, the amount of CT is increased prior to mitosis, which might explain why CT\textsubscript{H9251}-deficient livers contain fewer hepatocytes than do control livers. It is possible that hepatocytes lacking CT\textsubscript{H9251} cannot produce enough PC for normal cell division. If this were the case, the liver-specific CT\textsubscript{H9251} knockout mouse model might provide in vivo evidence for the requirement of CT\textsubscript{H9251} in hepatic cell division. Thus, the results presented herein further support the hypothesis suggested by previous studies that PEMT activity cannot substitute for CT\textsubscript{H9251} in maintaining the cell cycle. First, overexpression of PEMT in

\textsuperscript{2} R. L. Jacobs and D. E. Vance, unpublished data.
Chinese hamster ovary-MT58 cells (these cells have a temperature-sensitive mutation in CT that prevents PC biosynthesis at 40 °C) did not rescue growth of MT58 cells at 40 °C, because insufficient PC was produced for cellular replication (58). Second, induction of hepatocyte proliferation after partial hepatectomy of rats is accompanied by a reduction in the amount of PEMT mRNA, protein, and activity (59, 60). This down-regulation of PE methylation coincided with maximal DNA synthesis and an elevation of CT activity, illuminating the importance of the CDP-choline pathway in rapidly dividing hepatocytes.

Conclusions—We have generated a liver-specific CTα knockout mouse to investigate the function of CTα-derived PC in the liver. The experiments presented in this study demonstrate that CTα plays an important role in regulating plasma levels of both HDL and VLDL. One surprising observation is that livers deficient in CTα have fewer hepatocytes, but of larger size, than do control livers, suggesting a vital role for CTα in hepatic cell division. However, more in vivo data linking CTα and hepatic cell division are required. Our data clearly demonstrate that impairment in the hepatic CDP-choline pathway alters the metabolism of both hepatic and circulating lipids and lipoproteins.

Acknowledgments—We thank Dr. Mark A. Magnuson for the albuminCre mouse, Dr. Suzanne Jackowski for antibodies to CT, and Dr. Jean Vance for helpful discussions. We thank Audric Moses, Sandra Ungar, and Pricilla Gao for excellent technical assistance, Laura Hargraves and Jennifer Witmer for maintenance of the mouse colonies, and Dr. Mark Lee for analysis of histological data.

REFERENCES
Role of Hepatic CTα in Lipoprotein Metabolism

47410

Amsterdam

Targeted Deletion of Hepatic CTP:phosphocholine Cytidylyltransferase α in Mice Decreases Plasma High Density and Very Low Density Lipoproteins
René L. Jacobs, Cecilia Devlin, Ira Tabas and Dennis E. Vance

doi: 10.1074/jbc.M404027200 originally published online August 25, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M404027200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 34 of which can be accessed free at http://www.jbc.org/content/279/45/47402.full.html#ref-list-1