An Unexpected Requirement for Phosphatidylethanolamine-Transferase in the Secretion of Very Low Density Lipoproteins*  

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Phosphatidylethanolamine-Transferase (PEMT) catalyzes the conversion of phosphatidylethanolamine to phosphatidylcholine (PC). We investigated whether there was diminished secretion of lipoproteins from hepatocytes derived from mice that lacked PEMT, compared with wild-type mice. Hepatocytes were incubated with 0.75 mM oleate, the media were harvested, and triglyceride (TG), PC, apolipoprotein (apo) B100, and apoB48 were isolated and quantified. Compared with hepatocytes from wild-type mice, hepatocytes from PEMT-deficient mice secreted 50% less TG, whereas secretion of PC was unaffected. Fractionation of the secreted lipoproteins on density gradients demonstrated that the decrease in TG was in the very low density lipoprotein (VLDL)/low density lipoprotein fractions. The secretion of apoB100 was decreased by ~70% in VLDL/low density lipoproteins, whereas there was no significant decrease in apoB48 secretion in any fraction. Transfection of McArdle hepatoma cells (that lack PEMT) with PEMT cDNA enhanced secretion of TG in the VLDLs. Because the levels of PC in the hepatocytes and hepatoma cells were unaffected by the lack of PEMT expression, there appears to be an unexpected requirement for PEMT in the secretion of apoB100-containing VLDLs.

The nutritional importance of choline was described 70 years ago in a classic study (1). A major phenotype of choline deficiency was the accumulation of lipid in the liver. Subsequently, a defect in the secretion of lipid from the livers of choline-deficient animals was reported (reviewed in Ref. 2). This apparent defect in lipoprotein secretion would be expected to account for at least some of the accumulating hepatic triglyceride (TG)3 in choline-deficient animals and was proposed to be due to impaired phosphatidylcholine (PC) biosynthesis via the CDP-choline pathway. Advances in hepatocyte culture and lipoprotein isolation permitted a more rigorous evaluation of the role of PC biosynthesis in lipoprotein secretion in the 1980s. Experiments have demonstrated that indeed PC biosynthesis was required for secretion of very low density lipoproteins (VLDLs), but not high density lipoproteins, from primary cultures of rat hepatocytes (3). In the absence of choline, there was a decrease in secretion of lipids, apolipoprotein (apo) B48, and apoB100 associated with VLDL. A striking decrease in apoB100 and apoB48 in VLDL was also observed in the plasma of choline-deficient rats (4). Subsequent studies demonstrated that the requirement for choline was specific, and neither monomethylethanolamine nor dimethylethanolamine would substitute (5). However, lyso-PC was able to substitute for choline for the secretion of apoB48, TG, and PC (6). The requirement for PC biosynthesis was observed only when the apoB-containing particles contained a neutral lipid core because PC biosynthesis was not required for secretion of truncated apoBs, such as apoB18 or apoB23, that are secreted from hepatoma cells without significant neutral lipid (7). Mechanistic studies indicated that choline deficiency reduced the number of nascent VLDL particles in the Golgi, but not in the endoplasmic reticulum (ER) (8). These nascent VLDL particles were subsequently shown to be deficient in PC in the Golgi and to a lesser extent in the ER (9). The data suggested that many of the nascent particles were degraded in a post-ER compartment (9).

In addition to the CDP-choline pathway, the liver has a secondary pathway for PC biosynthesis in which phosphatidylethanolamine (PE) is converted to PC by three successive transmethylation reactions catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) (10). Several approaches have indicated that ~30% of the PC in liver is made via the PEMT pathway, whereas the remainder is synthesized via the CDP-choline pathway (11-13). The PEMT pathway may substitute for the CDP-choline pathway in lipoprotein secretion because supplementation of choline-deficient hepatocytes with methionine (required for the methylation reactions) restored the secretion of TG, apoB48, and apoB100 (3). The reciprocal question (can the CDP-choline pathway substitute for the PEMT pathway in VLDL secretion?) has been addressed by the use of inhibitors of PE methylation. An inhibitor of transmethylation reactions involving S-adenosylmethionine is 3-deazaadenosine (DZA) (14). DZA effectively inhibits the PEMT reaction in hepatocytes (15) without altering the secretion of labeled apoB48 or apoB100 (16). Thus, it seemed that the methylation pathway for PC biosynthesis was not required for VLDL secretion and that the CDP-choline pathway was sufficient. However, the interpretation of these results is complex.

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† The abbreviations used are: TG, triglyceride; apo, apolipoprotein; DMEM, Dulbecco’s modified Eagle’s medium; DZA, 3-deazaadenosine; ER, endoplasmic reticulum; MTP, microsomal triglyceride transfer protein; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; VLDL, very low density lipoprotein.
DZA potently inhibited the labeling of PC from PE derived from the CDP-ethanolamine pathway in both cells and secreted lipoproteins (16). In addition, although DZA effectively inhibited the labeling of PC from PE derived from the mitochondrial decarboxylation of phosphatidylserine in hepatocytes, the amount of labeled PC secreted into the medium was not significantly decreased by DZA treatment of hepatocytes (17). Consequently, we were unable to conclude that the PEMT pathway could be completely compensated by the CDP-choline pathway in providing PC for lipoprotein secretion. Other inhibitors of PEMT, the peroxisomal proliferators bezafibrate and clofibrlic acid, did not inhibit the secretion of apoB100, but not apoB48, in McArdle cells (20). 

To decipher the unexpected role in apoB100 secretion from hepatocytes, we investigated lipoprotein secretion from hepatocytes derived from Pemt−/− mice. The results show that in the absence of PEMT, the secretion of apoB100, but not apoB48, particles from cultured hepatocytes is decreased by ~70%. Furthermore, reconstitution of PEMT activity in McArdle RH-7777 hepatoma cells stimulated TG and apoB100 secretion, validating the results observed in the Pemt−/− hepatocytes. Because the CDP-choline pathway is enhanced in Pemt−/− mice (20) and normally expressed in the McArdle cells, we suggest that the PEMT pathway for PC biosynthesis has an unexpected role in apoB100 secretion from hepatocytes.

**EXPERIMENTAL PROCEDURES**

**Materials**—Hank’s balanced salt solutions, Dulbecco’s modified Eagle’s medium (DMEM), methionine-deficient DMEM, and fetal bovine serum were obtained from Invitrogen. Collagen, collagenase, insulin, bovine serum albumin, oleate, phospholipase C from Clostridium welchii, and protein A-Sepharose CL-4B were purchased from Sigma. Silica Gel G60 plates for thin-layer chromatography were from Merck, and protein A-Sepharose CL-4B were purchased from Sigma. Collagenase perfusion technique as described previously (21, 22). The agents were purchased from standard commercial sources. Roche Molecular Biochemicals. McArdle RH-7777 cells were obtained from Amersham Biosciences. The sheep anti-human apoB antibody used in immunoprecipitations was purchased from Roche Molecular Biochemicals. McArdle RH-7777 cells were obtained from the American Type Culture Collection. Other chemicals and reagents were purchased from standard commercial sources.

Isolation of Hepatocytes—The Pemt−/− mice had a mixed genetic background of 129/J and C57BL/6 (20) and were maintained via homozygous breeding in a reversed 12-h light/12-h dark cycle on a regular background of 129/J and C57BL/6 (20) and were maintained in medium containing 200 μg/ml G418. Stable colonies were selected in culture medium containing 400 μg/ml G418 and maintained in medium containing 200 μg/ml G418.

**PEMT and Protein Assays**—Protein determination was performed using the Pierce BCA kit or the Coomassie Blue system (Bio-Rad), with bovine serum albumin as a standard. PEMT assays were performed as described previously using phosphatidylmonomethyl ethanolamine as a substrate (24).

**Measurement of Lipids**—Hepatocytes were isolated from two mice of each genotype, pooled, and plated together. Cells were incubated in 2 ml of serum- and hormone-free DMEM in either the presence of 0.75 mM oleate + 1% (w/v) albumin or 1% albumin alone. Cells and media were harvested at 0, 6, and 12 h. For each sample, four dishes were pooled. Lipids were extracted from the media using a modified Bligh and Dyer protocol (25). Subsequently, the samples were loaded onto thin-layer plates and resolved using chloroform/methanol/acetic acid/water (25:15:4:2) until the solvent reached halfway up the plate. The solvent was evaporated, and the neutral lipids were separated using heptane/diisopropyl ether/acetic acid (60:40:4) that migrated to the top of the plate. PC was determined via a lipid phosphorus assay (26), and TG was measured using the hydroxylamine procedure (27). The cells were dispersed into 8 ml of phosphate-buffered saline (pH 4) and centrifuged, and the cellular pellet was resuspended in 4 ml of phosphate-buffered saline and sonicated. Lipids were extracted from cells containing 1 mg of protein and resolved by thin-layer chromatography.

McArdle cells at a density of 3 × 10^6 cells/100-mm dish were incubated in serum-free DMEM containing 0.1 mM ethanolamine and 0.4 mM oleate–bovine serum albumin for 20 h. The lipids were extracted and digested with phospholipase C, and the resulting triacylglycerol was analyzed by gas liquid chromatography.

**Radiolabeling and Density Fractionation of Secreted Lipids**—Hepatocytes from one mouse of each genotype were isolated. After an overnight incubation, the cells were rinsed twice overnight with serum- and hormone-free DMEM. Cells were radiolabeled with 10 μCi [3H]oleate/dish in the presence of 0.75 mM oleate and 1% albumin for 12 h. Media and cells were harvested, and lipoproteins were isolated by ultracentrifugation (28). Briefly, 1.4 ml of media was mixed with 0.7 g of KBr and then loaded at the bottom of a Quick-seal tube and overlaid to the top with 3.5 ml of 0.9% NaCl (29). The samples were centrifuged at 416,000 × g for 1 h in a VT-65 Beckman rotor (29). Afterward, 0.5-ml volumes were collected from the bottom, and lipids were extracted and fractionated by thin-layer chromatography. Bands corresponding to TG and PC were scraped, and radioactivity was determined.

For McArdle cells, duplicate or triplicate 60-mm dishes of cells (1 × 10^6 cells/dish) were seeded 20 h before the start of the experiment. The cells were incubated in serum-free DMEM containing 0.1 mM ethanolamine and 0.4 mM oleate–bovine serum albumin for 30 min. The medium was replaced with DMEM that contained either 10 μCi of [3H]glycerol or 10 Ci of [3H]-H-methionine in the presence of 0.1 mM ethanolamine and 0.4 mM oleate–bovine serum albumin for 2 h. The lipids were extracted and fractionated by thin-layer chromatography, and radioactivity was determined.

For fractionation of secreted lipoproteins from McArdle cells, the procedure was as described above for hepatocytes, except that a Beckman Ti 90 rotor was used, and the samples were centrifuged at 100,000 × g for 1 h.

**Secretion of ApoB from Pemt−/− and Pemt+/−**—Hepatocytes—Hepatocytes from one mouse for each genotype were incubated overnight and rinsed twice over a 2-h period with serum- and methionine-free DMEM. Cells were then incubated with 200 μCi [3H]-methionine/dish for 4 h in the presence of either 0.75 mM oleate + 1% albumin or 1% albumin alone in methionine-free DMEM. Media and cells were harvested, and lipoproteins were isolated by ultracentrifugation as described above, except that 1.3 ml medium and 1.3 ml of fresh murine or rat plasma as carrier to prevent loss of radiolabeled VLDLs. ApoB48 and apoB100 were isolated by immunoprecipitation using anti-human apoB antibody. To each 500-μl fraction, 55 μl of 10× immunoprecipitation buffer (1.5 mM NaCl, 0.5 mM Tris-HCl, pH 7.4, 50 μM EDTA,
5% Triton X-100 (v/v), and 15% SDS (w/v) (29) and 5 μl of anti-human apoB were added, and the samples were mixed overnight at 4°C. Samples (50 μl) were mixed with 50 μl of protein A-Sepharose CL-4B in phosphate-buffered saline for 1 h. The Sepharose was pelleted for 5 min at maximum speed in a microfuge and washed twice with 1× immunoprecipitation buffer. The pellets were boiled in sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS (w/v), 20% glycerol (v/v), 10% β-mercaptoethanol (v/v), and 0.02% bromphenol blue (30) and resolved by SDS-5% polyacrylamide gel electrophoresis. The gels were stained with Coomasie Blue, soaked in Amplify (Amersham Biosciences), dried, and exposed to film. Quantitative densitometry of the bands was performed using Image Gauge v3.0 software by Fuji.

For experiments on apoB secretion from McArdle cells, the cells were plated at 1 × 10⁶ cells/60-mm dish for 24 h. The cells were incubated in serum-free and methionine-deficient DMEM containing 0.1 mM ethanolamine and 0.4 mM oleate-bovine serum albumin for 30 min. Subsequently, the cells were incubated with serum-free, methionine-deficient DMEM containing 200 μCi [³⁵S]methionine/dish in the presence of 0.1 mM ethanolamine and 0.4 mM oleate-albumin for 2 h. The medium was harvested, concentrated 5-fold by ultrafiltration, and fractionated by density gradient ultracentrifugation as described above. ApoB from each fraction was immunoprecipitated, and radioactivity was determined as described for the protocol with primary hepatocytes.

RESULTS

PEMT Activity in Hepatocytes Decreases with Time of Culture—Previous studies indicated that PEMT activity is not stable in primary hepatocytes derived from rat liver. Therefore, we undertook a preliminary study to determine the role of PEMT in VLDL secretion, which was performed using rat primary hepatocytes isolated from Pemt+/+ mice. The next morning, the cells were rinsed, incubated for 0–18 h with serum-free DMEM, harvested, and assayed for PEMT activity. PEMT activity was 1.6 nmol/min/mg cell protein at 0 h and decreased by 50% after 12 h. By 18 h, PEMT activity had decreased to 0.7 nmol/min/mg cell protein. Therefore, none of the following experiments extended beyond 12 h. Hepatocytes derived from Pemt−/− mice contained no PEMT activity (data not shown; Ref. 20).

TG Secretion Is Impaired from Pemt−/− Hepatocytes—Hepatocytes isolated from Pemt+/+ and Pemt−/− mice were incubated in the presence or absence of 0.75 mM oleate for 0, 6, or 12 h. As shown in Fig. 1A, the absence of PEMT activity resulted in an ~50% decrease in TG secretion compared with hepatocytes isolated from Pemt+/+ animals. PC secretion from hepatocytes from Pemt−/− mice was not impaired (Fig. 1B).

Although TG secretion was decreased by ~50% in the Pemt−/− hepatocytes compared with the Pemt+/+ hepatocytes, there was no observable accumulation of TG in the Pemt−/− cells at any time point (range, 200–275 μg TG/mg cell protein). Also, no significant changes were observed in total cellular PC (range, 100–115 μg PC/mg cell protein), suggesting that the CDP-choline pathway is sufficient to maintain normal PC levels in Pemt−/− hepatocytes.

Hepatocytes were isolated from Pemt−/− and Pemt+/+ mice and incubated for 12 h in serum-free DMEM with 0.75 mM oleate, 1% albumin, and 10 μCi [³H]oleate/dish. The media were collected and separated into lipoprotein fractions by ultracentrifugation. Labeled TG secreted in the VLDL fraction from cells isolated from Pemt−/− animals was significantly decreased compared with that from Pemt+/+ mice (Fig. 2A). We did not observe a shift in the distribution of radiolabeled TG to more dense particles secreted by the Pemt−/− hepatocytes. Although we observed no change in secreted PC, separation of lipoprotein fractions revealed a significant decrease in [³H]oleate-labeled PC in the VLDL fraction secreted by Pemt−/− hepatocytes (Fig. 2B). The majority of radiolabeled PC was present in the fractions with density > 1.15 gm/ml in which there were no significant differences between the two genotypes of mice. The incorporation of [³H]oleate into hepatocyte lipids derived from both genotypes of mice was the same, indicating no alteration in [³H]oleate uptake or TG or PC synthesis (Fig. 2C).

ApoB100 Secretion Is Impaired from Pemt−/− Hepatocytes Incubated with Oleate—We next determined whether the secretion of apoB100 and/or apoB48 from Pemt−/− hepatocytes was also impaired. Hepatocytes were incubated with 0.75 mM oleate for 4 h in the presence of 200 μCi [³⁵S]methionine/dish. Afterward, the medium was centrifuged to separate the lipoproteins into different density fractions, and the apoB proteins were immunoprecipitated and separated by electrophoresis on SDS-polyacrylamide gels. Fluorograms were quantitated after exposure to x-ray film. ApoB100 secretion from Pemt−/− hepatocytes was decreased, whereas apoB48 secretion was unchanged, compared with that in Pemt+/+ hepatocytes (Fig. 3). Quantitation of the results from four experiments demonstrates that apoB100 secretion from hepatocytes incubated with oleate was 70% less than that from Pemt−/− hepatocytes (Fig. 3B). In the absence of oleate, we observed a decrease in apoB100 secretion from Pemt−/− hepatocytes that approached statistical significance (p = 0.054) (Fig. 4, A and B). No differences in apoB48 secretion were observed between Pemt+/+ and Pemt−/− hepatocytes, regardless of the presence of oleate in the medium (Figs. 3C and 4C).

Expression of PEMT in McArdle Cells Stimulates VLDL Secretion—Because it appears that the absence of PEMT caused the defect in VLDL secretion, we reasoned that expression of

**D. E. Vance, unpublished results.**

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FIG. 1. Triacylglycerol secretion is impaired from hepatocytes derived from Pemt−/− mice. Hepatocytes from Pemt−/− and Pemt+/+ mice were isolated. The next day, the dishes were incubated with 1% albumin ± 0.75 mM oleate in serum- and hormone-free media for 0, 6, or 12 h. At each time point, the media from four dishes were combined, the lipids were extracted, TG (A) and PC (B) were isolated by thin-layer chromatography, and the amounts were determined. Results are expressed as the averages of three separate experiments ± S.E. *+, p < 0.005; **+, p < 0.05 (based on a Student’s t test where Pemt+/+ is compared with Pemt−/−).
PEMT in cells that lacked PEMT activity might stimulate VLDL secretion. Because McArdle rat hepatoma cells have only trace levels of PEMT activity (31), we transfected these cells with a cDNA encoding PEMT behind the cytomegalovirus promoter. Stable cell lines of McArdle cells were constructed that expressed PEMT (Fig. 5, A and B). The parent McArdle cells or vector-transfected cells contained negligible PEMT activity.

**Fig. 2.** Secretion of labeled TG and PC in VLDL is impaired from Pemt

-**hepatocytes.** Hepatocytes from Pemt

-**/+/+ and Pemt

-**/− mice were isolated and allowed to settle overnight. The cells were incubated for 12 h in serum- and hormone-free DMEM that contained 0.75 mM oleate, 1% albumin, and 10 μCi [3H]oleate/dish. Secreted lipoproteins were fractionated according to density by ultracentrifugation on a salt gradient, the lipids were extracted and purified by thin-layer chromatography, and the radioactivity associated with TG (A) and PC (B) was determined. Radiolabel incorporation into cellular TG and PC was analyzed (C). The results are expressed as averages ± S.E. of three experiments, each performed in duplicate. *, p < 0.05 based on a Student’s t test.

**Fig. 3.** ApoB secretion from oleate-treated hepatocytes. Hepatocytes from Pemt

-**/− and Pemt

-**/+/+ mice were incubated for 4 h in serum-, hormone-, and methionine-free DMEM + 0.75 mM oleate, 1% albumin, and 200 μCi [35S]methionine/dish. The medium was fractionated by density-gradient centrifugation, and apoB100 and apoB48 were immunoprecipitated. The immunoprecipitates were resolved by electrophoresis on 5% SDS-polyacrylamide gels. The amount of 35S in apoB was assessed by densitometric scanning. A, an example of a fluorograph of apoB100 and apoB48 secreted from Pemt

-**/− and Pemt

-**/+/+ hepatocytes. B and C, quantitative analysis of the apoB bands was performed using Image Gauge v3.0 software from Fuji, and units were normalized to cellular protein. The data are the averages ± S.E. of four separate experiments with duplicates in each experiment. *, p < 0.05 based on a Student’s t test.
And no immunoreactivity was detected for PEMT (Fig. 5A). In contrast, cell lines that were transfected with PEMT showed high levels of PEMT activity (Fig. 5A) comparable with that seen in primary murine hepatocytes (1.6 nmol/min/mg protein). Moreover, strong PEMT immunoreactivity was seen for each of the cell lines that expressed PEMT (Fig. 5B). In addition, we confirmed that the cultured cells expressed PEMT activity in culture. The transfected McArdle cells were incubated with $[^3H]methylmethionine$ for 2 h, and then the cells were harvested, and the radioactivity incorporated into PC was measured. As evident from Fig. 5C, radiolabel was incorporated into PC in the cells that expressed PEMT, whereas in the vector-transfected control cells, minimal radioactivity was incorporated into PC. Thus, we established McArdle cell lines that stably expressed PEMT.

We next evaluated the ability of these cells to secrete TG. The cells were incubated for 20 h in the presence of 0.4 mM oleate, and the amounts of TG in the cells and medium were measured. Whereas the amount of intracellular TG was the same in cell lines that contained or lacked PEMT (Fig. 6A), cells expressing PEMT secreted approximately twice as much TG as cells transfected with vector alone (Fig. 6B).

The secretion of apoB100 from PEMT-expressing McArdle cells was evaluated by incubating cells with $[^35S]$methionine for 2 h in the presence of oleate. The medium was collected and fractionated into four different densities. ApoB was immunoprecipitated, and autoradiography was performed after SDS-polyacrylamide electrophoresis. As shown in Fig. 6C, 1.6 times (estimated by densitometric scanning) more apoB100 was se-
creted in the VLDL (lowest density) fraction from cells expressing PEMT than from control cells not expressing PEMT. This result is consistent with the observation that the TG secreted by the cells expressing PEMT was greater than that from the vector-transfected control cells.

We also incubated the transfected McArdle cells with [3H]-glycerol for 2h in the presence of 0.4 mM oleate, harvested the cells, and measured the amount of labeled TG in the cells, and the lipoproteins were fractionated by density gradient centrifugation. The gel was dried and exposed to film at −80 °C. The experiment was repeated twice with similar results.

**DISCUSSION**

The major finding in this study is that cultured hepatocytes from Pemt−/− mice incubated with oleate secrete ~50% less TG and ~70% less apoB100 than hepatocytes from Pemt+/+ mice. In contrast, there was no impairment in the secretion of apoB48 from Pemt−/− hepatocytes. The total amount of PC secreted was not altered in Pemt−/− hepatocytes, but PC recovered in the VLDL fraction was decreased, consistent with the reduction in secreted apoB100. The theory that the defect in VLDL secretion was due to a lack of PEMT was substantiated by stable expression of PEMT in McArdle cells in which TG secretion in VLDL was enhanced. Thus, PEMT has a special function that is required for normal secretion of TG and apoB100 particles from hepatocytes.

**Comparisons to Studies with Primary Rat Hepatocytes and PEMT Inhibitors**—There are several potential explanations why the Pemt−/− hepatocytes were defective in VLDL secretion, yet VLDL secretion from rat hepatocytes was not inhibited by DZA, a potent inhibitor of transmethylation reactions (16). First, in the present studies, a more pronounced decrease in apoB100 and TG secretion was observed in Pemt−/− hepatocytes when oleate was added to the medium. However, oleate was not present in the medium of the experiments using DZA (16, 19). Second, in the present studies, we used male mice, whereas the DZA studies were performed with hepatocytes derived from female rats. Third, a PE methylation reaction
that was insensitive to DZA produced some of the PC that was used for VLDL secretion (17), whereas in the current experiments, all PE methylation activity was completely abolished. Finally, the fact that DZA is a nonspecific inhibitor affecting many pathways may also have influenced the results. Our data also contrast with studies using fibres as inhibitors of PEMT in which apoB48 in the VLDL fraction was decreased. However, in these studies (18, 19), oleate was not added to the medium, and the hepatocytes were derived from female rats. It is also possible that the fibres elicited other nonspecific effects unrelated to inhibition of PEMT.

**PC Biosynthesis and VLDL Secretion**—Although total cellular PC was unchanged in the *Pemt*−/− hepatocytes (data not shown), it is possible that phospholipid ratios have been disrupted within specific organelles important for apoB secretion such as the ER and the Golgi. Previous studies performed on choline-deficient rats demonstrated that in the absence of sufficient levels of PC, the VLDL particles were degraded in a post-ER compartment (8). In-depth analysis of phospholipid levels in the livers after subcellular fractionation showed that PC/PE ratios had been significantly disrupted in the ER and the Golgi of these choline-deficient animals, resulting in the formation of abnormal VLDL particles (9).

However, there are several reasons to think that the reduction in apoB100 secretion from *Pemt*−/− hepatocytes is not simply a result of decreased PC biosynthesis or amount of PC. First, in the livers of *Pemt*−/− mice, the CDP-choline pathway is up-regulated, and PC levels are normal (20). Also, the total amount of PC secreted by hepatocytes derived from these livers is not decreased (Fig. 1B). Finally, if insufficient PC were made by the CDP-choline pathway, then the secretion of both apoB100 and apoB48 would be expected to be decreased, as was observed in choline/methionine-deficient hepatocytes (3).

A number of recent studies suggest that not only the quantity of PC but also the targeting, turnover, and fatty acid composition of PC are important for optimum VLDL assembly. Hepatocytes isolated from apoB transgenic/phospholipid transfer protein-deficient mice also exhibited reduced VLDL secretion (32). From these studies, it was hypothesized that transfer of phospholipid to apoB in the Golgi was a necessary remodeling step prior to VLDL secretion. In another study, the prevention of PC hydrolysis in hepatoma cells with an inhibitor of Ca2+-independent phospholipase A2 also resulted in an impaired secretion of VLDL particles (33). Pretreatment of these cells with oleate, but not with n-3 fatty acids, restored normal VLDL secretion, suggesting that remodeling of the acyl chains of the PC molecule itself is important for VLDL assembly (33). Other experiments in hepatoma cells indicated that PC turnover via the actions of phospholipase D was necessary for the addition of TG to apoB (34). It is important to note, however, that in all these studies, secretion of both apoB100 and apoB48 was impaired (32–34), whereas in our studies on PEMT-deficient hepatocytes, we observed an inhibition of apoB100 secretion, but not apoB48 secretion.

**Comparisons between *Pemt*−/− Mice and Mice That Lack Microsomal TG Transfer Protein**—One model system that has a defect in apoB100 secretion similar to that observed in *Pemt*−/− mice is in which the microsomal TG transfer protein (MTP) gene was ablated in murine liver (35, 36). MTP has been shown to transfer TG and PC between membranes in vitro (37). In the absence of hepatic MTP activity, the major defect was in the secretion of apoB100-containing lipoproteins, with only a small decrement in the amount of apoB48 secreted. Thus, perhaps a common defect in the livers of *Pemt*−/− mice and MTP-deficient mice is the inability to transfer a specific pool of PC to nascent apoB100 for VLDL assembly. Alternatively, the lipoprotein secretion defect in MTP−/− and *Pemt*−/− hepatocytes might result from a reduction in the availability of TG. This hypothesis is supported by the observation that the secretion defect in *Pemt*−/− hepatocytes is more pronounced when the culture medium is supplemented with oleate. Furthermore, it is well established that MTP plays a role in the maintenance of TG pools accessible for VLDL secretion (35, 36). MTP has been localized to lipid droplets in rat liver (39), thus MTP and MTP might act cooperatively in maintaining these pools of TG.

Nevertheless, *Pemt*−/− and MTP−/− hepatocytes do not exhibit exactly the same defect in lipoprotein secretion. In the absence of MTP, the secretion of apoB100 is virtually completely abolished, whereas apoB100 secretion from hepatocytes from *Pemt*−/− mice is decreased 70%. Moreover, the density (i.e. the extent of lipification) of apoB48 particles secreted from *Pemt*−/− hepatocytes is the same as that in MTP−/− hepatocytes, whereas in MTP−/− hepatocytes, the apoB48-containing particles are less lipidated than those in MTP−/− hepatocytes. Therefore, the observations outlined in this study are novel and may provide information concerning differences in the mechanism of apoB100 and apoB48 VLDL assembly and secretion.

**Conclusion**—We have demonstrated that PEMT can specifically limit the secretion of apoB100-containing VLDL from primary murine hepatocytes and McArdle rat hepatoma cells. PC is the most abundant lipid in the surface monolayer of VLDL particles. Thus, we speculate that PC derived from PEMT plays an important role in providing the surface of VLDL that is required for stabilizing the hydrophobic lipid core of the particles. As discussed, many potential mechanisms might explain these observations. Moreover, the observation that VLDL secretion is inhibited in PEMT-deficient mice provides strong evidence that the PEMT requirement is not simply a cell culture phenomenon. The unanticipated wrinkle in these studies is that the requirement for PEMT for optimal VLDL secretion is observed only in the male *Pemt*−/− mice.

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