Biochimica et Biophysica Acta 1831 (2013) 626–632

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

Physiological roles of phosphatidylethanolamine *N*-methyltransferase ☆

Dennis E. Vance *

Group on the Molecular and Cell Biology of Lipids, University of Alberta, Edmonton, Alberta, Canada
Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada

ARTICLE INFO

Article history:
Received 18 June 2012
Received in revised form 20 July 2012
Accepted 23 July 2012
Available online 31 July 2012

Keywords:
Phosphatidylcholine
Phosphatidylethanolamine
Obesity
Steatosis
Atherosclerosis
Choline

ABSTRACT

Phosphatidylethanolamine *N*-methyltransferase (PEMT) catalyzes the methylation of phosphatidylethanolamine to phosphatidylcholine (PC). This 22.3 kDa protein is localized to the endoplasmic reticulum and mitochondria associated membranes of liver. The supply of the substrates AdoMet and phosphatidylethanolamine, and the product AdoHcy, can regulate the activity of PEMT. Estrogen has been identified as a positive activator, and Sp1 as a negative regulator, of transcription of the PEMT gene. Targeted inactivation of the PEMT gene produced mice that had a mild phenotype when fed a chow diet. However, when *Pemt*$^{-/-}$ mice were fed a choline-deficient diet steatohepatitis and liver failure developed after 3 days. The steatohepatitis was due to a decreased ratio of PC to phosphatidylethanolamine that caused leakage from the plasma membrane of hepatocytes. *Pemt*$^{-/-}$ mice exhibited attenuated secretion of very low-density lipoproteins and homocysteine. *Pemt*$^{-/-}$ mice bred with mice that lacked the low-density lipoprotein receptor, or apolipoprotein E were protected from high fat/high cholesterol-induced atherosclerosis. Surprisingly, *Pemt*$^{-/-}$ mice were protected from high fat diet-induced obesity and insulin resistance compared to wildtype mice. If the diet were supplemented with additional choline, the protection against obesity/insulin resistance in *Pemt*$^{-/-}$ mice was eliminated. Humans with a Val-to-Met substitution in PEMT at residue 175 may have increased susceptibility to nonalcoholic liver disease. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.

1. Introduction

Phosphatidylethanolamine *N*-methyltransferase (PEMT) catalyzes the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) by the transfer of 3 methyl groups from *S*-adenosylmethionine to PE. In 1941 it was demonstrated that methyl groups from methionine could be utilized for the formation of choline [1]. In that same year, Stetten demonstrated that [^{15}N]ethanolamine was methylated to form choline which was then converted into PC [2]. It was not until 1960 that Bremer and Greenberg demonstrated that PE was the methyl group acceptor [3,4]. Subsequently, they characterized PEMT activity on rat liver microsomes [5].

2. Purification and characterization of PEMT

PEMT is tightly associated with the membranes of the endoplasmic reticulum (ER). Hence, attempts at purification were plagued by pitfalls and problems. Purification of membranous enzymes has been characterized as "masochistic enzymology" [6]. However, persistence and dedication by N. Ridgway resulted in the purification of a small protein (22.3 kDa) from Triton X-100 solubilized rat liver microsomes [7]. The pure protein catalyzed all 3 transmethylation reactions for the conversion of PE to PC. It appeared that this protein accounted for all the methylation of PE that occurs in liver. However, this was not conclusively demonstrated until the gene encoding PEMT was disrupted in mice [8,9]. *Pemt*$^{-/-}$ mice had no residual activity in the conversion of PE to PC [9].

The pure enzyme used PE, phosphatidyl-monomethylethanolamine and phosphatidyl-dimethylethanolamine as substrates [10]. Kinetic studies suggest a common substrate binding site for the 3 phospholipid substrates [11]. The pH optimum for all 3 reactions catalyzed by PEMT is 10.5 [12]. Since the pH of the cytoplasm in cells is usually 7.4, PEMT activity would normally be well below its potential. The physiological significance, if any, of the high pH optimum is not clear.

A crystallographic or NMR structure for PEMT has not yet been reported. Topographical studies of the enzyme on the ER membrane suggest that four transmembrane regions span the membrane such that both the C-terminus and N-terminus face the cytosolic side of

Abbreviations: AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; CT, CTP:phosphocholine cytidylyltransferase; DHA, docosahexaenoic acid; ER, endoplasmic reticulum; Hcy, homocysteine; LCTαKO, liver-specific CTα knockout; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; TG, triacylglycerol; VLDL, very low density lipoproteins

☆ This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.

* Group on Molecular and Cell Biology of Lipids, 328 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada T6G2S2. Tel.: +1 780 492 8286; fax: +1 780 492 3383.
E-mail address: dennis.vance@ualberta.ca.

1388-1981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2012.07.017

the ER (Fig. 1) [13]. Two separate AdoMet binding motifs were identified on the 3rd and 4th transmembrane sequences near the cytosolic surface of the ER [14]. It is remarkable that such a small protein can bind one of three phospholipid substrates as well as AdoMet. It will be interesting to determine the structure of PEMT to show how this small protein can bind these substrates.

Three novel forms of PEMT mRNA were identified in human liver by cloning of the cDNAs [15]. These forms differ from each other in the 5′-region with the point of divergence being 15 nucleotides upstream of the codon for initiation of translation. Each of the 3 PEMT transcripts was detected in human liver [16] whereas the heart expressed one of the transcripts. The testis expressed the same transcript as the heart as well as a small amount of another transcript. The three cDNAs were ectopically expressed in human embryonic kidney (HEK)-293 cells. One of the transcripts (designated as PEMT-L) had the N-terminal domain in the lumen of the ER and this domain was N-glycosylated with mannose oligosaccharides [17]. The specific activity of PEMT-L was much lower than that of the other two expressed transcripts (designated as PEMT-S). The PEMT-S forms were not glycosylated.

PEMT activity is highly expressed only in liver [18]. Nevertheless, low activities have been found in other tissues (e.g., heart, adipocytes). There is now evidence that PEMT activity might be important in lipid droplet biosynthesis in adipocyte cells and white adipose tissue [19].

3. Cloning and expression of *Pemt* cDNA

The purification of PEMT allowed us to obtain enough sequence of the protein to permit cloning and expression of *Pemt* cDNA [20]. The encoded protein contains 199 amino acids. An antibody was raised against the carboxyl terminus of PEMT. Subcellular fractions of rat liver were prepared, PEMT activity was measured and immunoblotting of the protein was performed [20]. Whereas most of the activity was found on the ER, there was no immunoreactivity with the antibody to PEMT on the ER. Rather, the antibody reacted with a protein on mitochondrial-associated membranes (MAM) [21], a subfraction of ER that associates with mitochondria. Subsequent attempts to distinguish the difference between PEMT on the ER and the MAM have been unsuccessful.

4. Regulation of the conversion of PE to PC

In an earlier review we summarized evidence that the supply of substrates for PEMT, and one product, *S*-adenosylhomocysteine, were major regulators of PEMT activity [18,22,23]. More recent studies have focused on transcriptional regulation of the expression of PEMT. The proximal promoter region for the PEMT gene does not contain a TATA box preceding the transcriptional start site in mice or humans [8,16]. Potential transcription factor binding sites were identified for hepatic nuclear factor, activating factor-1 (AP-1), and Sp1.

Estrogen was implicated as a positive regulator of PEMT transcription in primary cultures of mouse and human hepatocytes [24]. However, it is not clear if the observed changes in *Pemt* mRNA levels were caused by increased mRNA stability or alterations in gene transcription. Nevertheless, subsequent studies suggest that in some women the estrogen binding site in the PEMT promoter region is ablated, which has been proposed to put these women at risk for hepatic steatosis due to choline deficiency syndrome [25]. Moreover, pre-menopausal women are less likely to develop signs of muscle and liver damage when fed a choline-deficient diet, compared to postmenopausal women and to men [26]. The observed estrogen regulation of PEMT expression is consistent with the report that female mice have higher expression of PEMT than do male mice [27].

A major difficulty in studying the transcriptional regulation of PEMT expression was identification of a suitable cell line that expressed PEMT. Even though primary hepatocytes express PEMT, studies in these cells are difficult because the expression of PEMT begins to decline after culturing. Moreover, there is no suitable hepatoma cell line that shows significant expression of PEMT. This problem was overcome when 3T3-L1 cells were shown to increase the expression of PEMT during differentiation into adipocytes [28]. 5′-Deletion analyses of PEMT promoter-luciferase constructs stably expressed in 3T3-L1 cells implicated a regulatory region between −471 and −371 bp relative to the transcription start site. This region of the proximal promoter contains a binding site for Sp1. During the differentiation of 3T3-L1 cells into adipocytes, the level of Sp1 protein decreased just before the expression of PEMT increased. These and other experiments directly implicate Sp1 as a negative regulator of PEMT transcription [28]. In contrast, Sp1 is a positive regulator of the expression of CTP:phosphocholine cytidylyltransferase (CT) the rate-limiting enzyme of the CDP-choline pathway for PC synthesis [29–32]. Moreover, the CDP-choline pathway is up-regulated in liver when PEMT expression is decreased during hepatic regeneration and growth [33–35]. Therefore, Sp1 may have an important role in the reciprocal regulation of PEMT and CT.

5. Targeted deletion of the mouse *Pemt* gene

Until the technology was developed for specifically inactivating genes in mice, approaches to understand the roles of specific genes in mouse physiology were limited. Hence, it was of great interest to inactivate the *Pemt* gene in mice. At the time this was a rather difficult assignment because we needed to obtain information on the structure of the PEMT gene so that we could generate targeting vectors. Nevertheless, sequence of the *Pemt* gene in mice was established by C. Walkey in 1996 [8] and subsequently the *Pemt* gene was disrupted in mice [9]. The mice appeared overtly normal and the levels of PE and PC were minimally affected by elimination of PEMT. The observation that PC levels were not affected is possibly due to a 60% increase in activity of CT in the livers of the $Pemt^{-/-}$ mice (another example of reciprocal expression of the PEMT and the CDP-choline pathways).

It was not too surprising that $Pemt^{-/-}$ mice did not exhibit an obvious phenotype since the mice still had the more quantitatively significant CDP-choline pathway that is estimated to be responsible for 70% of the PC made in liver [22,36]. Thus, we stressed the $Pemt^{-/-}$ mice by feeding them a choline-deficient diet. A remarkable phenotype resulted and the mice developed severe liver failure (Fig. 2) after 3 days, and would have died within the next 24–48 h had they not been sacrificed [37]. The experiments suggested that the PEMT pathway was selected during evolution to provide a distinct advantage for animals during fasting/starvation. Under those conditions, choline would not be ingested from the diet and the animal would rely on PEMT to convert PE to PC that could be degraded as a source of choline (see Section 8 for more on PEMT as a source of choline).

The rapidity of liver failure in $Pemt^{-/-}$ mice fed the choline-deficient diet was initially an enigma. A major pathway for utilization of hepatic PC is the secretion of bile into the intestine. The liver of a 20 g mouse contains ~20 mg of PC and secretes ~23 mg of PC into bile each day [38]. In 1993, Smit et al. disrupted the gene (*Mdr2*) responsible for transfer of PC into the bile [39]. Upon breeding the $Pemt^{-/-}$ mice with $Mdr2^{-/-}$ mice, we generated mice in which both genes were disrupted. Subsequently, we fed the double knock-out mice a choline-deficient diet. As expected the $Pemt^{-/-}$ mice showed rapid liver failure in 3 days. In contrast, the $Pemt^{-/-}/Mdr2^{-/-}$ mice survived for >90 days [40]. Thus, it appears that the acute lethality in $Pemt^{-/-}$ mice fed the choline-deficient diet is due to the rapid depletion of hepatic PC via biliary PC secretion.

The $Pemt^{-/-}$ mice exhibited both steatosis and steatohepatitis that led to liver failure when fed the choline-deficient diet whereas $Pemt^{-/-}/Mdr2^{-/-}$ mice developed only severe steatosis [41]. The liver failure was shown to be due to a loss of membrane integrity due to a ratio of PC to PE that decreased below 1 (normal ratio is ~1.8). This abnormal PC/PE ratio in hepatocytes led to leakage of

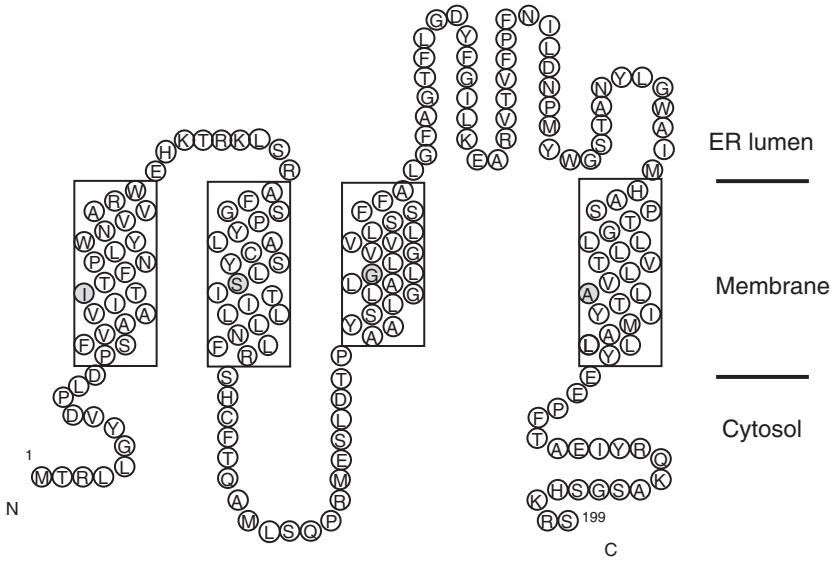

Fig. 1. Proposed orientation for PEMT in the membrane of the endoplasmic reticulum. N refers to amino terminal of the protein and C to the carboxyl terminal of PEMT. This figure was kindly supplied by Dr. Joanne Lemieux and TuoHuang Zhang, University of Alberta.

proteins (e.g., alanine aminotransferase) through the plasma membrane, and cell death. In contrast, the *Pemt*$^{-/-}$/*Mdr2*$^{-/-}$ mice maintained a near normal PC/PE ratio and cell membrane integrity was maintained. The PC/PE ratio thus appears to be a key regulator of cell membrane permeability and a disturbance in this ratio may have an important role in the progression of steatosis into steatohepatitis. In addition, the studies demonstrate that PEMT is an important source of PC for hepatic membranes.

In subsequent studies we tested the hypothesis that PEMT was required for optimal secretion of bile from the livers of mice. However, PC secretion into bile was not altered in *Pemt*$^{-/-}$ mice under normal choline supplementation [42]. Hence, the CDP-choline pathway is sufficient for normal PC secretion into bile even when bile secretion is stimulated by infusion of bile salts.

Pemt$^{-/-}$ mice are susceptible to hepatic steatosis and abnormally low levels of choline metabolites in the liver when fed a normal amount of choline in their drinking water (6.4 mmol/L) [43,44]. Nevertheless, even when the choline content of the drinking water was increased to 29 mmol/L, the *Pemt*$^{-/-}$ mice still exhibited steatosis. Thus, it seems that PEMT activity is quantitatively a very important source of choline. In other studies, PEMT has been implicated as an important source of PC species containing arachidonic acid and docosahexaenoic acid (DHA) that are secreted into plasma [44]. In line with these findings, brains from *Pemt*$^{-/-}$ mice at gestational day 17 contained 25–50% less DHA in the phospholipids than did *Pemt*$^{+/+}$ mice [45]. Supplementation of the diet of *Pemt*$^{-/-}$ mice with DHA increased fetal brain phospholipid-DHA to levels similar to those in *Pemt*$^{+/+}$ mice. Clinical studies have also implicated DHA content of plasma PC as a potential marker of PEMT activity in humans [46].

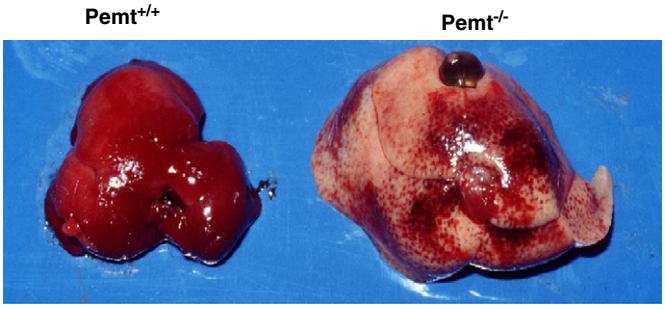

Fig. 2. Photo of livers from *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice that consumed a choline deficient diet for 3 days. The gall bladder can be observed in the liver from the *Pemt*$^{-/-}$ mouse.

6. PEMT, lipoprotein secretion and atherosclerosis

When very low density lipoproteins (VLDL) are secreted from the liver they are enriched in PC compared to hepatocytes [47]. Studies in the 1980s demonstrated that either choline or methionine was required for normal secretion of VLDL from rat primary hepatocytes [48–50]. Whereas choline is a precursor of PC produced via the CDP-choline pathway, methionine is converted to AdoMet that is the methyl donor for the conversion of PE to PC via PEMT. Studies in mice in which CTα (rate-limiting enzyme for PC biosynthesis via the CDP-choline pathway [51]) was eliminated specifically in the liver (hepatocytes) demonstrated that the secretion of VLDL was decreased despite a 2-fold increase in the expression of PEMT in these livers [52]. Similarly, hepatocytes from mice that lacked PEMT had decreased secretion of VLDL compared to *Pemt*$^{+/+}$ mice [53]. In partial agreement with these observations, PEMT deficiency decreased the hepatic secretion of VLDL in male, but not female, *Pemt*$^{-/-}$ mice [27]. Thus, it seems that both the CDP-choline and the PEMT pathways are required for optimal secretion of VLDL from livers of mice. Why one pathway cannot compensate for the other is not clear. Nor do we understand the mechanism underlying the gender-specific effects on the secretion of VLDL from *Pemt*$^{-/-}$ mice.

The availability of *Pemt*$^{-/-}$ mice with decreased VLDL secretion allowed us to address the question of whether or not *Pemt*$^{-/-}$ mice were protected from atherosclerosis. C57Bl6 mice are resistant to diet-induced atherosclerosis. Studies are, thus, often performed with gene-targeted mice that are prone to this disease. Two commonly used models are mice lacking LDL receptors (*Ldlr*$^{-/-}$) that are fed a high-fat, high-cholesterol diet, and chow-fed mice that lack apolipoprotein E (*Apoe*$^{-/-}$) [54]. We, therefore, cross bred the *Pemt*$^{-/-}$ mice with mice having either of these two genetic backgrounds.

PEMT deficiency strikingly protected *Ldlr*$^{-/-}$ mice from atherosclerosis. Compared to *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice fed the high-fat, high-cholesterol diet for 16 weeks, *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice developed ~80% fewer atherosclerotic lesions (Fig. 3) [55]. This finding was consistent with the atheroprotective plasma lipoprotein profile in which VLDL/low density lipoprotein lipids were significantly lower in the *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice (TG reduced by ~70%, cholesterol by ~56%, phospholipid by ~45% and cholesteryl esters by ~69%) [55]. The levels of plasma apo B100 and apo B48 were reduced by 30% and

60% respectively. VLDL secretion was attenuated in the *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice compared to *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice consistent with the decreased plasma lipid levels. In addition, the VLDL particles in plasma of *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice had a significant 34% lower content of PC than did VLDL in the plasma of *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice [55]. The in vivo clearance of VLDLs from *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice was also much more rapid than from *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice, likely due to the alteration in VLDL structure caused by decreased lipid content. Thus, the protection from atherosclerosis observed in *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice compared to *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice is due to impaired PC biosynthesis in the liver that leads to diminished secretion of PC-deficient VLDL that is more rapidly cleared compared to VLDL produced by *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice.

PEMT deficiency in *Apoe*$^{-/-}$ mice fed a chow diet for 1 year also provided protection from the development of atherosclerosis [56]. In this study, cardiac function was also assessed. In vivo echocardiography demonstrated a ~50% improvement in systolic function in the *Pemt*$^{-/-}$/*Apoe*$^{-/-}$ mice compared to *Pemt*$^{+/+}$/*Apoe*$^{-/-}$ mice. In addition, there was a significant 34% reduction in TG in the hearts of *Apoe*$^{-/-}$ mice that lacked PEMT. Thus, our data suggest that disruption of the *Pemt* gene decreases atherosclerosis and attenuates the development of a lipotoxic cardiomyopathy in mice that lack apo E.

7. The PEMT reaction is a major source of plasma homocysteine

Mild hyper-homocysteinemia is an independent risk factor for cardiovascular and atherosclerotic diseases [57–59]. A 50% increase in plasma homocysteine (Hcy) is linked to a 60% increase in risk of coronary artery disease for men, and 80% increase for women. Hcy is a non-protein amino acid that is a breakdown product of AdoHcy which is generated by transmethylation reactions in which AoMet is the methyl donor [57]. Hcy can be converted to methionine or cysteine, or can be secreted. A potential major source of Hcy is AdoHcy generated by the 3 successive methylation reactions catalyzed by PEMT during the conversion of PE to PC. In studies with *Pemt*$^{-/-}$ mice, we showed that the plasma levels of Hcy were decreased by 50% compared to those in *Pemt*$^{+/+}$ mice [60]. Moreover, hepatocytes isolated from *Pemt*$^{-/-}$ mice secreted 50% less Hcy than did *Pemt*$^{+/+}$ hepatocytes [60]. In companion experiments, McArdle RH7777 hepatoma cells stably transfected with a cDNA encoding PEMT resulted in an almost 3-fold higher amount of Hcy in the medium after a 24 h incubation compared to hepatoma cells transfected with vector alone [60].

To gain further insight into the physiological role of PEMT as a modulator of plasma Hcy, we studied Hcy formation in mice that lacked hepatic CTα (LCTαKO). In these mice, the activity of PEMT is double that in mice that express CTα [52,61] and plasma Hcy was 20–40% higher in the LCTαKO mice [61]. Furthermore, hepatocytes isolated from LCTαKO mice secreted 40% more Hcy than did control hepatocytes. Thus, PEMT is not only important for producing PC in the liver but is also a significant source of plasma Hcy in mice.

There are at least 50 AdoMet-dependent methylation reactions in mammals [62]. The biosynthesis of creatine has been estimated to utilize ~70% of AdoMet in humans [63]. With the new information available on the quantitative significance of the PEMT reaction in the utilization of AdoMet, it is likely that the contribution of creatine biosynthesis to AdoMet utilization may be less than previously estimated [64,65].

8. No one predicted a relationship between PEMT, obesity and type 2 diabetes

Perhaps one of the most rewarding aspects of biomedical research is that we expect the unexpected. An excellent example is the discovery that mice that lack PEMT are strikingly protected from high fat-induced obesity and insulin resistance [66]. When the Norwegian Jon Bremer first characterized PEMT activity in microsomes [5], a relationship between this membrane-bound enzyme and obesity would not have entered his mind. There were no clues to this connection between PEMT and obesity until *Pemt*$^{-/-}$ mice were fed a high-fat diet. Unlike *Pemt*$^{+/+}$ mice that gained 10 g over 10 weeks when fed the high-fat diet, the PEMT-deficient mice held their weight constant at 30 g (Fig. 4) [66]. The *Pemt*$^{-/-}$ mice were also protected against insulin resistance that developed in the *Pemt*$^{+/+}$ mice fed the high-fat diet, as reflected by their intolerance to glucose and insulin [66]. On the other hand, the *Pemt*$^{-/-}$ mice developed steatosis (TG in livers from *Pemt*$^{+/+}$ mice was 184 μg/mg protein compared to 790 μg/mg protein in *Pemt*$^{-/-}$ mice). In the *Pemt*$^{-/-}$ mice, plasma alanine aminotransferase (a marker for liver disease) was 10-fold higher than in *Pemt*$^{+/+}$ mice (7.2 units/L compared to 71.2 units/L).

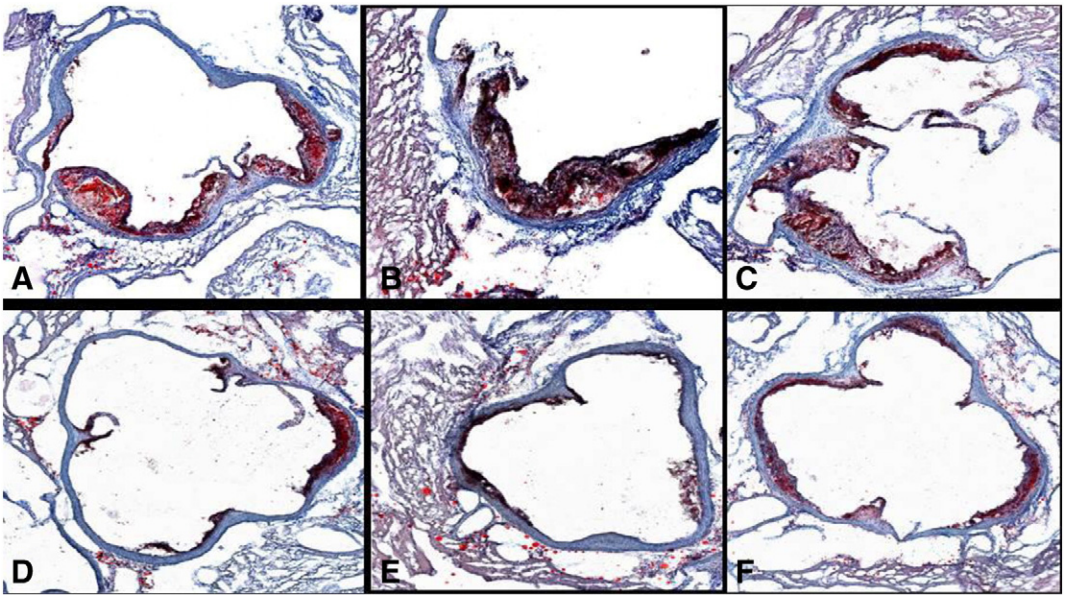

Fig. 3. Atherosclerosis in aortic root regions from *Pemt*$^{+/+}$/*LDLr*$^{-/-}$ mice (top 3 panels) and from *Pemt*$^{-/-}$/*LDLr*$^{-/-}$ mice (bottom 3 panels). Stained with oil red O (red) and hematoxylin (light blue).

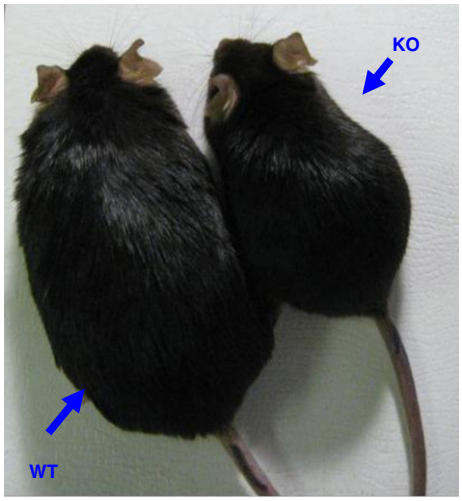

Fig. 4. Photo of a *Pemt*$^{+/+}$ mouse (WT) and a *Pemt*$^{-/-}$ mouse (KO) that have been fed a high fat diet for 10 weeks.

How does the lack of PEMT protect against obesity and insulin resistance (type 2 diabetes)? One explanation is that the *Pemt*$^{-/-}$ mice fed the high fat diet are hypermetabolic, as reflected in their increased oxygen consumption. Moreover, these mice rely more on glucose, than fat, as a source of energy compared to *Pemt*$^{+/+}$ mice [66].

We considered the possibility that the significant decrease in hepatic PC from 76 nmol/mg protein in *Pemt*$^{+/+}$ mice to 57 nmol/mg protein in *Pemt*$^{-/-}$ mice might lead to decreased obesity and increased insulin sensitivity. Hence, we determined if the hepatic specific CTα knockout (LCTαKO) mice were also protected from obesity/insulin resistance. This seemed a reasonable hypothesis since the CT pathway accounts for ~70% of hepatic PC biosynthesis [22,67]. However, compared to floxed control mice, the LCTαKO mice gained a similar 10 g of weight when fed the high fat diet for 10 weeks [66]; oxygen consumption and glucose tolerance test were the same in the two strains of mice. Thus, the amount of PC in the liver does not appear to be a factor in protecting *Pemt*$^{-/-}$ mice from obesity/insulin resistance.

We have focused on the role of PEMT in PC production particularly in the liver. What is generally not appreciated is that PC can be degraded via phospholipases C/D to generate phosphocholine/choline [68]. Thus, LCTαKO mice (that contain PEMT) can synthesize choline de novo whereas *Pemt*$^{-/-}$ mice cannot. The high fat diet we have used contains 1.3 g of choline/kg diet. Hence, the mice are not choline-deficient. But perhaps the *Pemt*$^{-/-}$ mice were not choline sufficient. Hence, we supplemented the high-fat diet with an additional 2.7 g of choline/kg diet and were astonished to find that the benefits of reduced obesity and increased insulin sensitivity in *Pemt*$^{-/-}$ mice were abolished. Thus, the *Pemt*$^{-/-}$ mice fed the high-fat/high-choline diet gained weight, developed glucose intolerance and showed other metabolic features characterisitic of *Pemt*$^{+/+}$ mice fed the high-fat diet [66]. These results strongly imply that the deficiency of PC in the liver per se had not provided the protection observed in *Pemt*$^{-/-}$ mice fed the high-fat diet, but rather the deficiency in choline biosynthesis provided the beneficial effect. Further evidence for this hypothesis was obtained when *Pemt*$^{+/+}$ mice were fed the choline-deficient diet for 12 weeks; these mice were also protected from diet-induced obesity/insulin resistance [66]. These observations are consistent with the report that when obese mice were fed either a choline-deficient, or a choline-supplemented/high fat diet, for 4 weeks insulin resistance was lower in the choline deficient mice [69]. During this period no difference was observed in weight gain between mice fed the two diets [69]. Moreover, when male ob/ob mice (lack leptin and become very obese) were fed a high fat diet ± choline for two months, the choline deficient mice gained less weight and exhibited improved insulin and glucose tolerance compared to the choline-supplemented mice [70].

How does a decreased supply of choline produce an anti-obesity/anti-insulin resistance phenotype in mice? While current data strongly suggest that the liver is involved in determining the phenotype, recent studies with 3T3-L1 adipocytes suggest that PEMT in adipose tissue might also be involved [19]. PEMT is not expressed in 3T3-L1 fibroblasts but 4 days after the cells are differentiated into adipocytes, PEMT is expressed. Subsequent experiments demonstrated that PEMT had an important role in the stabilization of lipid droplets in 3T3-L1 adipocytes [19]. Hence, although the activity of PEMT is relatively low in adipose tissue compared to liver, the conversion of PE to PC appears to be important for normal fat deposition. Thus, lack of PEMT in adipose tissue might contribute to the lower fat mass in mice fed the high-fat diet for 10 weeks.

9. The human connection

With the progress in DNA sequencing it is now possible to gain insight into human physiology through studies on genetic polymorphisms. Thus, Zeisel and co-workers found that a Val-to-Met substitution at residue 175 of the human PEMT protein was associated with non-alcoholic fatty liver disease in a group composed mainly of Caucasians [71]. cDNAs encoding the Val isoform and the Met form of PEMT were expressed in McArdle hepatoma cells and the Met isoform of PEMT had ~40% lower specific activity than the Val form [71]. When the polymorphism for residue 175 was examined in 59 control subjects and 28 humans with nonalcoholic liver disease, the Met/Met substitution occurred in 68% of the subjects with non-alcoholic liver disease but in only 41% of control subjects. Thus, the V175M polymorphism was suggested to confer susceptibility to nonalcoholic liver disease in humans. In agreement with this conclusion, the V175M variant was found to be more frequent in patients with non-alcoholic steatohepatitis than in healthy subjects [72]. In contrast, no association between the V175M mutation and steatosis was observed in the Dallas Heart study that involved multiple races [73]. When only Caucasians were considered in the Dallas Heart study a relationship between the V175M polymorphism and liver disease became apparent [74]. Thus, it appears that Caucasians have a different distribution of this polymorphism than Hispanics and African Americans. Zeisel also suggests that the V175M polymorphism may be associated with fatty liver only if the secretion of TG into VLDL is decreased and/or TG synthesis increased [74].

A single nucleotide polymorphism (G to C) was also identified in the promoter region of the PEMT gene [75]. Of the 23 subjects that had the C allele, 18 developed “organ dysfunction” when fed a low choline diet. Subsequently, a dose–response relationship also was revealed between the C allele and organ dysfunction in subjects fed a very-low-choline diet. In pre-menopausal women, those with 2 C alleles were much more likely to develop organ problems than were women with 1 or 0 C alleles [76]. Post-menopausal women who received estrogen were much less likely to develop organ dysfunction when fed a low choline diet [76]. The authors concluded that post-menopausal women with low estrogen levels have a higher dietary requirement for choline than do pre-menopausal women. Moreover, the C allele increased the requirement for choline in both groups of women [76].

10. Future directions

Fig. 5 provides a summary of the different functions of PEMT in mouse liver. Although there has been significant progress in understanding the roles of PEMT in the past two decades, there are still many important questions to be addressed. Of key importance is to determine the mechanism(s) by which large supplements of choline can override the protection from obesity/insulin resistance observed in *Pemt*$^{-/-}$ mice fed a high fat diet. Second, pharmaceutical inhibition of PEMT might protect humans from atherosclerosis, obesity and type

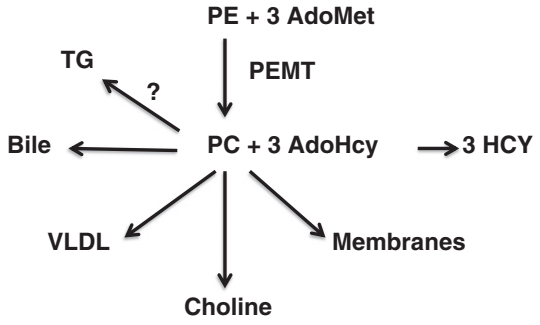

Fig. 5. Physiological roles for PEMT in mouse liver. Phosphatidylethanolamine (PE) + 3 *S*-adenosylmethionines (AdoMet) is converted to phosphatidylcholine (PC) and 3 *S*-adenosylhomocysteines (AdoHcy) by PEMT. PC is utilized for: the structure of all membranes in the hepatocye, the formation of choline, secretion of very low density lipoproteins (VLDL), secretion into bile and possibly the formation of triacylglycerols (TG). AdoHcy is converted to homocysteine (Hcy) that is secreted into the plasma.

2 diabetes, although a complication is the development of steatosis/steatohepatitis in $Pemt^{-/-}$ mice fed the high fat diet. Thus, it important to determine if steatosis also occurs in animal models, such as the guinea pig or hamster, that have a lipid metabolic profile closer to that in humans. If steatosis also occurred in these animals, one would need to think creatively about mechanisms by which steatosis might be relieved when PEMT was inhibited. A third consideration is that PC in hepatocyes [77], and PC associated with low density lipoproteins [78] and high density lipoproteins [79,80] that are supplied to hepatocytes, is a source of significant amounts of TG. It is not yet known if PC made via PEMT is an important source of hepatic TG.

Acknowledgements

I enthusiastically thank my co-workers and collaborators who have contributed to the research from my lab that has been summarized here. I am very grateful to the Canadian Institutes of Health Research, the Alberta Heritage Foundation for Medical Research (now Alberta Innovates-Health Solutions), the Canadian Diabetes Association and the Heart and Stroke Foundation of Alberta for financial support. I appreciate the very helpful comments of Drs. René Jacobs and Jean Vance for helpful comments on the manuscript and Dr. Joanne Lemieux and Tuo Huang Zhang for supplying Fig. 1.

References

[1] V. du Vigneaud, M. Cohn, J.P. Chandler, J.R. Schenck, S. Simmonds, The utilization of the methyl group of methionine in the biological synthesis of choline and creatine, J. Biol. Chem. 140 (1941) 625–641.
[2] D. Stetten, Biological relationships of choline, ethanolamine and related compounds, J. Biol. Chem. 138 (1941) 437–438.
[3] J. Bremer, P.H. Figard, D.M. Greenberg, The biosynthesis of choline and its relation to phospholipid metabolism, Biochim. Biophys. Acta 43 (1960) 477–488.
[4] J. Bremer, D.M. Greenberg, Biosynthesis of choline in vitro, Biochim. Biophys. Acta 37 (1960) 173–175.
[5] J. Bremer, D.M. Greenberg, Methyl transferring enzyme system of microsomes in the bioysnthesis of lecithin (phosphatidylcholine), Biochim. Biophys. Acta 46 (1961) 205–216.
[6] D.E. Vance, Phosphatidylcholine metabolism: masochistic enzymology, metabolic regulation and lipoprotein assembly, Biochem. Cell Biol. 68 (1990) 1151–1165.
[7] N.D. Ridgway, D.E. Vance, Purification of phosphatidylethanolamine *N*-methyltransferase from rat liver, J. Biol. Chem. 262 (1987) 17,231–17,239.
[8] C.J. Walkey, Z. Cui, L.B. Agellon, D.E. Vance, Characterization of the murine phosphatidylethanolamine *N*-methyltransferase-2 gene, J. Lipid Res. 37 (1996) 2341–2350.
[9] C.J. Walkey, L.R. Donohue, R. Bronson, L.B. Agellon, D.E. Vance, Disruption of the murine gene encoding phosphatidylethanolamine *N*-methyltransferase, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 12,880–12,885.
[10] N.D. Ridgway, Z. Yao, D.E. Vance, Phosphatidylethanolamine levels and regulation of phosphatidylethanolamine *N*-methyltransferase, J. Biol. Chem. 264 (1989) 1203–1207.
[11] N.D. Ridgway, D.E. Vance, Kinetic mechanism of phosphatidylethanolamine *N*-methyltransferase, J. Biol. Chem. 263 (1988) 16,864–16,871.
[12] F. Audubert, D.E. Vance, Pitfalls and problems in studies on the methylation of phosphatidylethanolamine, J. Biol. Chem. 258 (1983) 10,695–10,701.
[13] D.J. Shields, R. Lehner, L.B. Agellon, D.E. Vance, Membrane topography of human phosphatidylethanolamine *N*-methyltransferase, J. Biol. Chem. 278 (2003) 2956–2962.
[14] D.J. Shields, J.Y. Altarejos, X. Wang, L.B. Agellon, D.E. Vance, Molecular dissection of the AdoMet binding site of phosphatidylethanolamine *N*-methyltransferase, J. Biol. Chem. 278 (2003) 35,826–35,836.
[15] C.J. Walkey, D.J. Shields, D.E. Vance, Identification of three novel cDNAs for human phosphatidylethanolamine *N*-methyltransferase and localization of the human gene on chromosome 17p11.2, Biochim. Biophys. Acta 1436 (1999) 405–412.
[16] D.J. Shields, L.B. Agellon, D.E. Vance, Structure, expression profile and alternative processing of the human phosphatidylethanolamine *N*-methyltransferase (PEMT) gene, Biochim. Biophys. Acta 1532 (2001) 105–114.
[17] S.Y. Morita, A. Takeuchi, S. Kitagawa, Functional analysis of two isoforms of phosphatidylethanolamine *N*-methyltransferase, Biochem. J. 432 (2010) 387–398.
[18] D.E. Vance, N.D. Ridgway, The methylation of phosphatidylethanolamine, Prog. Lipid Res. 27 (1988) 61–79.
[19] G. Horl, A. Wagner, L.K. Cole, R. Malli, H. Reicher, P. Kotzbeck, H. Kofeler, G. Hofler, S. Frank, J.G. Bogner-Strauss, W. Sattler, D.E. Vance, E. Steyrer, Sequential synthesis and methylation of phosphatidylethanolamine promote lipid droplet biosynthesis and stability in tissue culture and in vivo, J. Biol. Chem. 286 (2011) 17,338–17,350.
[20] Z. Cui, J.E. Vance, M.H. Chen, D.R. Voelker, D.E. Vance, Cloning and expression of a novel phosphatidylethanolamine *N*-methyltransferase. A specific biochemical and cytological marker for a unique membrane fraction in rat liver, J. Biol. Chem. 268 (1993) 16,655–16,663.
[21] J.E. Vance, Phospholipid synthesis in a membrane fraction associated with mitochondria, J. Biol. Chem. 265 (1990) 7248–7256.
[22] R. Sundler, B. Akesson, Regulation of phospholipid biosynthesis in isolated rat hepatocytes. Effect of different substrates, J. Biol. Chem. 250 (1975) 3359–3367.
[23] D.R. Hoffman, D.W. Marion, W.E. Cornatzer, J.A. Duerre, *S*-Adenosylmethionine and S-adenosylhomocystein metabolism in isolated rat liver. Effects of L-methionine, L-homocystein, and adenosine, J. Biol. Chem. 255 (1980) 10,822–10,827.
[24] M. Resseguie, J. Song, M.D. Niculescu, K.A. da Costa, T.A. Randall, S.H. Zeisel, Phosphatidylethanolamine *N*-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes, FASEB J. 21 (2007) 2622–2632.
[25] M.E. Resseguie, K.A. da Costa, J.A. Galanko, M. Patel, I.J. Davis, S.H. Zeisel, Aberrant estrogen regulation of PEMT results in choline deficiency-associated liver dysfunction, J. Biol. Chem. 286 (2011) 1649–1658.
[26] L.M. Fischer, K.A. da Costa, L. Kwock, P.W. Stewart, T.S. Lu, S.P. Stabler, R.H. Allen, S.H. Zeisel, Sex and menopausal status influence human dietary requirements for the nutrient choline, Am. J. Clin. Nutr. 85 (2007) 1275–1285.
[27] A.A. Noga, D.E. Vance, A gender-specific role for phosphatidylethanolamine *N*-methyltransferase-derived phosphatidylcholine in the regulation of plasma high density and very low density lipoproteins in mice, J. Biol. Chem. 278 (2003) 21,851–21,859.
[28] L.K. Cole, D.E. Vance, A role for Sp1 in transcriptional regulation of phosphatidylethanolamine *N*-methyltransferase in liver and 3T3-L1 adipocytes, J. Biol. Chem. 285 (2010) 11,880–11,891.
[29] C. Banchio, L.M. Schang, D.E. Vance, Activation of CTP:phosphocholine cytidylyltransferase alpha expression during the S phase of the cell cycle is mediated by the transcription factor Sp1, J. Biol. Chem. 278 (2003) 32,457–32,464.
[30] C. Banchio, L.M. Schang, D.E. Vance, Phosphorylation of Sp1 by cyclin-dependent kinase 2 modulates the role of Sp1 in CTP:phosphocholine cytidylyltransferase alpha regulation during the S phase of the cell cycle, J. Biol. Chem. 279 (2004) 40,220–40,226.
[31] C. Banchio, S. Lingrell, D.E. Vance, Sp-1 binds promoter elements that are regulated by Rb and regulate CTP:phosphocholine cytidylyltransferase-alpha transcription, J. Biol. Chem. 282 (2007) 14,827–14,835.
[32] H. Sugimoto, C. Banchio, D.E. Vance, Transcriptional regulation of phosphatidylcholine biosynthesis, Prog. Lipid Res. 47 (2008) 204–220.
[33] E. Sesca, G.P. Perletti, V. Binasco, M. Chiara, L. Tessitore, Phosphatidylethanolamine *N*-methyltransferase 2 and CTP–phosphocholine cytidylyltransferase expressions are related with protein kinase C isozymes in developmental liver growth, Biochem. Biophys. Res. Commun. 229 (1996) 158–162.
[34] M. Houweling, Z. Cui, L. Tessitore, D.E. Vance, Induction of hepatocyte proliferation after partial hepatectomy is accompanied by a markedly reduced expression of phosphatidylethanolamine *N*-methyltransferase-2, Biochim. Biophys. Acta 1346 (1997) 1–9.
[35] Z. Cui, Y.J. Shen, D.E. Vance, Inverse correlation between expression of phosphatidylethanolamine *N*-methyltransferase-2 and growth rate of perinatal rat livers, Biochim. Biophys. Acta 1346 (1997) 10–16.
[36] C.J. DeLong, Y.-J. Shen, M.J. Thomas, Z. Cui, Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway, J. Biol. Chem. 274 (1999) 29,683–29,688.
[37] C.J. Walkey, L. Yu, L.B. Agellon, D.E. Vance, Biochemical and evolutionary significance of phospholipid methylation, J. Biol. Chem. 273 (1998) 27,043–27,046.
[38] F. Kuipers, R.P. Oude Elferink, H.J. Verkade, A.K. Groen, Mechanisms and (patho) physiological significance of biliary cholesterol secretion, Subcell. Biochem. 28 (1997) 295–318.
[39] J.J. Smit, A.H. Schinkel, R.P. Oude Elferink, A.K. Groen, E. Wagenaar, L. van Deemter, C.A. Mol, R. Ottenhoff, N.M. van der Lugt, M.A. van Roon, M.A. van der Valk, G.J.A. Offerhaus, A.J.M. Berns, P. Borst, Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease, Cell 75 (1993) 451–462.

[40] Z. Li, L.B. Agellon, D.E. Vance, Phosphatidylcholine homeostasis and liver failure, J. Biol. Chem. 280 (2005) 37,798–37,802.
[41] Z. Li, L.B. Agellon, T.M. Allen, M. Umeda, L. Jewell, A. Mason, D.E. Vance, The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis, Cell Metab. 3 (2006) 321–331.
[42] H.J. Verkade, R. Havinga, D.J. Shields, H. Wolters, V.W. Bloks, F. Kuipers, D.E. Vance, L.B. Agellon, The phosphatidylethanolamine *N*-methyltransferase pathway is quantitatively not essential for biliary phosphatidylcholine secretion, J. Lipid Res. 48 (2007) 2058–2064.
[43] X. Zhu, J. Song, M.H. Mar, L.J. Edwards, S.H. Zeisel, Phosphatidylethanolamine *N*-methyltransferase (PEMT) knockout mice have hepatic steatosis and abnormal hepatic choline metabolite concentrations despite ingesting a recommended dietary intake of choline, Biochem. J. 370 (2003) 987–993.
[44] S.M. Watkins, X. Zhu, S.H. Zeisel, Phosphatidylethanolamine-*N*-methyltransferase activity and dietary choline regulate liver-plasma lipid flux and essential fatty acid metabolism in mice, J. Nutr. 133 (2003) 3386–3391.
[45] K.A. da Costa, K.S. Rai, C.N. Craciunescu, K. Parikh, M.G. Mehedint, L.M. Sanders, A. McLean-Pottinger, S.H. Zeisel, Dietary docosahexaenoic acid supplementation modulates hippocampal development in the *Pemt*$^{-/-}$ mouse, J. Biol. Chem. 285 (2010) 1008–1015.
[46] K.A. da Costa, L.M. Sanders, L.M. Fischer, S.H. Zeisel, Docosahexaenoic acid in plasma phosphatidylcholine may be a potential marker for in vivo phosphatidylethanolamine *N*-methyltransferase activity in humans, Am. J. Clin. Nutr. 93 (2010) 968–974.
[47] J.E. Vance, K. Adeli, Assembly and secretion of triacylglycerol-rich lipoproteins, in: Biochemistry of Lipids, Lipoproteins and Membranes, 5th edn, Elsevier B.V., San Diego, CA, 2008, pp. 507–531.
[48] Z. Yao, D.E. Vance, The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes, J. Biol. Chem. 263 (1988) 2998–3004.
[49] Z. Yao, D.E. Vance, Head group specificity in the requirement of phosphatidylcholine biosynthesis for very low density lipoprotein secretion from cultured hepatocytes, J. Biol. Chem. 264 (1989) 11,373–11,380.
[50] A. Kulinski, D.E. Vance, J.E. Vance, A choline-deficient diet in mice inhibits neither the CDP-choline pathway for phosphatidylcholine synthesis in hepatocytes nor apolipoprotein B secretion, J. Biol. Chem. 279 (2004) 23,916–23,924.
[51] D.E. Vance, J.E. Vance, Phospholipid biosynthesis in eukaryotes, in: D.E. Vance, J.E. Vance (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes, 5th edn, Elsevier B.V., San Diego, CA, 2008, pp. 214–244.
[52] R.L. Jacobs, C. Devlin, I. Tabas, D.E. Vance, Targeted deletion of hepatic CTP: phosphocholine cytidylyltransferase a in mice decreases plasma high density and very low density lipoproteins, J. Biol. Chem. 279 (2004) 47,402–47,410.
[53] A.A. Noga, Y. Zhao, D.E. Vance, An unexpected requirement for phosphatidylethanolamine *N*-methyltransferase in the secretion of very low density lipoproteins, J. Biol. Chem. 277 (2002) 42,358–42,365.
[54] A.S. Plump, J.L. Breslow, Apolipoprotein E and the apolipoprotein E-deficient mouse, Annu. Rev. Nutr. 15 (1995) 495–518.
[55] Y. Zhao, B. Su, R.L. Jacobs, B. Kennedy, G.A. Francis, E. Waddington, J.T. Brosnan, J.E. Vance, D.E. Vance, Lack of phosphatidylethanolamine *N*-methyltransferase alters plasma VLDL phospholipids and attenuates atherosclerosis in mice, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1349–1355.
[56] L.K. Cole, V.W. Dolinsky, J.R. Dyck, D.E. Vance, Impaired phosphatidylcholine biosynthesis reduces atherosclerosis and prevents lipotoxic cardiac dysfunction in *ApoE*$^{-/-}$ mice, Circ. Res. 108 (2011) 686–694.
[57] K. Robinson, Homocysteine and coronary artery disease, in: R. Carmel, D.W. Jacobson (Eds.), Homocysteine in Health and Disease, Cambridge University Press, Cambridge, 2001, pp. 371–383.
[58] H. Refsum, P.M. Ueland, O. Nygard, S.E. Vollset, Homocysteine and cardiovascular disease, Annu. Rev. Med. 49 (1998) 31–62.
[59] C.J. Boushey, S.A. Beresford, G.S. Omenn, A.G. Motulsky, A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes, JAMA 274 (1995) 1049–1057.
[60] A.A. Noga, L.M. Stead, Y. Zhao, M.E. Brosnan, J.T. Brosnan, D.E. Vance, Plasma homocysteine is regulated by phospholipid methylation, J. Biol. Chem. 278 (2003) 5952–5955.
[61] R.L. Jacobs, L.M. Stead, C. Devlin, I. Tabas, M.E. Brosnan, J.T. Brosnan, D.E. Vance, Physiological regulation of phospholipid methylation alters plasma homocysteine in mice, J. Biol. Chem. 280 (2005) 28,299–28,305.
[62] J.E. Katz, M. Dlakic, S. Clarke, Automated identification of putative methyltransferases from genomic open reading frames, Mol. Cell. Proteomics 2 (2003) 525–540.
[63] S.H. Mudd, J.R. Poole, Labile methyl balances for normal humans on various dietary regimens, Metabolism 24 (1975) 721–735.
[64] L.M. Stead, J.T. Brosnan, M.E. Brosnan, D.E. Vance, R.L. Jacobs, Is it time to reevaluate methyl balance in humans? Am. J. Clin. Nutr. 83 (2006) 5–10.
[65] S.H. Mudd, J.T. Brosnan, M.E. Brosnan, R.L. Jacobs, S.P. Stabler, R.H. Allen, D.E. Vance, C. Wagner, Methyl balance and transmethylation fluxes in humans, Am. J. Clin. Nutr. 85 (2007) 19–25.
[66] R.L. Jacobs, Y. Zhao, D.P. Koonen, T. Sletten, B. Su, S. Lingrell, G. Cao, D.A. Peake, M.S. Kuo, S.D. Proctor, B.P. Kennedy, J.R. Dyck, D.E. Vance, Impaired de novo choline synthesis explains why phosphatidylethanolamine *N*-methyltransferase-deficient mice are protected from diet-induced obesity, J. Biol. Chem. 285 (2010) 22,403–22,413.
[67] R. Sundler, B. Akesson, Biosynthesis of phosphatidylethanolamines and phosphatidylcholines from ethanolamine and choline in rat liver, Biochem. J. 146 (1975) 309–315.
[68] S.H. Zeisel, Phosphatidylcholine: endogenous precursor of choline, in: I. Hanin, G.B. Ansell (Eds.), Lecithin: Technological, Biological and Therapeutic Aspects, Plenum Press, New York, 1987, pp. 107–120.
[69] P.J. Raubenheimer, M.J. Nyirenda, B.R. Walker, A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet, Diabetes 55 (2006) 2015–2020.
[70] G. Wu, L. Zhang, T. Li, G. Lopaschuk, R.L. Jacobs, D.E. Vance, Choline deficiency attenuates weight gain and improves glucose tolerance in ob/ob mice, J. Obes. 2012 (2012) 319172.
[71] J. Song, K.A. da Costa, L.M. Fischer, M. Kohlmeier, L. Kwock, S. Wang, S.H. Zeisel, Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD), FASEB J. 19 (2005) 1266–1271.
[72] H. Dong, J. Wang, C. Li, A. Hirose, Y. Nozaki, M. Takahashi, M. Ono, N. Akisawa, S. Iwasaki, T. Saibara, S. Onishi, The phosphatidylethanolamine *N*-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population, J. Hepatol. 46 (2007) 915–920.
[73] S. Romeo, J.C. Cohen, H.H. Hobbs, No association between polymorphism in PEMT (V175M) and hepatic triglyceride content in the Dallas Heart Study, FASEB J. 20 (2006) 2180.
[74] S.H. Zeisel, People with fatty liver are more likely to have the *PEMT* rs7946 SNP, yet populations with the mutant allele do not have fatty liver, FASEB J. 20 (2006) 2181–2182.
[75] K.A. da Costa, O.G. Kozyreva, J. Song, J.A. Galanko, L.M. Fischer, S.H. Zeisel, Common genetic polymorphisms affect the human requirement for the nutrient choline, FASEB J. 20 (2006) 1336–1344.
[76] L.M. Fischer, K.A. da Costa, L. Kwock, J. Galanko, S.H. Zeisel, Dietary choline requirements of women: effects of estrogen and genetic variation, Am. J. Clin. Nutr. 92 (2010) 1113–1119.
[77] D. Wiggins, G.F. Gibbons, Origin of hepatic very low density lipoprotein triacylglycerol: the contribution of cellular phospholipid, Biochem. J. 320 (1996) 673–679.
[78] C. Minahk, K.W. Kim, R. Nelson, B.L. Trigatti, R. Lehner, D.E. Vance, Conversion of low density lipoprotein-associated phosphatidylcholine to triacylglycerol by primary hepatocytes, J. Biol. Chem. 283 (2008) 6449–6458.
[79] J.C. Robichaud, J.N. van der Veen, Z. Yao, B. Trigatti, D.E. Vance, Hepatic uptake and metabolism of phosphatidylcholine associated with high density lipoproteins, Biochim. Biophys. Acta 1790 (2009) 538–551.
[80] J.N. van der Veen, S. Lingrell, D.E. Vance, The membrane lipid, phosphatidylcholine, is an unexpected source of triacylglycerol in the liver, J. Biol. Chem. 287 (2012) 23418–23426.