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Review

Phosphatidylcholine biosynthesis and lipoprotein metabolism ☆

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ABSTRACT

Phosphatidylcholine (PC) is the major phospholipid component of all plasma lipoprotein classes. PC is the only phospholipid which is currently known to be required for lipoprotein assembly and secretion. Impaired hepatic PC biosynthesis significantly reduces the levels of circulating very low density lipoproteins (VLDLs) and high density lipoproteins (HDLs). The reduction in plasma VLDLs is due in part to impaired hepatic secretion of VLDLs. Less PC within the hepatic secretory pathway results in nascent VLDL particles with reduced levels of PC. These particles are recognized as being defective and are degraded within the secretory system by an incompletely defined process that occurs in a post-endoplasmic reticulum compartment, consistent with degradation directed by the low-density lipoprotein receptor and/or autophagy. Moreover, VLDL particles are taken up more readily from the circulation when the PC content of the VLDLs is reduced, likely due to a preference of cell surface receptors and/or enzymes for lipoproteins that contain less PC. Impaired PC biosynthesis also reduces plasma HDLs by inhibiting hepatic HDL formation and by increasing HDL uptake from the circulation. These effects are mediated by elevated expression of ATP-binding cassette transporter A1 and hepatic scavenger receptor class B type 1, respectively. Hepatic PC availability has recently been linked to the progression of liver and heart disease. These findings demonstrate that hepatic PC biosynthesis can regulate the amount of circulating lipoproteins and suggest that hepatic PC biosynthesis may represent an important pharmaceutical target. This article is part of a Special Issue entitled Triglyceride Metabolism and Disease.

1. Overview and scope

Phosphatidylcholine (PC) was originally described in 1847 as a constituent of egg yolk and was named lecithin based on the Greek equivalent *lekithos* [1]. Shortly thereafter, Diakonow and Strecker demonstrated that PC contains two fatty acids esterified to a glycerol backbone, as well as a phosphodiester linkage connecting the third hydroxyl group to choline [2–4]. PC molecules contain a range of fatty acyl chains which vary in length and position of double bonds [5]. In the liver, PC typically contains a saturated fatty acyl chain at the *sn-1* position (e.g., 16:0 palmitic acid) and a polyunsaturated fatty acid (e.g., 20:4, arachidonic acid) at the *sn-2* position [5]. PC is physiologically important as the principal component of eukaryotic cellular membranes, as a precursor of signalling molecules [6,7], and as a key element of lipoproteins [8], bile [9] and lung surfactant [10,11]. This review is restricted to the role of PC in lipoprotein metabolism.

The liver is a major site for both the synthesis of PC and the generation of plasma lipoproteins. Phospholipids and cholesterol form a monolayer on the lipoprotein surface surrounding the hydrophobic core of triacylglycerols (TG) and cholesteryl esters [12]. PC is by far the most abundant phospholipid component in all the lipoprotein classes with levels ranging from 60 to 80 mol% of total phospholipid [8]. For example, PC comprises ~70% (mol%) of total phospholipids of rat plasma very low density lipoproteins (VLDLs) with sphingomyelin (11%), lyso-PC (3%), phosphatidylethanolamine (PE) (4%), and phosphatidylinositol (3%) [13]. Since PC is a quantitatively significant component of lipoproteins, it was not unexpected that reduced levels of hepatic PC impair the secretion of VLDLs from the liver [14–17]. The requirement of PC for VLDL secretion has been demonstrated in both cell [17,18] and animal models [19–23]. Currently, there is no evidence that any other phospholipid is required for VLDL assembly and secretion. However, since the PE content of newly-secreted VLDLs, and VLDLs isolated from the lumen of the Golgi of rat liver, is several fold higher than that of circulating VLDLs, it is possible that PE is required for VLDL assembly and/or secretion. In addition, PC on the

Abbreviations: apo, apolipoprotein; ABCA1, ATP-binding cassette transporter subfamily member A1; CD, choline-deficient; CS, choline-supplemented; CT, CTP:phosphocholine cytidylyltransferase; ER, endoplasmic reticulum; ERAD, ER-associated degradation; HDL, high density lipoprotein; LCTα, liver-specific CTα knock out; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; NAFLD, non-alcoholic fatty liver disease; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, PE *N*-methyltransferase; PLTP, phospholipid transfer protein; TG, triacylglycerol; VLDL, very low density lipoprotein

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surface of circulating VLDL and its derivative low density lipoprotein (LDL) comes into contact with cell surface enzymes and receptors that could affect the rate of lipoprotein removal from the circulation [24]. This review will focus on the role of PC biosynthesis in hepatic VLDL secretion and metabolism in the circulation. Moreover, since PC is also a major component of high density lipoproteins (HDLs), constituting up to 40% of the total lipoprotein lipid mass [8], we shall also address the role of hepatic PC in the assembly and clearance of HDLs.

2. PC biosynthesis

In mammalian species two pathways synthesize PC de novo. The major pathway, which occurs in all nucleated cells, is the CDP-choline pathway (Fig. 1) which was first described in the 1950s by Eugene Kennedy and therefore is often referred to as the "Kennedy pathway" [25]. The CDP-pathway requires choline and consists of three enzymatic steps: choline kinase catalyses the phosphorylation of choline using ATP; CTP:phosphocholine cytidylyltransferase (CT) catalyses the reaction between phosphocholine and CTP to form CDP-choline; CDP-choline:1,2-diacylglycerol cholinephosphotransferase catalyses the exchange of CMP for diacylglycerol to form PC. PC can also be generated endogenously in a second pathway via three sequential methylations of PE by phosphatidylethanolamine *N*-methyltransferase (PEMT) (Fig. 1) [26]. The PEMT pathway is quantitatively significant only in the liver where it contributes approximately 30% of total hepatic PC synthesis [27]. Subcellular fractionation revealed that both PEMT [28] and the final enzyme of the CDP-choline pathway [29] reside on the endoplasmic reticulum (ER), underscoring the importance of this organelle in PC biosynthesis.

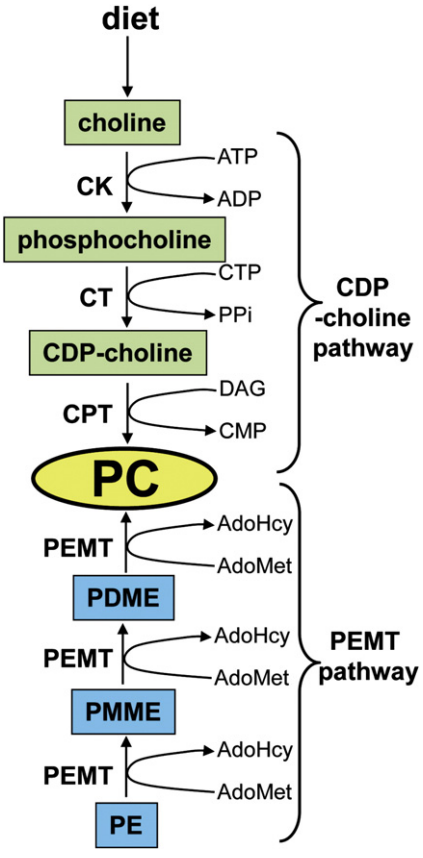

Fig. 1. Pathways involved in phosphatidylcholine biosynthesis. The enzymes indicated are choline kinase (CK), CTP:phosphocholine cytidylyltransferase (CT), CDP-choline:1,2-diacylglycerol:cholinephosphotransferase (CPT), and phosphatidylethanolamine *N*-methyltransferase (PEMT). The intermediates of the PEMT pathway are phosphatidylmonomethylethanolamine (PMME) and phosphatidyldimethylethanolamine (PDME). Other abbreviations are: DAG, diacylglycerol; AdoMet, *S*-adenosylmethionine; and AdoHcy, *S*-adenosylhomocysteine.

3. Hepatic VLDL secretion

3.1. Hepatic PC biosynthesis and regulation of VLDL secretion

The first evidence that reduced availability of PC impairs hepatic secretion of lipoproteins came from experiments performed in mammals that were fed a choline-deficient (CD) diet. This diet restricts the supply of choline required for the synthesis of PC via the CDP-choline pathway. In classic experiments in 1932, Best and Huntsman identified the importance of dietary choline in preventing the accumulation of fat in the liver [30]. Subsequent studies demonstrated that rats fed a CD diet for 3 days had lower levels of hepatic PC (25%) and elevated amounts of TG (650%) compared to choline-supplemented (CS) rats (0.4% choline w/w) [20]. Plasma TG and apolipoprotein (apo) B were reduced by a similar magnitude indicating that choline deficiency impaired hepatic VLDL secretion [20,31].

To address the role of PC biosynthesis in VLDL secretion directly, hepatocytes isolated from rats fed a CD diet for 3 days were incubated in medium deficient in L-methionine and choline for 7–16 h [14,15,17,32]; L-methionine is the precursor of *S*-adenosylmethionine (Fig. 1), the methyl donor for PEMT, and thereby methionine deficiency attenuates flux through the PEMT pathway [27,33–35]. When the culture medium lacked both choline and L-methionine, the level of hepatocyte PC was reduced and the amounts of TG, PC, and apo B secreted into the medium in VLDLs were significantly lower than in hepatocytes cultured with either choline or L-methionine [17]. The rapid (<1 h) and simultaneous normalization of VLDL secretion and cellular PC levels following the addition of choline or L-methionine to the medium suggested that active synthesis of PC by either pathway is required to supply adequate amounts of hepatic PC for VLDL secretion [17]. In support of this model, mice fed a methionine- and choline-deficient diet for 4 weeks developed fatty liver, in part due to reduced hepatic VLDL secretion [23].

The generation of mice lacking hepatic PC biosynthetic enzymes has provided valuable insights into the role of PC in regulating VLDL secretion. In both PEMT knock-out mice and liver-specific CTα knock-out (LCTα) mice, the hepatic secretion of VLDLs into plasma was significantly reduced (~50% decrease in apo B100) compared to control animals [22,36]. The impairment of VLDL secretion in these mouse models was a direct result of reduced PC biosynthesis since in vivo restoration of hepatic CTα activity by adenovirus-mediated expression of CTα, or by dietary supplementation of choline increased plasma TG levels and reduced TG accumulation in the liver [19,37,38]. Thus, impaired PC biosynthesis attenuates the secretion of VLDLs from the liver.

3.2. The role of different PC biosynthetic pathways

Studies in the mid-1980s indicated that specific pools of PC may be preferred for lipoprotein secretion [39,40]. Specifically, in primary cultures of rat hepatocytes there was discrimination against the secretion of PC made from the methylation of [^{3}H]ethanolamine-derived PE, and a preference for PC made from [^{3}H]choline or from PE produced by decarboxylation of phosphatidylserine [40]. The relative importance of PE methylation for VLDL secretion was questioned when inhibition of cellular methyltransferase activity by 3-deazaadenosine did not reduce the amount of apo B secreted from isolated hepatocytes [39]. One of the first indications that PE methylation could regulate VLDL secretion was the observation that L-methionine normalized TG secretion in CD rat hepatocytes [17].

The development of genetically engineered mice demonstrated that impairment of either pathway for PC biosynthesis attenuates VLDL secretion [22,36,38]. Livers of mice lacking either PEMT or CTα secrete significantly less VLDL (~50% less apo B100) in vivo than do

wild-type livers [22,36]. Notably, the in vivo reduction in VLDL secretion in PEMT-deficient mice occurred only when the mice were challenged with a high-fat diet [36,38]. Nevertheless, experiments with hepatocytes from chow-fed *Pemt*$^{-/-}$ mice demonstrated that VLDL (TG and apo B) secretion was reduced compared to that from hepatocytes from *Pemt*$^{+/+}$ mice. However, TGs did not accumulate in isolated hepatocytes or in intact mouse livers [18,36,38]. The liver-specific CTα knock-out (LCTα) mice exhibited both impaired VLDL secretion and significant hepatic TG accumulation even when fed a chow diet suggesting that CTα is more robustly required for VLDL secretion than is PEMT [22]. The difference in the degree to which VLDL secretion is reduced in the PEMT versus the LCTα mouse model is likely due to the relative contribution of PEMT (30%) and the CDP-choline pathway (~70%) to total hepatic PC biosynthesis [27]. Currently, it is generally accepted that both PEMT and CTα are required for normal hepatic VLDL secretion.

3.3. Phospholipid composition of membranes of the secretory system

Nascent hepatic VLDLs are assembled on the ER, the site of PC synthesis, indicating that the processes of hepatic PC synthesis and lipoprotein assembly and secretion might be coupled. When rats were fed a CD diet, subcellular membranes and luminal lipoproteins within the hepatic secretory pathway (ER and Golgi) contained significantly less PC (reduced by ~27% in membranes and ~37% in luminal lipoproteins) than in rats fed a CS diet [14].Thus, PC biosynthesis determines the phospholipid content of the membranes within the secretory pathway as well as nascent VLDL particles [14]. The level of PC was reduced to a similar extent in the Golgi (27%) and the ER (26%) in the livers of rats fed a CD diet [14]. Based on this result it was unclear which part of the hepatic secretory pathway was primarily responsible for altering the phospholipid content of the nascent VLDL particles. It would be interesting to determine whether PC is reduced uniformly within the hepatic secretory pathway (Golgi and ER) of *Pemt*$^{-/-}$ and LCTα mice. It is likely that a reduction of PC on the surface of nascent VLDLs enhances degradation of the VLDLs within the secretory pathway (see Section 3.4) [14,15]. Correlations have been established among the reduction in the PC content of the ER, the number of nascent particles within the secretory pathway, and the amount of VLDLs (apo B) secreted from isolated hepatocytes [15,17]. Decreased PC levels in the ER also accurately predict impaired VLDL secretion in whole animal models. For example, the absence of PEMT reduced hepatic microsomal PC levels (by ~20%) as well as VLDL secretion in vivo in mice lacking the LDL receptor (LDLR) [41]. Thus, depletion of PC from the ER membrane appears to reduce VLDL secretion in animal and cells models of impaired PC biosynthesis.

3.4. Proposed mechanism(s) for PC-mediated regulation of VLDL secretion

Regulation of hepatic VLDL secretion is mediated by the availability of its lipid components. Most studies indicate that the bulk of TG is added to apo B in the ER lumen [42,43] whereas some studies have suggested that the majority of TG is assembled with apo B in the lumen of the Golgi [44]. Under most metabolic conditions apo B is synthesized constitutively and in excess of its requirement for secretion. Generally, changes in levels of apo B mRNA do not modulate apo B secretion. For example, the amount of apo B secreted can be altered over a 7-fold range without any change in the amount of apo B mRNA [45]. When insufficient lipid is available for formation of stable apo B-containing particles, the excess apo B is not secreted but is targeted for intracellular degradation [46–49]. Consistent with the view that the rate of apo B synthesis does not regulate apo B secretion [45], the rate of [^{3}H]leucine incorporation into intracellular apo B was similar in CD and CS hepatocytes [17]. Increased lipid supply, particularly of fatty acids and TGs, can promote apo B translocation across the ER membrane and into the secretory pathway. Thus, CD-mediated reduction of apo B secretion is linked to post-translational intracellular degradation by one or more of the established mechanisms.

At least three distinct apo B degradation pathways have been identified: ER-associated co-translational degradation (ERAD) [49–52], post-ER pre-secretory proteolysis [53,54] and receptor-mediated, pre-secretory re-uptake and degradation [55,56]. In addition, the non-proteasomal degradation of apo B within the lumen of the ER has been reported to be mediated by the ER-associated protein ER60 which has both protease and chaperone activities [57].

3.4.1. ER-associated co-translational degradation (ERAD)

ERAD is the major mechanism involved in intracellular apo B degradation. Like all secretory proteins apo B undergoes co-translational translocation into the ER lumen from membrane bound ribosomes [46]. During translation of apo B, phospholipids, cholesterol, cholesteryl esters and TGs are recruited to the apo B from the ER membrane to promote appropriate folding of apo B and its translocation into the ER lumen [58]. In the absence of sufficient lipid, less apo B is translocated into the lumen and/or the apo B becomes mis-folded, leading to cytosolic exposure of the apo B and degradation via the ubiquitin-proteasome pathway [50–52]. In contrast, the absence of appropriate levels of newly synthesized TG results in the co-translational degradation of apo B both in HepG2 cells cultured with an inhibitor of TG synthesis (Triascin C) [59] and in mice lacking the TG synthesis enzyme acyl-CoA: diacylglycerol acyltransferase 2 [60]. Currently there is no evidence that co-translocational degradation of apo B occurs as a result of decreased PC levels in the ER membrane. The amount of newly-made microsomal apo B that was protected from trypsin digestion (i.e. luminal apoB) was similar in CD and CS hepatocytes [15].

3.4.2. Post-ER pre-secretory proteolysis

The process whereby impaired PC biosynthesis stimulates VLDL degradation occurs after the apo B-containing lipoproteins have exited the ER and this process preferentially decreases the secretion of highly-lipidated apo B-containing lipoproteins rather than poorly-lipidated particles [14,61]. The characteristics of this type of degradation are similar to a process that has been termed “post-ER pre-secretory proteolysis” [61,62]. Primary cultures of hepatocytes from *Pemt*$^{-/-}$ mice and LCTα mice secrete ~50% less TG, and 50–70% less apo B, than do hepatocytes from wild-type control mice indicating that reduction in PC synthesis targets lipidated apo B-containing particles for degradation [18,19]. For example, choline deficiency in McArdle RH-7777 rat hepatoma cells prevented the secretion of only highly lipidated apo B-containing particles [16]. Moreover, impairment of hepatic PC biosynthesis increased the degradation of highly-lipidated apo B-containing particles as shown by analysis of the particles by density gradient centrifugation. Fewer of the lowest density (<1.06 g/ml) apo B-containing lipoproteins were secreted from *Pemt*$^{-/-}$ hepatocytes than from *Pemt*$^{+/+}$ hepatocytes, whereas secretion of the higher density (1.06–1.2 g/ml) apo B-containing particles was not altered [18].

Substantial evidence indicates that a post-ER degradation step is involved in PC-mediated regulation of VLDL secretion. One study in McArdle RH-7777 cells stably expressing carboxyl-truncated forms of apo B demonstrated that choline deficiency inhibits the secretion of only apo B variants that are longer than 23% (designated as apo B23) of the full-length protein (apo B100) [16]. Interestingly, only apo B fragments longer than apo B23 contain protein domains necessary to acquire a significant amount of TGs and assemble a neutral lipid core [58,63]. In agreement with the view that impaired PC biosynthesis promotes apo B degradation in a post-ER compartment, the number of apo B-containing particles was lower in the lumina of the Golgi (by 40–50%), but not in the ER, of CD, compared to CS, rat livers [15]. Additional evidence for a post-ER degradation of these apo B-containing particles was provided when vesicular trafficking from the ER to Golgi was blocked by brefeldin A in primary cultures of hepatocytes [14]. The authors found similar levels of apo B in the

ER lumen of CD and CS hepatocytes [14]. Based on these results it was proposed that a quality control protease within a post-ER compartment facilitates VLDL degradation in response to choline deficiency [15]. Presumably, this protease recognizes and degrades misfolded apo B in particles with an abnormal phospholipid composition which may include a decrease in the availability of PC and/or an increase in another phospholipid such as PE. However, despite numerous experiments with various inhibitors of proteolysis (chloroquine, soybean trypsin inhibitor, NH_4Cl, leupeptin, phenylmethylsulfonyl fluoride and *N*-ethylmaleimide) the participating protease was not identified [14]. In addition, the intracellular compartments responsible for degrading apo B and disposing of the lipid core remain unclear.

One mechanism proposed for post-ER pre-secretory proteolysis is the formation of double membrane intracellular vesicles (autophagosomes) which surround apo B-containing lipoproteins and target them for destruction in lysosomes [64,65]. The induction of autophagy in response to impaired PC biosynthesis has not been investigated although convincing data suggest that both polyunsaturated fatty acids and insulin inhibit hepatic apo B secretion by this process [53,54,61,65–67]. In response to polyunsaturated fatty acids, VLDL degradation is preceded by the formation of reactive oxygen species which facilitate the oxidation and subsequent aggregation of apo B particles [54]. Thus, nascent lipoproteins containing an abnormal phospholipid composition and misfolded apo B might promote a similar mechanism. Fast et al. proposed that aggregation of apo B, caused by reduced PC availability, contributes to the CD-specific degradation process [14]. However, the molecular determinants responsible for selecting apo B for degradation are poorly understood. Similarly, it is unclear how apo B that is on particles with inadequate PC would be specifically targeted. Alternatively, autophagosome nucleation might occur in response to impaired PC biosynthesis as a result of decreased PC/PE ratio in membranes of the secretory system. Autophagosome formation requires conjugation of a ubiquitin-like protein (LC3) with PE [64,68]. Thus, it is possible that increased PE availability in membranes that harbour components for autophagosome nucleation induces autophagy in mice with impaired PC biosynthesis.

Consistent with the view that choline-deficiency promotes post-ER presecretory proteolysis of apo B are data implicating phospholipid transfer protein (PLTP) in this intracellular degradative process [69,70]. Primary cultures of hepatocytes isolated from mice lacking PLTP have impaired apo B secretion due to post-ER oxidation (and presumably aggregation) of apo B [70]. However, because the function of PLTP in regulating VLDL secretion is complex, it remains unclear whether or not activation of post-ER pre-secretory proteolysis of apo B is distinct from receptor-mediated pre-secretory degradation of apo B [see Section 3.4.3] [69]. In addition, the mechanism responsible for adding phospholipids to apo B during VLDL assembly has not been established despite localization of PLTP to the ER and Golgi [69,70]. Interestingly, *Drosophila* microsomal triacylglycerol transfer protein, which has functional PL, but not TG, transfer activity, is sufficient for the assembly and secretion of apo B lipoproteins in COS cells [71]. Inhibitors of PLTP activity have recently been shown to reduce hepatic apo B secretion and should serve as useful tools in determining the role of phospholipids in regulating intracellular VLDL degradation [72,73].

3.4.3. Receptor-mediated pre-secretory degradation

The LDLR is capable of binding nascent apo B-containing particles both at the cell surface and within the secretory pathway [55,56]. The rate of secretion of apo B is higher in LDLR knock-out mice than in wild-type mice [74]. The LDLR directs VLDLs to degradation in a post-ER compartment [55] in a manner that is similar to the effect of impaired PC synthesis. However, VLDL degradation in the absence of PEMT is at least partially independent of the LDLR since hepatic TG secretion was significantly lower (by ~30%) in $Pemt^{-/-}/Ldlr^{-/-}$ mice than in $Pemt^{+/+}/Ldlr^{-/-}$ mice [41]. Based on the current literature, it is difficult to ascertain whether or not LDLR-mediated degradation of apo B contributes partially to the degradation of PC-deficient apo B-containing particles. For example, VLDL secretion in $Pemt^{-/-}/Ldlr^{-/-}$ mice was assessed under experimental conditions that were different from those used for the $Pemt^{-/-}/Ldlr^{+/+}$ mice [36,41,75]. Thus, it will be important to investigate the direct involvement of LDLR-mediated pre-secretory degradation in the reduction of VLDL secretion in response to impaired PC synthesis.

3.4.4. The role of apolipoprotein E

Apo E is synthesized in the liver, is a component of VLDL particles, and is a ligand for the LDLR. The presence of apo E on nascent VLDL particles promotes VLDL secretion [76]. It has been demonstrated in vivo that mice lacking PEMT (i.e. $Pemt^{-/-}$ mice and $Pemt^{-/-}/Ldlr^{-/-}$ mice) secrete significantly less VLDL (TG and apo B) than do $Pemt^{+/+}$ and $Pemt^{+/+}/Ldlr^{-/-}$ mice, respectively [36,41]. Consistent with previous reports [77], our laboratory has found that apo E-deficient mice secrete ~50% less hepatic VLDL-TG than do wild-type C57BL/6 mice (Fig. 2). However, VLDL secretion (TG and apo B) was not further reduced in $Apoe^{-/-}$ mice by elimination of PEMT, suggesting that apo E might be required for PEMT deficiency to impair VLDL secretion in vivo (Fig. 2). This observation might be explained by the timing of the involvement of apo E in VLDL secretion. Apo E appears to facilitate an early lipidation step in VLDL assembly [78]. Alternatively, the potential antioxidant properties [79] of apo E may afford protection of apo B from lipid peroxidation and subsequent formation of aggregates in post-ER pre-secretory proteolysis [53]. Impaired PC biosynthesis might influence apo E association with VLDLs directly since the phospholipid species that bind to apo E can dramatically change the protein conformation and potentially alter the biological activity of apo E [80].

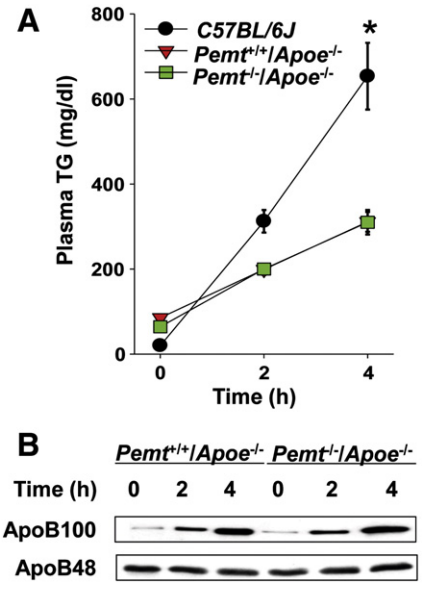

Fig. 2. PEMT deficiency does not impair hepatic secretion of TG or Apo B in $Apoe^{-/-}$ mice. (A) Male wild-type C57BL/6 J mice (circles), $Pemt^{+/+}/Apoe^{-/-}$ mice (triangles) and $Pemt^{-/-}/Apoe^{-/-}$ mice (squares) were fed a chow diet for 6 months after which they were fasted overnight. Blood was collected at time = 0 and at indicated times after intraperitoneal injection of Poloxamer 407. The accumulation of TG in plasma reflects newly-secreted VLDLs because Poloxmer 407 blocks lipolysis of lipoproteins [111]. Plasma TG levels were quantitated by gas–liquid chromatography [81]. Values are means ± SEM. *$P<0.05$ vs. $Pemt^{+/+}/Apoe^{-/-}$ and $Pemt^{-/-}/Apoe^{-/-}$ (n = 4 to 6 mice). (B) Plasma apo B48 and apo B100 protein levels were detected in $Pemt^{+/+}/Apoe^{-/-}$ mice and $Pemt^{-/-}/Apoe^{-/-}$ mice by immunoblotting [81] at indicated times following intraperitoneal injection of Poloxmer 407. Data are representative of 4 experiments.

4. Clearance of VLDLs from circulation

Increased clearance of circulating VLDLs also significantly contributes to the hypolipidemic effect of impaired hepatic PC biosynthesis. This unexpected phenomenon was first revealed in *Ldlr*$^{-/-}$ mice [41] in which the absence of PEMT decreased plasma TG and cholesterol by ~70%. This reduction was primarily attributed to enhanced VLDL clearance from the plasma [41]. For example, radiolabeled VLDLs ([^{3}H]-TG or [^{125}I]-apo B) generated by *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice were preferentially removed from the circulation of both *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ and *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice in vivo compared to VLDLs isolated from *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice [41]. It is likely, therefore, that enhanced particle clearance from the plasma is also responsible for the hypolipidemic effect of PEMT deficiency in *Apoe*$^{-/-}$ mice since hepatic secretion of VLDLs (apo B and TG) was similar in *Pemt*$^{+/+}$/*Apoe*$^{-/-}$ mice and *Pemt*$^{-/-}$/*Apoe*$^{-/-}$ mice [81].

Convincing evidence demonstrates that reduction in the amount of PC on the surface of nascent VLDL particles promotes their clearance. The total amount of PC secreted on nascent VLDLs was 34% lower in *Pemt*$^{-/-}$/*Ldlr*$^{-/-}$ mice than in *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ animals, presumably because of inadequate levels of PC in membranes of the secretory system [41]. This observation is consistent with significant reductions in the amount of PC associated with VLDLs secreted into the plasma or medium in various animal and cell models of impaired hepatic PC biosynthesis [14,17,19,22,36,38,41,81]. An explanation for the increased clearance of PC-deficient VLDLs is that phospholipids on the surface of circulating lipoproteins come into contact with cell surface enzymes and receptors and can modulate the rate of lipoprotein removal [41,82]. Thus, apo B-containing particles with reduced PC content might interact abnormally with these enzymes/receptors. Interestingly, decreased amounts of PC in VLDL particles result in lipoproteins of increased size which may additionally affect how they interact with cell surface proteins [17,41].

Enhanced VLDL uptake is promoted by other mechanisms in addition to the reduction in VLDL PC content caused by impaired PC biosynthesis. For example, VLDL particles isolated from *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice have normal PC content yet are removed from plasma more rapidly in mice lacking PEMT (*Pemt*$^{-/-}$/*Ldr*$^{-/-}$ mice) than in *Pemt*$^{+/+}$/*Ldlr*$^{-/-}$ mice [41]. One possible explanation for why PEMT deficiency increases VLDL clearance is that expression and/or activity of cell surface receptors and enzymes might be different in individual organs (e.g., liver, muscle, adipose).

5. The role of PC in HDL metabolism

The generation of mice lacking specific PC biosynthetic enzymes has established a relationship between hepatic PC levels and HDL metabolism. The levels of both PC and cholesterol in HDLs were lower in both *Pemt*$^{-/-}$ mice (by 25–45%) and LCTα mice (by 40–50%) than in their respective wild-type controls [19,22,36]. This observation was initially unexpected since earlier studies had demonstrated that choline deficiency did not alter plasma HDL levels in rats [20], or the amount of cholesterol and PC secreted by primary cultures of rat hepatocytes [17]. Since the liver is a major contributor to both the formation and clearance of HDLs [83,84] both PC biosynthetic pathways were investigated for possible links between cellular PC availability and HDL metabolism. Nascent HDL particles are formed when phospholipids and cholesterol are exported from the cell to lipid-poor apo A1 in the plasma [85,86]. In primary cultures of hepatocytes isolated from LCTα mice, the apo A1-dependent efflux of PC and cholesterol was significantly reduced compared to that in control littermates [19]. This reduction was likely due to a 50% decrease in expression of the hepatic ATP-binding cassette transporter subfamily member A1 (ABCA1) [19] which facilitates the transfer of phospholipids and cholesterol to apo A1 [87–89]. Since the level of cellular cholesterol, a known regulator of ABCA1 expression, was unchanged in LCTα mice, the amount/molecular species of PC might be directly involved in regulating ABCA1 expression [19,86]. In support of this hypothesis, adenoviral expression of CTα in vivo in LCTα mice restored hepatic PC mass, ABCA1 expression and plasma HDLs to near control levels [19]. The importance of PC species in HDL formation was suggested by the observation that hepatic PC in LCTα mice had significantly higher (2-fold) arachidonate content compared to that in control mice [19]. Furthermore, Wang and Oram [90,91] demonstrated that increased incorporation of arachidonate into phospholipids decreases the stability of ABCA1 protein through a phospholipase D/protein kinase Cδ-dependent mechanism.

Interestingly, in contrast to CTα deficiency, PEMT deficiency in mice does not affect HDL formation and has little effect on the arachidonate content of hepatic PC [92,93]. Apo1-dependent lipid efflux from hepatocytes and hepatic ABCA1 expression were similar in PEMT-deficient and wild-type mice [92]. However, the uptake of radiolabeled HDL particles ([^{3}H]-cholesterol or [^{3}H]-PC) into hepatocytes was increased by PEMT deficiency [92] because hepatic expression of a receptor for HDL, the scavenger receptor class B, type 1 was increased [92,94]. Expression of scavenger receptor B1 and HDL uptake were also elevated in hepatocytes from LCTα mice compared to littermate controls (Robichaud et al., unpublished data). However, the mechanism by which impaired hepatic PC biosynthesis alters the amount of scavenger receptor B1 has not yet been elucidated. Nor is it known if a reduced PC content of HDLs alters the affinity of the HDLs for binding to cell surface receptors such as the scavenger receptor B1.

6. Hepatic PC biosynthesis, lipoprotein metabolism and disease

Significant advances have been made in understanding the relationship between hepatic PC biosynthesis and lipoprotein metabolism. The physiological importance of this link has been recently underscored in *Pemt*$^{-/-}$ mice which develop non-alcoholic fatty liver disease (NAFLD) but are protected from cardiovascular disease [38,41,81].

6.1. Cardiovascular disease

Established risk factors for cardiovascular disease include elevated plasma levels of VLDLs and reduced plasma HDLs [95,96]. As discussed above, reduced levels of hepatic PC (caused by a deficiency of either PEMT or CTα) reduce the amount of TG-rich VLDL particles in the circulation [22,36,41]. Consistent with the hypolipidemic effect of PEMT deficiency, plaque formation was reduced by PEMT deficiency in two well-known animal models of atherosclerosis: *Ldlr*$^{-/-}$ mice [41] and *Apoe*$^{-/-}$ mice [81]. In addition, the absence of PEMT in *Apoe*$^{-/-}$ mice prevented both the accumulation of TG in the heart and lipid-induced cardiomyopathy [81]. Because myocardial lipid accumulation is regulated in part by the supply of circulating TGs [7], the hypolipidemic effect of PEMT deficiency is likely responsible for reduced TG levels in the heart [97]. Since PEMT deficiency did not alter plasma HDL levels in mice with either the *Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ genetic backgrounds [41,81], the beneficial effect of lack of PEMT does not appear to involve HDLs.

6.2. Non-alcoholic fatty liver disease (NAFLD)

NAFLD includes a wide range of chronic, hepatic pathologies that occur in the absence of alcohol abuse [98]. Typically, NAFLD begins with the development of a simple fatty liver (steatosis) characterized by the accumulation of hepatic TGs [98,99]. The current consensus is that hepatic steatosis sensitizes the liver to a variety of metabolic injuries that promote inflammation (steatohepatitis) and eventually liver failure [100]. Animal models of impaired PC biosynthesis accumulate hepatic TGs as a result of impaired VLDL secretion [15,20,22,23,36,38]. Thus, PC-mediated regulation of VLDL secretion has the potential to alter the susceptibility to severe forms of NAFLD. Indeed, a link between the absence of PEMT activity and the development of steatohepatitis

(lipid accumulation and inflammation) has been established in two distinct diet-induced animal models: choline-deficiency [101–103] and high-fat feeding [38]. A pivotal study demonstrated that when hepatic PC levels decreased to a level below which the PC/PE ratio was significantly reduced, the integrity of the plasma membrane was no longer maintained so that pro-inflammatory molecules (e.g., cytokines) leaked into the hepatocytes causing a molecular insult that initiated the progression to steatohepatitis [102,103]. Interestingly, PEMT activity might be an important predictor of NAFLD in humans [103–108]. A genetic variant of human *PEMT* has been identified that results in partial loss of PEMT activity and occurs more frequently (1.7-fold) in humans with NAFLD than in unaffected individuals [104,107].

It has also recently been demonstrated that PC directly contributes to hepatic TG synthesis and thus may promote the development of steatosis [109,110]. In mice, ~65% of the total hepatic pool of TG appears to be derived from hepatic PC (van der Veen et al., unpublished data). The conversion of PC to TG occurs in a non-lysosomal compartment by a process that involves a PC-specific phospholipase C and acyl-CoA:diacylglycerol acyltransferase-2 [109,110]. The PC-phospholipase C cleaves the phosphocholine moiety from PC releasing diacylglycerol that can be acylated to TG. This pathway appears to be highly specific since acyl-CoA:diacylglycerol acyltransferase-1 and several intracellular lipases, including phospholipase A_2 and phospholipase D, are not involved [109,110]. A significant amount of PC delivered to the liver from circulating HDLs (~25%) and LDLs (~50%) is converted to TG in mouse hepatocytes [109,110]. underscoring the quantitative importance of the uptake of lipoprotein-associated PC for hepatic TG production.

7. Concluding remarks

The liver is a major site for both the synthesis of PC and the metabolism of plasma lipoproteins. When hepatic PC biosynthesis is impaired, by either choline/methionine deficiency or by the absence of specific PC biosynthetic enzymes (PEMT or CTα), plasma levels of VLDLs and HDLs are reduced. This hypolipidemia is the result of reduced particle secretion by the liver, combined with enhanced uptake of mature lipoproteins from the circulation. Hepatic PC has a significant impact on lipoprotein metabolism by influencing the biological activity and expression of key components that regulate hepatic lipoprotein production (apo B degradation, as well as expression of ABCA1 and the scavenger receptor B1). This review has outlined the progress made in identifying the specific factors involved in regulation of lipoprotein metabolism and how these factors are linked to hepatic PC availability. We have also described recent progress in understanding the contribution of impaired PC biosynthesis to the progression of liver and heart disease. Experiments which further characterize the link between hepatic PC metabolism and lipoprotein metabolism will provide additional insights into the mechanisms involved, and will evaluate the possibility that inhibition of PC biosynthesis represents a therapeutic target for liver and heart disease.

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