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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.

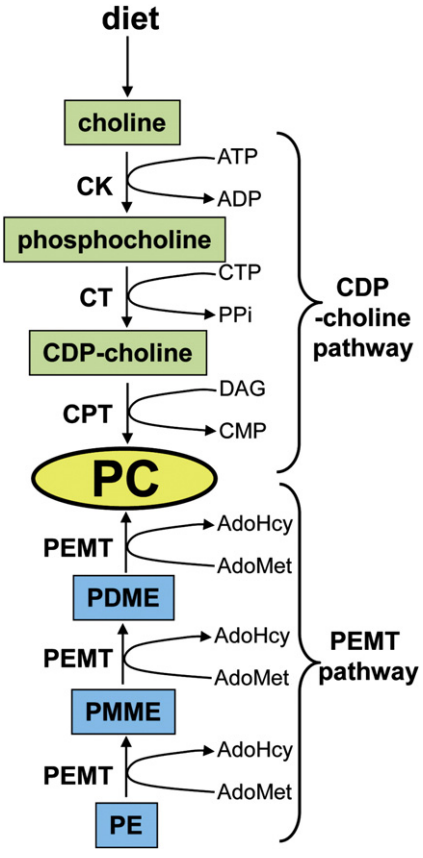


**Fig. 1.** Pathways involved in phosphatidylcholine biosynthesis. The enzymes indicated are choline kinase (CK), CTP:phosphocholine cytidylyltransferase (CT), CDP-choline:1,2-diacylglycerol:cholinephosphotransferase (CPT), and phosphatidylethanolamine *N*-methyltransferase (PEMT). The intermediates 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