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A mini review on the role of phosphatidylcholine metabolism in obesity

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ABSTRACT

Phosphatidylcholine (PC) is one of the major building blocks of biological membranes in mammalian cells. In PC, two fatty acyl chains are linked to the sn-1 and sn-2 positions of the glycerol backbone, and a head group containing a phosphate and choline is linked to the sn-3 position. The length and saturation of the fatty acyl chains of PC vary significantly and determine the biophysical and chemical properties of PC. In mammalian cells, active metabolism of PC takes place primarily through *de novo* synthesis, the remodelling cycle of deacylation and reacylation, and further modification of lysophosphatidylcholine. PC metabolism undergoes remarkable changes in obesity. In this mini review, we discuss PC metabolism and summarize recent advances about the important involvement of PC metabolism in obesity and obesity-related diseases.

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1. Introduction

The phospholipid bilayer, the essential structure of the biological membrane in mammalian cells, encapsulates and defines the cell as a distinct unit from its surrounding environment. The majority of phospholipids in mammalian cells are glycerophospholipids, which include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidic acid (PA)[1, 2]. PC is the most abundant (40-50%) and PE is the second most abundant (15-25%) lipid species in the cellular membrane[1, 3]. In PC, the sn-1 and sn-2 positions of the glycerol backbone are linked to hydrophobic fatty acyl chains, whereas the sn-3 position of glycerol is linked to a hydrophilic head group containing a phosphate and choline (Figure 1). The length and saturation of PC fatty acyl chains largely define the fluidity, subdomain organization, and curvature of the cellular membrane. In addition to its structural function, PC serves as a source of signalling molecules to mediate a series of signalling pathways[4].

The dynamics of PC are primarily mediated by *de novo* biosynthesis, the remodelling cycle of deacylation and reacylation, and further modification of lysophosphatidylcholine (LPC). Increasing evidence has indicated that PC metabolism plays an important role in health and disease. In this mini review, we will focus on the impact of PC metabolism on obesity and obesity-related diseases.

2. Overview of PC metabolism

2.1 *de novo* biosynthesis of PC

The primary pathway for *de novo* synthesis of PC in mammalian cells is the CDP-choline pathway (Figure 1)[1, 2], first described in 1956 by Eugene Kennedy and his colleagues[5] and often referred to as the Kennedy pathway, In this pathway, choline is first taken up by cells via choline transporters. In the cytosol, choline is phosphorylated to phosphocholine by choline kinase (CK). The active CK consists of either the homo- or hetero-dimers of CKα and CKβ, which are encoded by the *Chka* and *Chkb* genes, respectively (Table 1)[6].

Subsequently, CTP:phosphocholine cytidylyltransferase (CT) mediates the conversion of CTP and phosphocholine to CDP-choline, which is a rate-limiting reaction for the Kennedy pathway[1, 2]. CT has two isoforms: CTα (encoded by the *Pcyt1a* gene, Table 1) and CTβ (encoded by the *Pcyt1b* gene, Table 1). The *Pcyt1a* gene is expressed in most tissues, whereas the *Pcyt1b* gene is mostly transcribed in the brain[7].

Finally, CDP-choline:1,2-diacylglycerol choline phosphotransferase (CPT, encoded by the *Chpt1* gene, Table 1) or CDP-choline:1,2-diacylglycerol choline/ethanolamine phosphotransferase (CEPT, encoded by the *Cept1* gene, Table1) transfers phosphocholine from CDP-choline to diacylglycerol (DAG) to generate PC. This Kennedy pathway is generally active in most mammalian cells.

Besides the Kennedy pathway, the liver has an additional pathway to synthesize PC (Figure 1)[8]. In this pathway, the ethanolamine moiety of PE undergoes three sequential methylations to generate PC via the enzyme PE N-methyltransferase (PEMT, encoded by the *Pemt* gene, Table 1). This pathway

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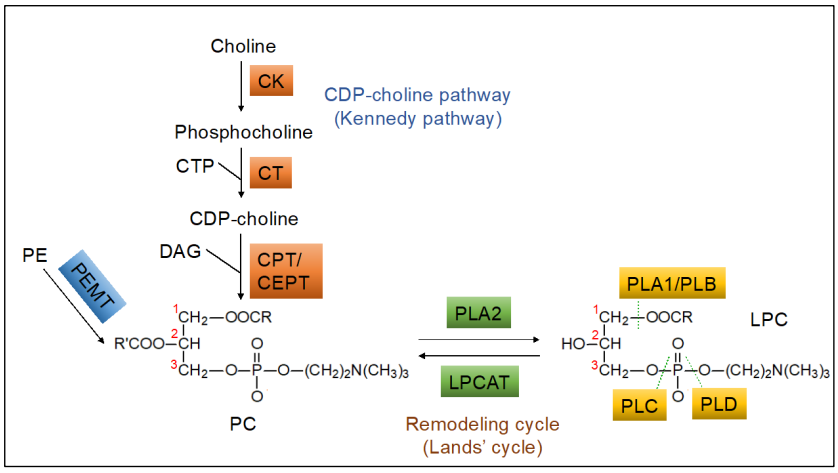

Figure 1. Overview of PC metabolism.

Table 1 Summary of enzymes involved in PC metabolism

Full name	Abbreviation	Isoforms	Gene symbols in *Mus Musculus*
choline kinase	CK	Homo or hetero-dimer of CKα, CKβ	*Chka, Chkb*
CTP: phosphocholine cytidylyltransferase	CT	CTα, CTβ	*Pcyt1a, Pcyt1b*
CDP-choline:1,2-diacylglycerol choline phosphotransferase	CPT		*Chpt1*
CDP-choline:1,2-diacylglycerol choline/ethanolamine phosphotransferase	CEPT		*Cept1*
phosphatidylethanolamine N-methyltransferase	PEMT		*Pemt*
phospholipase A2	PLA2	15 members	
Lysophosphatidylcholine acyltransferase	LPCAT	LPCAT1, LPCAT2, LPCAT3, LPCAT4	*Lpcat1, Lpcat2, Lpcat3, Lpcat4*
autotaxin	ATX		*Enpp2*

accounts for 30% of PC synthesis in the rodent liver[2]. Emerging evidence shows that this pathway is active in other metabolic tissues (discussed below).

2.2 Remodelling cycle of PC

The composition of the fatty acyl chains in PC is regulated through a remodelling cycle of deacylation and reacylation, also called the Lands' cycle (Figure 1), named for William Lands who first proposed that phospholipids are actively metabolized through deacylation and reacylation[9, 10]. It is known now that the fatty acyl chain at the sn-2 position of PC are cleaved by phospholipase A2 (PLA2) to generate LPC. LPC acyltransferase (LPCAT) links another fatty acyl chain at the sn-2 position of LPC to generate a new PC species. This active remodelling cycle of deacylation and reacylation makes a significant contribution to the diversity and dynamics of PC species[1, 2].

At least 15 PLA2s have been identified and classified into five subtypes: secretory PLA2s, cytosolic PLA2s, calcium-independent PLA2s, platelet-activating factor acetylhydrolases, and lysosomal PLA2s[11]. Four LPCAT isoforms, LPCAT1, 2, 3, and 4 (encoded by the *Lpcat1, 2, 3,* and *4* genes, respectively, Table 1), have been identified with distinct enzymatic activities, substrate preference, and tissue distribution[2].

2.3 Modification of LPC

For glycerophospholipids, different phospholipases target different bonds to generate a variety of downstream products[12]. For example, phospholipase A1 (PLA1) hydrolyzes the sn-1 fatty acyl chain of glycerophospholipids. PLB cleaves either the sn-1 or sn-2 fatty acyl chain. PLC hydrolyzes the glycerophosphate bond to remove the whole phosphate-containing polar head group. PLD cleaves the bond between the phosphate and head group. Therefore, LPC theoretically can be further degraded by PLA1, PLB, PLC, and PLD (Figure 1)[13].

Phospholipase B-like 1 purified from human neutrophils demonstrated PLA1 activity that removes the fatty acyl chain at the sn-1 position of LPC[14]. Of note, this enzyme can also remove the fatty acid chain at the sn-2 position of PC[14] and, therefore, is more related to PLB activity. No PLC has been reported to hydrolyze the glycerophosphate bond in LPC. Autotaxin (ATX, encoded by the *Enpp2* gene, Table 1) was originally identified as a motility-stimulating protein in the culture medium of human melanoma A2058 cells[15]. To date, ATX is recognized as a major PLD in serum that cleaves the bond between the phosphate and head group in LPC to generate lysophosphatidic acid (LPA)[16].

3. Role of PC metabolism in obesity

Obesity is defined as excessive accumulation of body fat that causes adverse effects on health. The prevalence of obesity has rapidly increased worldwide, as well as in the United States (U.S.). In 2016, over one billion people worldwide and 107 million people in the U.S. were considered obese[17, 18]. Obesity increases the risk of developing heart disease, stroke, liver disease, and type 2 diabetes[19]. Genetic evidence, lipidomics analysis, and human studies have pointed to a critical role of PC metabolism in obesity and obesity-related diseases.

3.1 Liver

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive lipid accumulation in the liver in humans with little or no alcohol consumption[20]. It is a hepatic manifestation of dysregulated metabolic homoeostasis often associated with obesity. The disease can progress from simple fatty liver (steatosis) to non-alcoholic steatohepatitis (NASH) with symptoms of steatosis, inflammation, and fibrosis, and eventually to end-stage cirrhosis and/or hepatocellular carcinoma. Aberrant hepatic PC metabolism has been linked to NAFLD. In animal studies, when the *Pcyt1a* gene (encoding CTα) was specifically deleted in liver, the resulting tissue-specific knockout (ko) mice demonstrated a reduced hepatic PC/PE ratio and increased steatosis and hepatic inflammation after high-fat diet (HFD) feeding compared to their wildtype (wt) littermates[21, 22]. The decreased hepatic PC/PE ratio correlated with the severity of NAFLD in these mice. *Pemt*$^{-/-}$ mice displayed a similar phenotype - the ko mice had a lower hepatic PC/PE ratio and more severe NAFLD compared to the wt control mice after 2 weeks or 10 weeks of HFD feeding[21, 23]. Choline supplementation corrected the hepatic PC/PE ratio in HFD-fed *Pemt*$^{-/-}$ mice[21], likely due to the enhanced CDP-choline pathway. Interestingly, choline supplementation did not affect steatosis, but suppressed inflammation in the liver. Specific knockdown of the *Lpcat3* gene in the liver using an adenoviral shRNA vector decreased hepatic triacylglycerol level and steatosis in ob/ob mice[24]. Deletion of the *Enpp2* gene (encoding ATX) protected mice from HFD-induced hepatic steatosis[25].

In NASH patients, both the total hepatic PC level and the ratio of hepatic PC/PE decreased[26, 27]. A polymorphism (V175M) of the PEMT gene in humans associated with decreased enzymatic activity occurred more frequently in NAFLD patients, compared to healthy control subjects[28].

3.2 Adipose tissue

Lipid droplets, the key mechanisms for storing lipids in adipocytes, have a neutral lipid core surrounded by a phospholipid monolayer[29]. PC is the major lipid species (50-60%) in the membrane of lipid droplets[30]. Upon differentiation of mouse 3T3-L1 cells or human preadipocytes to mature adipocytes, the CTα level was increased, accompanied by enhanced PC synthesis[31]. Knockdown of the *Pcyt1a* gene in 3T3-L1 cells reduced the size of the lipid droplets but had no effects on adipogenesis or triacylglycerol storage.

As noted earlier, PEMT-mediated conversion from PE to PC is primarily active in the liver. Interestingly, both the mRNA and protein levels of the *Pemt* gene increased during adipogenesis of 3T3-L1 cells[32, 33] and in mouse adipose tissue after HFD challenge[33]. Knockdown of the *Pemt* gene in 3T3-L1 adipocytes led to enhanced hydrolysis of basal triacylglycerol[33]. When the *Pemt*$^{-/-}$ mice were fed with a HFD for 10 weeks, they gained less weight, had increased energy expenditure, and maintained normal insulin sensitivity, compared to their wt littermates[23]. Despite these improved metabolic parameters, these HFD-fed *Pemt*$^{-/-}$ mice had severe NAFLD symptoms (mentioned in 3.1). The adipocytes of *Pemt*$^{-/-}$ mice were smaller than those of their wt littermates[23, 33]. Supplementation of additional choline in the diet largely reversed the phenotypes of HFD-fed *Pemt*$^{-/-}$ mice[23].

Knockout of the *Lpcat3* gene in 3T3-L1 cells impaired their adipogenesis[34]. A calcium-dependent PLA2 (AdPLA, encoded by the *Plaat3* gene) is mainly expressed in adipose tissue and its expression is enhanced in HFD-induced obesity[35,36]. The *Plaat3*$^{-/-}$ mice, compared to *Plaat3*$^{+/+}$ control mice, gained less weight and had increased lipolytic activity in adipose tissue upon HFD feeding[35].

The *Enpp2*$^{-/-}$ mice showed reduced cell size of adipocytes in both subcutaneous and visceral fat compared to their wt littermates when the mice were fed with a HFD, which is associated with reduced expression of inflammatory genes in adipose tissue[25]. However, deletion of the *Enpp2* gene did not affect body weight or fat mass upon HFD feeding. Overexpression of the *Enpp2* gene in mice led to increased adiposity when the mice were fed with a HFD[37].

In humans, expression of the *PCYT1A* and *PEMT* genes in white adipose tissue correlated with adiposity and body mass index and polymorphisms of the *PEMT* gene significantly associated with adiposity[38].

3.3 Skeletal muscle

Expression of the *Cept1* gene in skeletal muscle increased in HFD-fed mice. When the *Cept1* gene was specifically deleted in skeletal muscle, insulin sensitivity of muscle was improved when the mice were fed with a HFD[39]. Specific deletion of the *Pemt* gene in skeletal muscle raised the metabolic rate in both skeletal muscle and whole body[40]. As a result, these mice had decreased adiposity and remained insulin sensitive upon HFD feeding. Of note, these muscle-specific *Pemt* ko mice did not develop steatosis or inflammation in the liver.

In obese human subjects, transcription of the *CEPT1* gene was inversely correlated with insulin sensitivity[39]. After the obese patients underwent gastric bypass surgery, they experienced significant weight loss, accompanied by the decreased CEPT protein level in skeletal muscle[39].

4. Conclusions and Perspectives

The important role of PC metabolism in obesity and obesity-related diseases has begun to be recognized and appreciated. However, many outstanding questions have not been investigated. Although genetic manipulation of key enzymes in PC metabolism have demonstrated its importance in obesity, more studies are needed to elucidate the underlying mechanisms. The lipidomics data have shown that the levels of many different PC species are either upregulated or down-regulated in obesity. Therefore, it would be important to investigate the contributions of distinct PC species to metabolic regulation at the cellular and systemic levels. Dynamic changes of PC species are often considered to regulate fluidity and subdomain formation of the biological membrane. However, they can serve as a source of signalling molecules as well. Further studies on the signalling contribution of individual PCs would offer insights into some unprecedented functions of PCs and their derivatives in metabolic regulation in obesity.

5. References

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