Phosphatidylethanolamine-\(N\)-methyltransferase Activity and Dietary Choline Regulate Liver-Plasma Lipid Flux and Essential Fatty Acid Metabolism in Mice

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ABSTRACT Phosphatidylethanolamine-\(N\)-methyltransferase (PEMT) catalyzes the methylation of phosphatidylethanolamine to form phosphatidylcholine (PC) and represents one of the two major pathways for PC biosynthesis. Mice with a homozygous disruption of the PEMT gene are dependent on the 1,2-diacylglycerol choline-phosphotransferase (CDP-choline) pathway for the synthesis of PC and develop severe liver steatosis when fed a diet deficient in choline. The present study used quantitative lipid metabolite profiling to characterize lipid metabolism in PEMT-deficient mice fed diets containing varying concentrations of choline. Choline supplementation restored liver, but not plasma PC concentrations of PEMT-deficient mice to levels commensurate with control mice. Choline supplementation also restored plasma triglyceride concentrations in the PEMT-deficient mice to those equal to control mice. PEMT-deficient mice also had substantially diminished concentrations of docosahexaenoic acid [22:6(n-3)] and arachidonic acid [20:4(n-6)] in plasma, independent of choline status. Thus, choline supplementation rescued some but not all of the phenotypes induced by the knockout. These findings indicate that PEMT activity functions beyond its recognized role as a compensatory pathway for PC biosynthesis and that, in contrast, PEMT activity is involved in many physiologic processes including the flux of lipid between liver and plasma and the delivery of essential fatty acids to blood and peripheral tissues via the liver-derived lipoproteins. J. Nutr. 133: 3386–3391, 2003.

KEY WORDS: • essential fatty acids • lipid metabolism • metabolomics • phosphatidylcholine

Phosphatidylcholine (PC)\(^{1}\) is the most abundant phospholipid in mammalian cells; it is synthesized by the transfer of phosphocholine to the sn-3 position of 1,2-diacylglycerol (DAG) via cytidine diphosphate-choline (CDP-choline): 1,2-diacylglycerol cholinephosphotransferase activity (1) or by the methylation of phosphatidylethanolamine (PE) via phosphatidylethanolamine-\(N\)-methyltransferase (PEMT) activity (2). Although the production of phosphatidylcholine (PC) via the CDP-choline pathway is dependent on dietary choline, the production of PC by PEMT activity is not, and requires only PE and \(\text{S}-\text{adenosylmethionine (2). The CDP-choline and PEMT pathways operate in concert to maintain normal liver PC concentrations in response to variations in choline and methyl status (2–6). Many agents modulate the rate of liver PC biosynthesis including ethanol (7), estrogen (8), norepinephrine and propranolol (9) and fibrates (10–12), but the effects of these modulations on global lipid metabolism are largely unknown.

Although liver PC concentrations are maintained by both the PEMT and the CDP-choline pathways, recent work demonstrated that PEMT is essential for the incorporation of neutral lipid into VLDL particles in liver cells (13) and for normal concentrations and compositions of VLDL and VLDL components in plasma in mice (14,15). Moreover, PEMT-deficient mice had gender-specific differences in the concentrations of PC, triglyceride (TG) and cholesterol esters (CE) in plasma (15). Thus, it appears that PC synthesized by the two pathways does not fully mix within the cell, and that the two pathways have distinct physiologic roles not confined to compensating for decreased PC concentrations. Several studies evaluated the relative contribution of the PEMT and CDP-choline pathways to liver PC concentration and composition. NMR analysis of liver extracts from rats treated with \(^{13}\text{C}\)-labeled choline and ethanolamine demonstrated that \(\sim 30\%\) of liver PC was synthesized by PEMT (16). The composition of liver PC derived from PEMT activity was also shown to differ from that produced by the CDP-choline pathway. PC molecules produced from the CDP-choline pathway were comprised mainly of medium-chain, saturated fatty acids, whereas PC produced via PEMT activity were comprised of long-chain PUFA (17). These data suggest that changes in PEMT activity can cause changes in lipid metabolism and

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\(^{3}\) Abbreviations used: CD; choline-deficient; CDP-choline, cytidine diphosphate-choline; CE, cholesterol ester; CS, choline-supplemented; CT, control diet; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine-\(N\)-methyltransferase; TG, triglyceride.
TABLE 1
Lipid compositions of liver from phosphatidylethanolamine-N-methyltransferase (PEMT)-deficient and wild-type mice fed choline-deficient (CD), control (CT) or choline-supplemented (CS) diets for 12 d.1,2

<table>
<thead>
<tr>
<th></th>
<th>PEMT-deficient</th>
<th></th>
<th>Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol lipid/g liver</td>
<td>CD</td>
<td>CT</td>
<td>CS</td>
</tr>
<tr>
<td>PCabc</td>
<td>7.6 ± 1.0</td>
<td>9.6 ± 1.0</td>
<td>16.6 ± 2.6</td>
</tr>
<tr>
<td>PEbc</td>
<td>5.3 ± 1.3</td>
<td>7.2 ± 0.9</td>
<td>8.9 ± 0.6</td>
</tr>
<tr>
<td>PS/I</td>
<td>3.4 ± 0.4</td>
<td>4.1 ± 0.7</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>CL</td>
<td>1.2 ± 0.8</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>FFA</td>
<td>4.6 ± 2.0</td>
<td>5.4 ± 2.3</td>
<td>3.8 ± 1.4</td>
</tr>
<tr>
<td>TGabc</td>
<td>99.0 ± 26.5</td>
<td>117.2 ± 25.1</td>
<td>31.4 ± 20.8</td>
</tr>
<tr>
<td>CEa</td>
<td>6.4 ± 2.0</td>
<td>7.3 ± 4.0</td>
<td>3.3 ± 1.1</td>
</tr>
</tbody>
</table>

1 Values are means ± sd, n = 5/group. a Significant effect of PEMT status (P < 0.05; two-way ANOVA); b significant effect of choline status (P < 0.05; two-way ANOVA); c significant interaction between PEMT status and choline status (P < 0.05; two-way ANOVA).
2 Abbreviations: CE, cholesterol ester; CL, cardiolipin; FFA, free fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS/I, phosphatidylserine/inositol; TG, triglyceride.
Phosphatidylcholine concentrations. PEMT-deficient mice fed either CD or CT diets had significantly lower concentrations of PC in liver than control mice fed CD or CT diets, respectively (Table 1). However, PEMT-deficient mice fed the CS diet had PC concentrations that did not differ from those of control mice fed a CS diet (Table 1). Hence, the concentration of PC in PEMT-deficient mice was responsive to choline and PEMT status, and under conditions of choline supplementation, the CDP-choline pathway produced normal liver concentrations of PC. The choline content of the diet did not affect the PC concentration in liver in control mice (Table 1), indicating that the normal hepatic PC concentrations were not increased by supplementary choline, and that PEMT activity compensated for diminished CDP-choline synthesized PC in the choline-deficient state.

Plasma PC concentrations were lower in PEMT-deficient mice relative to control mice fed each of the three diets (Table 2). The concentration of PC in plasma from PEMT-deficient mice fed the CS diet was significantly greater than that in PEMT-deficient mice fed the CD or CT diets, demonstrating that plasma PC concentrations were responsive to choline status (Table 2). The choline-responsive nature of PC in plasma reflected that of PC in the liver; however, in contrast to liver, choline supplementation did not restore plasma PC concentrations to the level of their corresponding wild-type controls. These data demonstrate that although the CDP-choline pathway can compensate for diminished CDP-choline synthesized PC in plasma, it cannot fully compensate for PEMT deficiency in restoring the concentrations of PC in plasma.

TG and CE metabolism. PEMT-deficient mice fed all three diets had significantly greater concentrations of TG in liver relative to control mice (Table 1). Feeding the CS diet relative to the CD or CT diets significantly diminished the magnitude of the increased TG. Thus, choline supplementation partially relieved the TG accumulation induced by the PEMT deficiency. These data demonstrate that the accumulation of TG in liver is responsive to both choline and PEMT status. The concentration of TG in plasma was inversely related to the concentration of TG in liver because PEMT-deficient mice fed CD or CT diets had significantly lower plasma TG concentrations (Table 2), relative to their control groups fed the same diet. Additionally, PEMT-deficient mice fed CS diets had plasma TG concentrations that did not differ from those of their wild-type controls (Table 2), indicating that choline supplementation was sufficient to fundamentally restore the normal flux of TG between the liver and plasma.

Livers from PEMT-deficient mice fed all three diets had greater concentrations of CE relative to control mice (Table 1). Similar to TG, the magnitude of the increased CE was lower when the CS diet was fed relative to mice fed CD or CT diets. Thus, the hepatic CE concentrations were responsive to both PEMT and choline status. In plasma, CE concentrations were responsive to choline status in PEMT-deficient mice; increasing dietary choline concentrations increased plasma CE concentrations (Table 2). As with TG concentrations, plasma CE concentrations were inversely related to hepatic CE concentrations, indicating that choline status affected the flux of CE between liver and plasma in PEMT-deficient mice. In contrast to plasma TG concentrations, none of the three diets restored plasma CE concentrations to the levels of control mice.

The dysregulation of PEMT and the depletion of choline from the diet each decreased plasma CE concentrations and concomitantly increased hepatic CE concentrations. Thus, although CE concentrations demonstrated a reciprocal relationship between plasma and liver, CE concentrations in plasma were correlated directly with the concentration of plasma PC ($r = 0.86, P < 0.0001$). These data demonstrate a relationship between PC metabolism and CE metabolism that is different from that between PC and TG metabolism.

Other lipid classes. Hepatic PE concentrations in PEMT-deficient mice were altered by the three diets, with CD-fed mice having significantly lower concentrations of PE, and CS-fed mice significantly higher concentrations of PE relative to mice fed CT-diets (Table 1). Thus, there was an accumulation of PE in liver in response to PEMT deficiency, but only under conditions of choline supplementation. Hepatic PE concentrations were not affected by choline status in control mice (Table 1). The concentration of other lipid classes in liver including cardiolipin, free fatty acids and phosphatidylserine + inositol were not altered by either PEMT or choline status (Table 1).

Compositional analysis. Because the PEMT and CDP-choline pathways use distinct substrates for the synthesis of PC, the compositions of hepatic and plasma PC were analyzed to determine whether liver and plasma lipid compositions were affected by PEMT deficiency. Each of the fatty acids comprising hepatic PC was quantified independently and reported in
Figure 1 as a mol% composition of total PC fatty acids. PEMT deficiency caused a significant depletion of docosahexaenoic acid [22:6(n-3)] from both hepatic and plasma PC (Fig. 1). Additionally, plasma PC from PEMT-deficient mice was comprised of significantly less stearic acid (18:0) and arachidonic acid [20:4(n-6)] and significantly more linoleic acid [18:2(n-6)] than in control mice, independent of choline status (Fig. 1). The fatty acids 22:6(n-3) and 20:4(n-6) are essential for many biological processes including neural function and eicosanoid metabolism, respectively, and the substantial depletion of these fatty acids from plasma in the PEMT-deficient mice indicates a key role for PEMT in essential fatty acid metabolism.

Other lipid classes were investigated to determine what secondary effects the disruption of PEMT activity would elicit on fatty acid composition. In the absence of PEMT, hepatic PE is not converted to PC and thus may display an unusual fatty acid composition. A notable feature of the hepatic PE composition (Fig. 1) was a significant accumulation of 22:6(n-3) independent of choline status. Curiously, the concentration of 20:4(n-6) in hepatic PE was decreased in PEMT-deficient mice, indicating that the PEMT deficiency prevented 20:4(n-6) from entering the liver PE pool as well as PC. This observation requires further investigation because it is not obvious how PEMT activity would cause this change in 20:4(n-6) metabolism. Other fatty acids in hepatic PE influenced by the PEMT deficiency included palmitoleic acid [16:1(n-7)], 18:2(n-6) and dihomo-γ-linolenic acid [20:3(n-6)] (Fig. 1). The decreased concentrations of 16:1(n-7) and 18:2(n-6) were alleviated by feeding the PEMT-deficient mice CS diets, indicating that these fatty acids were responsive to both choline and PEMT status.

The composition of plasma CE from PEMT-deficient mice was significantly depleted of 22:6(n-3) and 20:4(n-6) relative to that from control mice (Fig. 1). Hepatic CE 22:6(n-3) and 20:4(n-6) concentrations did not differ.
Composition of hepatic and plasma TG. Hepatic TG from PEMT-deficient mice was comprised of significantly more 18:0, 22:6(n-3) and long-chain (n-6) PUFA than that from control mice when the mice were fed the CD diets. TG from PEMT-deficient mice fed CD diets contained significantly less 16:0, 16:1(n-7) and 18:1(n-9) than TG from their control mice. Except for a lower 18:1(n-9) concentration, hepatic TG from PEMT-deficient mice fed the CS diet was indistinguishable from the hepatic TG produced by the control mice (Fig. 1).

DISCUSSION

Two pathways, the CDP-choline pathway and the PEMT pathway, synthesize hepatic PC. These pathways use two previously acylated glycerolipids, 1,2-DAG in the CDP-choline pathway and PE in the PEMT pathway, as substrate for the biosynthesis of PC. Because 1,2-DAG and PE have widely dissimilar fatty acid compositions, the PC produced by the CDP-choline and PEMT pathways may also have distinct compositions. Delong et al. (17) presented evidence that hepatic PC synthesized by the PEMT pathway was comprised of more PUFA than PC produced by the CDP-choline pathway, whereas the molecular species of PC synthesized via the CDP-choline pathway were comprised mainly of saturated (e.g., 16:0/18:0) species. Other reports describe no acyl-chain–specific differences between bile PC synthesized exclusively by the CDP-choline pathway relative to PC synthesized by both the CDP-choline pathway and the PEMT pathway (22). This report presents quantitative evidence from PEMT-deficient mice that PEMT activity does in fact produce a PC molecular species that is distinct from the species produced by the CDP-choline pathway, and that PEMT activity is essential to maintain normal concentrations of 18:0 and 22:6(n-3) in hepatic PC and 18:0, 22:6(n-3) and 20:4(n-6) in plasma PC. This indicates that PEMT activity is critical for mobilizing the essential fatty acids 20:4(n-6) and 22:6(n-3) from liver into plasma.

Because PC is by far the most prevalent phospholipid in plasma, factors that influence hepatic PEMT activity influence the concentration of fatty acid components of plasma PC and their distribution to peripheral tissues. The results presented here show that the disruption of PEMT activity significantly diminishes the concentration of the essential fatty acid 22:6(n-3) in both hepatic and plasma PC. This depletion of 22:6(n-3) occurred independently of choline status, demonstrating the key role for PEMT in mobilizing essential fatty acids from the liver and plasma. The disruption of the PEMT pathway also caused an accumulation of 22:6(n-3) in hepatic PE, indicating that the conversion of 22:6(n-3)–containing PE to PC via PEMT activity is the normal pathway for incorporating 22:6(n-3) into PC. These data strongly suggest that PEMT activity is almost solely responsible for the mobilization of 22:6(n-3) into plasma, and factors that modulate PEMT activity may also alter the essential fatty acid status. This may have important implications for neurodevelopment, cardiovascular disease, immune dysfunction and other diseases related to tissue and plasma essential fatty acid concentrations. Furthermore, although the disruption of the PEMT pathway had little effect on the concentration of 20:4(n-6) in hepatic PC, 20:4(n-6) was significantly depleted from plasma PC in the PEMT-disrupted mice independent of choline status. These data indicated a critical role for PEMT in the mobilization of 20:4(n-6) into plasma and suggested that PEMT status may be important in phenotypes influenced by 20:4(n-6)–derived regulatory lipids, including eicosanoids, epoxides and diols.

The depletion of 22:6(n-3) from plasma PC also has secondary effects on essential fatty acid metabolism in plasma. The major carrier of 22:6(n-3) in plasma other than PC, CE, was also significantly depleted of 22:6(n-3). The basis for this depletion was likely a drastic decrease in the concentration of 22:6(n-3) in plasma PC because the enzyme responsible for esterifying cholesterol in plasma, lecithin:cholesterol acyltransferase, requires fatty acids from the sn-2 position of plasma PC as substrate. Thus, PEMT appears to exert substantial control over the concentration of 22:6(n-3) in plasma.

Another effect of the knockout was a large accumulation of PUFA in hepatic TG, a condition that is quite unusual. The depletion of PEMT caused a substantial and specific accumulation of 18:0, 22:6(n-3) and long-chain (n-6) PUFA in TG that was exacerbated by choline deficiency (Fig. 1). Interestingly, these fatty acids are major components of PE, but not PC, indicating a conversion of PE into (ultimately) TG via a phospholipase C or D pathway. This conversion of PE to neutral lipid may implicate phospholipases as a strategy for maintaining normal PE concentrations in liver. In the absence of the conversion of PE to PC and its subsequent export into plasma, several compensatory mechanisms, including decreased synthesis of PE via the CDP-ethanolamine and phosphatidyserine decarboxylase pathways and increased clearance of PE from membranes by phospholipase C or D reactions may be induced.

The present data indicate that choline supplementation is sufficient to restore normal hepatic PC concentrations, but not sufficient to restore plasma PC, CE or TG concentrations (Table 2). This finding is in partial disagreement with a recent report by Noga and Vance (15), who found no effect of the PEMT deficiency on plasma PC and CE concentrations in male mice fed a standard diet. It is not entirely clear why the present data differ from the previous report. However, it should be noted that decreases in PC and CE similar to those in the present study occurred in mice fed a high fat diet (15), indicating a relationship between PEMT activity and CE metabolism that is similar to that reported here. In the present study, the pattern of CE accumulation in the liver suggests that both the PEMT pathway and the CDP-choline pathway have to be active for proper flux of CE between liver and plasma. This stands in contrast with TG metabolism in which the disruption of PEMT activity or inhibition of the CDP-choline pathway alone was not sufficient to significantly diminish plasma TG concentrations. Thus, although choline supplementation may be sufficient to ameliorate TG accumulation in the liver, it may not be sufficient to ameliorate CE accumulation in the liver.

The quantitative profiling of lipid metabolites described in this study indicated that the PEMT pathway has several functions critical to normal physiologic function beyond its recognized role as a compensatory pathway for PC biosynthesis. These functions include a key role for PEMT in the flux of CE and TG between liver and plasma and the regulation of the distribution of 22:6(n-3) and 20:4(n-6) to tissues other than liver. The present data also suggest that diseases related to essential fatty acid metabolism could be caused by dysregulation of PEMT activity; under such conditions, dietary (n-3) fatty acids could fail to rescue this deficiency because PEMT activity is still required for the inclusion of 22:6(n-3) in lipoproteins. It is clear that the PEMT pathway plays a role in lipid metabolism beyond its recognized role as a compensatory pathway for PC biosynthesis under conditions of choline deficiency and that the pathway may prove central to many aspects of physiology.
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LITERATURE CITED


