

## Phosphatidylethanolamine *N*-Methyltransferase and Regulation of Homocysteine

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*Homocysteine is a non-protein, sulfur-containing amino acid derived from methionine metabolism and S-adenosylmethionine (SAM)-dependent transmethylation. Elevations in plasma homocysteine have been associated with increased risk of cardiovascular disease, atherosclerosis, birth defects, Alzheimer's disease, and osteoporosis. Of the many known methyltransferases that utilize SAM, phosphatidylethanolamine N-methyltransferase has recently received much attention for its possible role in the regulation of homocysteine.*

**Key words:** homocysteine; phosphatidylethanolamine *N*-methyltransferase; phospholipid; transmethylation

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Homocysteine is a non-protein, sulfur-containing amino acid that is a by-product of methyltransferase reactions, many of which occur predominantly in the liver. Methyltransferases catalyze the donation of a methyl group from *S*-adenosylmethionine (SAM) to a variety of biomolecules including lipids, protein, and DNA. All SAM-dependent methyltransferases result in the production of *S*-adenosylhomocysteine (SAH), which is then further converted to homocysteine (Figure 1). Homocysteine has a variety of fates. It can be remethylated to methionine by the folate-dependent enzyme methionine synthase or by the folate-independent enzyme betaine-homocysteine *S*-methyltransferase (BHMT). Alternatively, homocysteine can be irreversibly catabolized by the transsulfuration pathway, with cystathionine  $\beta$ -synthase being the first enzyme in that process. Catabolism of homocysteine is important in the production of a number of compounds including cysteine and glutathione. Excess homocysteine is excreted from the cell and enters the plasma circulation. This process is highly relevant because elevations in normal plasma homocysteine

concentrations of 5  $\mu$ M can increase the risk of coronary artery disease by 60% and 80% for men and women, respectively.<sup>1</sup>

In addition to remethylation and catabolism, homocysteine pools also reflect its production by SAM-dependent transmethylation reactions. There are many SAM-dependent methyltransferases involved in homocysteine production, including phosphatidylethanolamine *N*-methyltransferase (PEMT) and guanidinoacetate *N*-methyltransferase (GAMT). GAMT methylates guanidinoacetate, produced by arginine:glycine amidinotransferase in the kidney, to form creatine in the liver. In the studies of Mudd et al.,<sup>2,3</sup> it was estimated that GAMT consumes approximately 75% of the labile SAM methyl group pool, and about 15% of the remaining 25% is used by PEMT.

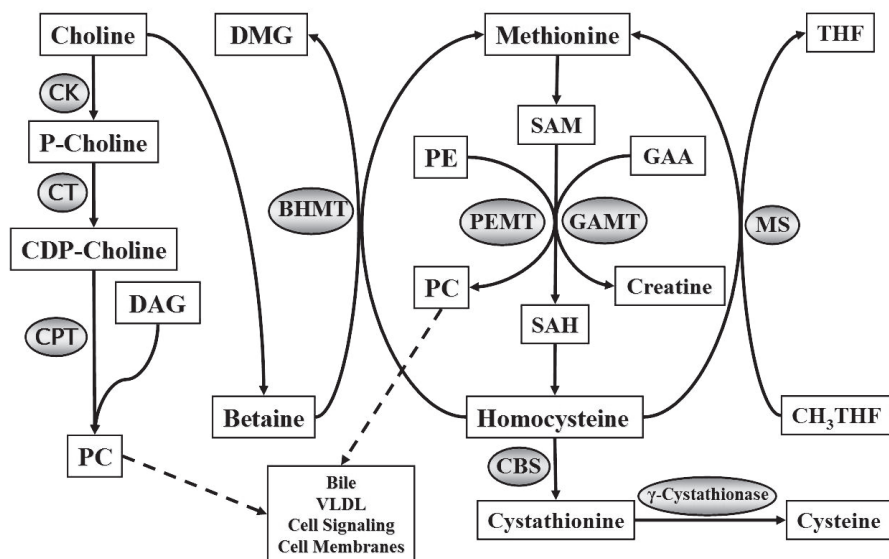
Phosphatidylcholine can be synthesized in the body by two different pathways: the CDP-choline pathway, which is present in all nucleated cells, and the PEMT pathway, which is found primarily in the liver. PEMT catalyzes the methylation of phosphatidylethanolamine to form phosphatidylcholine. The three methyl groups needed for this reaction are donated by SAM, and concomitantly this reaction produces three *S*-adenosylhomocysteine (SAH) molecules, which are then further converted to homocysteine. It is estimated that the PEMT pathway synthesizes 20% to 40% of the phosphatidylcholine in the liver.<sup>4</sup> The phosphatidylcholine from this reaction can also be used as a *de novo* source of choline.

There has been much research recently regarding the relative consumption of hepatic methyl groups by GAMT and PEMT and subsequent homocysteine production. Noga et al.<sup>5</sup> utilized *Pemt*<sup>-/-</sup> knockout mice and McArdle RH-7777 cells transfected with PEMT to study the contribution of PEMT to homocysteine levels. Plasma homocysteine concentrations in the *Pemt*<sup>-/-</sup> knockout mice were 50% of those in the wild-type mice. Similarly, hepatocytes isolated from the knockout mice secreted 50% less homocysteine than hepatocytes from wild-type mice. Furthermore, when McArdle RH-7777 cells, which have negligible PEMT activity, were transfected to stably express PEMT, there was more than a 2-fold increase in homocysteine secretion compared with

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**Figure 1.** Hepatic methyl group metabolism and CDP-choline pathways. BHMT, betaine-homocysteine *S*-methyltransferase; CBS, cystathionine  $\beta$ -synthase; CH<sub>3</sub>THF, 5-methyltetrahydrofolate; CK, choline kinase; CPT, choline phosphotransferase; CT, CTP-phosphocholine cytidyltransferase; DAG, diacylglycerol; DMG, dimethylglycine; GAA, guanidinoacetate; GAMT, guanidinoacetate *N*-methyltransferase; MS, methionine synthase; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; PC, phosphatidylcholine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

cells that were transfected with the vector alone. Based on the results of this study, along with estimates that a 20 g mouse secretes 30  $\mu$ mol of phosphatidylcholine into the bile every day<sup>6</sup> and that PEMT is responsible for synthesizing approximately one-third of the total phosphatidylcholine,<sup>2,3</sup> it was calculated that PEMT produces approximately 30  $\mu$ mol of SAH in the liver per day, an estimate that does not include the phosphatidylcholine synthesis needed to maintain cell membranes and lipoprotein secretion. It was further estimated that a 1 g liver would secrete about 5  $\mu$ mol of homocysteine per day, considerably less than the 30  $\mu$ mol of SAH estimated to be produced by PEMT. In contrast, hepatic methylation of guanidinoacetate to creatine to replace urinary creatinine losses has been estimated to be 3 to 15  $\mu$ mol/d.<sup>7,8</sup> Therefore, PEMT would produce from 2 to 10 times more SAH than GAMT, thereby presenting itself as a major determinant of homocysteine pools.<sup>9</sup>

McArdle RH-7777 cells not only have negligible PEMT activity, they also have negligible enzymatic activity of BHMT and choline oxidase, the latter being the enzyme responsible for the oxidation of choline to betaine. Moreover, it has been shown that methionine synthase is inactive in human hepatocarcinoma cells.<sup>10</sup> Therefore, hepatocarcinoma cells may be compromised in their ability to manage increased homocysteine production by transfected PEMT, resulting in an exaggerated increase in homocysteine secretion. Therefore, while this study demonstrated that PEMT has the ability to increase homocysteine levels, further research needs to be done to further elucidate the role of PEMT in homocysteine regulation under *in vivo* conditions.

A number of other relevant enzymes that contribute to the production and metabolism of homocysteine need to be evaluated as well. Future studies should utilize cell lines such as H4IIE rat hepatoma cells, which express glycine *N*-methyltransferase (GNMT)<sup>11</sup> and BHMT<sup>12</sup> and appear to be sensitive to many of the factors known to regulate them *in vivo*. Recently, it has been shown in GNMT knockout mice that GNMT is a critical protein required for methyl group homeostasis.<sup>13</sup>

As a follow-up to this study, Jacobs et al.<sup>14</sup> used liver-specific CTP:phosphocholine cytidyltransferase- $\alpha$  (CT $\alpha$ ) knockout mice. The CDP-choline pathway is regulated by the activity of cytidyltransferase, which has two isoforms, CT $\alpha$  and CT $\beta$ , of which CT $\alpha$  is the major form expressed in mice. For CT $\alpha$  knockout mice, hepatic cytidyltransferase activity was reduced to 15% of normal, thus diminishing the capacity of the CDP-choline pathway to contribute to hepatic phosphatidylcholine production. This reduction in phosphatidylcholine synthesis by the CDP-choline pathway led to a 2-fold induction of PEMT activity. Plasma homocysteine concentrations were increased 20% and 40% in knockout male and female mice, respectively, compared with their wild-type littermates. There was also an increase in homocysteine secretion from hepatocytes isolated from CT $\alpha$  knockout mice compared with hepatocytes from wild-type controls. This study also reported increased activities of BHMT and methionine adenosyltransferase, enzymes for homocysteine re-methylation and SAM synthesis, respectively. Theoretically, the induction of BHMT and methionine adenosyltransferase compensated for the increase in PEMT activity and may

have attenuated the increase in plasma homocysteine concentrations.

Taken together, the studies by Noga et al.<sup>5</sup> and Jacobs et al.<sup>14</sup> provide strong evidence that PEMT plays a significant role in the regulation of plasma homocysteine concentrations. However, both studies involved knockout mice models and thus the question of whether more moderate changes in PEMT activity will concomitantly be reflected in plasma homocysteine concentrations is not known. It is also not known how cells that exhibit normal expression levels of all of the relevant enzymes involved in remethylation and transsulfuration compensate for these changes to maintain homocysteine pools. Jacobs et al.<sup>14</sup> noted that there was increased activity and abundance of BHMT and methionine adenosyltransferase, although not enough to keep plasma homocysteine at control levels, leaving the possibility that, with a less drastic reduction in cytidylyltransferase activity, BHMT would be able to compensate for the increased homocysteine production.

In mice fed a choline-deficient diet for 3 weeks, fasting plasma homocysteine concentrations were not significantly different from mice fed a choline-sufficient or choline-supplemented diet; only post-methionine load homocysteine concentrations were significantly greater.<sup>15</sup> The same study found similar results in humans fed a choline-deficient diet. Cui and Vance<sup>16</sup> have shown that PEMT activity is elevated approximately 2-fold in rats fed a choline-deficient diet for 3 weeks. Therefore, under in vivo conditions, PEMT activity can be increased without resulting in increased fasting plasma homocysteine concentrations.

Although much remains to be elucidated, the studies of Noga et al.<sup>5</sup> and Jacobs et al.<sup>14</sup> have greatly advanced our understanding of how PEMT contributes to homocysteine production and its regulation. It will be important in future studies to ascertain the relative contributions of a number of enzymes involved in the metabolism of folate, methyl groups, homocysteine, and choline under a variety of physiologic and/or dietary conditions. A recent paper by Ratnam et al.<sup>12</sup> presented convincing evidence that BHMT plays an essential role in lowering plasma homocysteine levels in the diabetic state. Thus, it appears that the regulation of homocysteine likely reflects the collective action of more than one key enzyme. For example, it has recently been shown that although a diabetic state induced hepatic PEMT activity, plasma homocysteine concentrations were reduced.<sup>17</sup> Investigating homocysteine metabolism quantitatively in vivo under both normal and altered physiological states will be a key focus for future research and subsequent prevention of the numerous pathological conditions associated with hyperhomocysteinemia.

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