

Phosphatidylcholine and choline homeostasis

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Abstract Phosphatidylcholine (PC) is made in mammalian cells from choline via the CDP-choline pathway. Animals obtain choline primarily from the diet or from the conversion of phosphatidylethanolamine (PE) to PC followed by catabolism to choline. The main fate of choline is the synthesis of PC. In addition, choline is oxidized to betaine in kidney and liver and converted to acetylcholine in the nervous system. Mice that lack choline kinase (CK) α die during embryogenesis, whereas mice that lack CK β unexpectedly develop muscular dystrophy. Mice that lack CTP:phosphocholine cytidyltransferase (CT) α also die during early embryogenesis, whereas mice that lack CT β exhibit gonadal dysfunction. The cytidyltransferase β isoform also plays a role in the branching of axons of neurons. An alternative PC biosynthetic pathway in the liver uses phosphatidylethanolamine *N*-methyltransferase to catalyze the formation of PC from PE. Mice that lack the methyltransferase survive but die from steatohepatitis and liver failure when placed on a choline-deficient diet. Hence, choline is an essential nutrient. PC biosynthesis is required for normal very low density lipoprotein secretion from hepatocytes. Recent studies indicate that choline is recycled in the liver and redistributed from kidney, lung, and intestine to liver and brain when choline supply is attenuated.—Li, Z., and D. E. Vance. Phosphatidylcholine and choline homeostasis. *J. Lipid Res.* 2008. 49: 1187–1194.

Supplementary key words phosphatidylethanolamine *N*-methyltransferase • choline recycling • choline redistribution • phosphatidylethanolamine • lipoproteins

Phosphatidylcholine (PC) is an essential phospholipid in mammalian cells and tissues and is made in all nucleated cells via the choline pathway (Fig. 1). Choline was first identified in ox bile by Strecker (1) in 1862. The Greek word for bile is *chole*. After a long interlude, in 1932, Best and Huntsman (2) discovered the choline deficiency that results in fatty liver in rodents when insufficient choline is provided in the diet. In animals, choline can be acquired from the diet and via de novo biosynthesis: choline is produced through the methylation of phosphatidyleth-

anolamine (PE) to PC catalyzed by phosphatidylethanolamine *N*-methyltransferase (PEMT) (Fig. 1) (3, 4). Choline can then be generated from PC via the action of phospholipases. The PEMT/phospholipase reactions constitute the only known endogenous pathway for choline biosynthesis in animals, whereas in plants and some microbes, choline can be made from the methylation of phosphoethanolamine (5–7). Thus, choline is made from the methylation of the ethanolamine moiety of phosphoethanolamine or PE. Both exogenous and endogenous choline is converted into PC, which accounts for ~95% of the total choline pool in most animal tissues. The remaining 5% includes choline, phosphocholine, glycerophosphocholine, CDP-choline, and acetylcholine (8, 9). In animals, PEMT is quantitatively significant only in the liver (10), and it accounts for ~30% of hepatic PC biosynthesis in rodents (11–13). The other 70% of hepatic PC is made via the choline pathway.

THE CHOLINE PATHWAY FOR PC BIOSYNTHESIS

Choline kinase

Dietary choline is absorbed by the intestine in the form of lysophosphatidylcholine or choline, and uptake of the latter is mediated by choline transporters (14). Upon entry into the cell, choline is immediately phosphorylated to phosphocholine or oxidized to betaine in some cell types such as hepatocytes (15). The phosphorylation of choline is catalyzed by choline kinase (CK). Two genes encode CK. CK α 1 and CK α 2 are encoded by *Chka* on mouse chromosome 19 (16). CK β is encoded by *Chkb*, which is located

Abbreviations: AdoHcy, Sadenosylhomocysteine; AdoMet, Sadenosylmethionine; CD, choline-deficient; CK, choline kinase; CPT, CDP-choline:1,2-diacylglycerol cholinephosphotransferase; CT, CTP:phosphocholine cytidyltransferase; ER, endoplasmic reticulum; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase.

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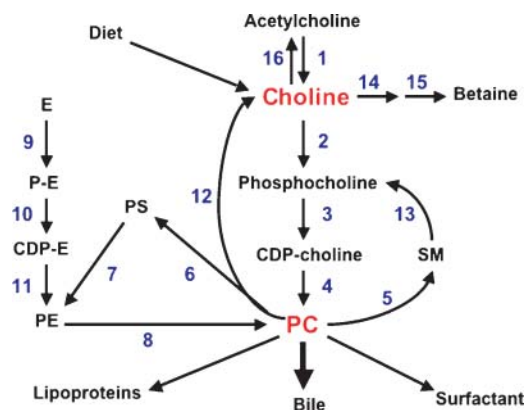


Fig. 1. Pathways involved in choline and phosphatidylcholine (PC) homeostasis. E, ethanolamine; P-E, phosphoethanolamine; CDP-E, CDP-ethanolamine; PE, phosphatidylethanolamine; SM, sphingomyelin. Enzyme names are indicated by numbers. 1, choline acetyltransferase; 2, choline kinase; 3, CTP:phosphocholine cytidyltransferase; 4, CDP-choline:1,2-diacylglycerol cholinephosphotransferase; 5, sphingomyelin synthase; 6, phosphatidylserine synthase 1; 7, phosphatidylserine decarboxylase; 8, phosphatidylethanolamine *N*-methyltransferase; 9, ethanolamine kinase; 10, CTP:phosphoethanolamine cytidyltransferase; 11, CDP-ethanolamine:1,2 diacylglycerol ethanolaminephosphotransferase; 12, various phospholipase and lysophospholipase activities; 13, sphingomyelinase; 14, choline oxidase; 15, betaine aldehyde dehydrogenase.

on chromosome 15. Mice that do not express either CK α isoform die at an early stage of embryogenesis, around day 3 (17). Mice that lack CK β are viable but develop rostrocaudal muscular dystrophy and neonatal bone deformity (18). The connection between PC synthesis and muscular dystrophy in *Chkb*^{-/-} mice remains unclear. The lack of understanding of how the lack of CK β could lead to muscular dystrophy reflects the almost complete lack of knowledge about PC biosynthesis and metabolism in muscle. CK α 1 contains 435 amino acids, and the splice variant, CK α 2, is identical except that an additional 18 amino acids are inserted after methionine-150 in the CK α 1 sequence (16). CK β contains 394 amino acids, and the sequence is 60% similar to that of CK α 1. The CKs can exist as either homodimers or heterodimers (16).

CTP:phosphocholine cytidyltransferase

Although CK catalyzes the initial and committed step in the conversion of choline to PC, CK is usually not considered to be rate-limiting or to regulate the rate of PC biosynthesis (19). Rather, the second reaction in the pathway, catalyzed by CTP:phosphocholine cytidyltransferase (CT), is usually rate-limiting (19, 20). CT in the mouse is also encoded by two genes (21–23). *Pcyt1a* encodes CT α 2 and the splice variant CT α 3 and is located on chromosome 16 (24). *Pcyt1b* encodes CT β 2 and CT β 3 and is located on the X chromosome (22). Mice that do not express CT α die during the early stages of embryogenesis (25). In contrast, mice that do not express CT β 2 survive but show gonadal dysfunction and reduced fertility (26). This phenotype reflects the relatively high expression of CT β 2 mRNA in testes and ovary (26). The mRNA for CT β 2 is not highly

expressed in most tissues but is found in brain, as will be discussed below, and in lung.

The regulation of CT activity has been studied intensely. A major mode of regulation involves the reversible movement of CT on and off the endoplasmic reticulum (ER) and/or the nuclear membrane (27–33). When bound to either of these membranes, CT is in its active form. Otherwise, CT is in a soluble form that is inactive. The membrane binding domain of CT is a long amphipathic α helix that enhances the binding of CT to membranes that are deficient in PC (31). The binding appears to activate CT by relieving an inhibitory restraint in the catalytic domain of the enzyme. Curiously, the soluble form of CT α has been localized to the nucleus in many, but not all, cell types, whereas soluble CT β , which lacks a nuclear localization signal, is cytoplasmic (24, 34, 35).

The transcriptional regulation of CT α expression has been investigated in detail and recently reviewed (36). CT α expression is largely governed by Sp1, Sp3, Rb, TEF4, Ets-1, and E2F, which enhance the expression of CT, and by Net, a factor that represses CT α expression. Key transcription factors involved in cholesterol or fatty acid metabolism (sterol-regulatory element binding proteins, liver X receptors, peroxisome proliferator-activated receptors) do not appear to play a major role in the transcriptional regulation of CT α . Rather than being linked to cholesterol or energy metabolism, the regulation of CT α at the level of gene expression is linked to the cell cycle, cell growth, and differentiation (36).

CDP-choline:1,2-diacylglycerol cholinephosphotransferase

The final reaction in the choline pathway for PC biosynthesis is catalyzed by CDP-choline:1,2-diacylglycerol cholinephosphotransferase (CPT). This enzyme has never been purified from any source, probably because it is an intrinsic membrane protein found primarily on the ER (19). With new molecular tools, such as the use of a tagged protein in an expression system, CPT purification can now be undertaken with a higher probability of success. Also, the gene(s) in mice that encodes CPT has not been characterized. However, two human CPT cDNAs have been cloned and expressed (37, 38). Most studies indicate that there is an excess of CPT activity in cells; hence, the amount of enzyme does not limit the rate of PC biosynthesis. However, *in vivo*, it seems clear that the CPT reaction is governed by the supply of both CDP-choline and diacylglycerol (19).

PEMT

The second pathway for PC biosynthesis, catalyzed by the ~20 kDa protein PEMT, is quantitatively significant only in the liver (10). PEMT is localized to the ER and mitochondria-associated membranes (a subfraction of the ER) (39). PEMT spans the membrane with four transmembrane sequences (40). The methyl donor for the methylation reactions is *S*-adenosylmethionine (AdoMet),

which binds to several residues of PEMT exposed on the cytosolic surface of the ER (41).

The PEMT gene resides on chromosome 11 in the mouse and spans 25 kb (42). Mice that lack PEMT (*Pemt*^{-/-}) exhibit no obvious phenotype when fed a chow diet (43). However, when fed a choline-deficient (CD) diet to restrict the availability of choline for PC synthesis, the mice rapidly develop steatosis, steatohepatitis, and die from liver failure after 3 days (44). The liver failure coincides with a 50% decrease in hepatic PC content; thus, it was speculated that this was the reason for the steatohepatitis and liver failure (44). Because other tissues did not show this dramatic change in PC levels, we hypothesized that the liver failure/decrease in hepatic PC levels might be attributable to the robust secretion of PC into bile [23 mg/day for a 20 g mouse (45), which is equivalent to the entire pool of PC in the liver]. This hypothesis was tested by breeding *Pemt*^{-/-} mice with mice that lacked MDR2/ABCB4, the protein that transports PC into bile. *Mdr2*^{-/-} mice secrete no PC and little cholesterol into bile but continue to secrete bile acids (46). Remarkably, when fed a CD diet, the *Pemt*^{-/-}/*Mdr2*^{-/-} mice survived for >90 days, whereas *Pemt*^{-/-}/*Mdr2*^{+/+} mice experienced end-stage liver failure after 3 days. Unexpectedly, the *Pemt*^{-/-}/*Mdr2*^{-/-} mice developed steatosis when fed the CD diet, and the PC levels also decreased by ~50%. Thus, the rapid liver failure in *Pemt*^{-/-} mice could not be attributed to the decrease in PC or the accumulation of triacylglycerol (47).

The question then became: what difference between *Pemt*^{-/-} mice and *Pemt*^{-/-}/*Mdr2*^{-/-} mice allows the double knockout to survive for 90 days when fed a CD diet, whereas the *Pemt*^{-/-} mice die after 3 days? One difference that became apparent was that the concentration of hepatic PE decreased in the double knockout mice but not in the *Pemt*^{-/-} mice (47). Thus, the ratio of PC to PE decreased from ~1.8 to ~0.8 in the livers of *Pemt*^{-/-} mice fed the CD diet for 3 days, whereas the ratio in *Pemt*^{-/-}/*Mdr2*^{-/-} mice decreased to only ~1.4 after being fed the CD diet for 21 days. No changes were observed in hepatic sphingomyelin or cholesterol content in either strain of mouse.

PC is a cylindrically shaped molecule, whereas PE usually has an inverted cone shape (48). Because the amount of PC decreased on the plasma membrane of livers from *Pemt*^{-/-} mice fed a CD diet (47), we considered that PC might be replaced by PE. In such a circumstance, the packing of the bilayer might become permeable, leading to steatohepatitis and liver failure. Subsequent studies showed that, indeed, there was increased PE on the hepatic cell surface of *Pemt*^{-/-} mice fed a CD diet as well as increased plasma alanine aminotransferase (normally only found in the liver), indicating membrane permeability (47). Moreover, the leakage of alanine aminotransferase was attenuated by inhibiting the biosynthesis of PE, thereby increasing the ratio of PC to PE in the livers or hepatocytes from *Pemt*^{-/-} mice fed the CD diet (47). These results provided strong support for the hypothesis that *Pemt*^{-/-} mice fed the CD diet develop steatohepatitis as a result of increased permeability of the hepatic plasma

membrane caused by a decreased ratio of PC to PE. In a preliminary study on liver biopsies from humans with nonalcoholic steatohepatitis, the PC-to-PE ratio was reduced markedly (47).

Thus, when the dietary supply of choline limits PC biosynthesis, PEMT plays an important role by providing PC/choline for normal liver function.

PC BIOSYNTHESIS, VLDL SECRETION, AND HOMOCYSTEINE

PC biosynthesis is required for the normal secretion of VLDL by hepatocytes. Elimination of choline and methionine (two precursors of PC synthesis) from hepatocyte culture medium reduced VLDL secretion (49). The requirement for choline was specific, and choline could not be replaced by dimethylethanolamine, monomethylethanolamine, or ethanolamine (50). In rodents, a CD diet markedly reduced plasma levels of apolipoprotein B, a major component of VLDL (51). If, however, only choline were removed from hepatocyte culture medium, VLDL secretion was not impaired because PC synthesis was not reduced (52). Consequently, these studies did not establish whether or not PC synthesis from the choline pathway is required for VLDL secretion. In subsequent studies, the choline pathway for PC biosynthesis was shown to be required for normal VLDL secretion in mice that lacked CT α specifically in hepatocytes (53). In CT α -deficient hepatocytes, there was an almost 2-fold increase in PEMT activity. However, this increased capacity for the generation of PC did not substitute for the attenuation of the CDP-choline pathway in CT α -deficient hepatocytes.

The generation of *Pemt*^{-/-} mice allowed the role of PEMT in VLDL secretion to be examined. When these mice were fed a chow diet, the concentration of hepatic PC was the same as in *Pemt*^{+/+} mice. Unexpectedly, the secretion of triacylglycerol from *Pemt*^{-/-} hepatocytes was 50% less than that from *Pemt*^{+/+} hepatocytes (54). Thus, both the choline pathway and the PEMT pathway are independently required for normal VLDL secretion. Exactly what role each pathway contributes to the assembly and secretion of VLDL is not clear.

In the process of converting PE to PC, the PEMT reaction generates three molecules of S-adenosylhomocysteine (AdoHcy). AdoHcy is hydrolyzed in the liver to adenosine and homocysteine by AdoHcy hydrolase. Increased plasma homocysteine is an independent risk factor for cardiovascular disease and myocardial infarction (55, 56). Because PEMT accounts for 30% of PC made in rat liver, and because three AdoMet molecules are made per PC produced, we expected that the PEMT reaction might generate much of the plasma homocysteine. Indeed, the plasma of *Pemt*^{-/-} mice contained 50% less homocysteine compared with that of *Pemt*^{+/+} mice (57). Moreover, hepatocytes from *Pemt*^{-/-} mice secreted less homocysteine than did those from *Pemt*^{+/+} mice. In liver-specific CT α knockout mice, PEMT activity was increased and plasma homocysteine was increased by 20–40% (58).

Thus, *Pemt*^{-/-} mice exhibit decreased secretion of VLDL and homocysteine. Because increased levels of both VLDL and homocysteine increase the risk of cardiovascular disease, we reasoned that *Pemt*^{-/-} mice might be protected from atherosclerosis. Mice that are fed a high-fat/high-cholesterol diet do not easily develop atherosclerosis, whereas mice that lack the low density lipoprotein receptor (*Ldlr*^{-/-}) are highly susceptible to diet-induced atherosclerosis (59). Consequently, *Pemt*^{-/-} mice were bred into the *Ldlr*^{-/-} mouse background and fed a high-fat/high-cholesterol diet for 16 weeks. Atherosclerotic lesion areas were 85% less in the mice that lacked PEMT compared with the *Ldlr*^{-/-}/*Pemt*^{+/+} mice (Y. Zhao and D. E. Vance, unpublished data).

A UNIQUE ROLE FOR CTβ IN NEURONS

PC was thought to be made in the cell body of neurons and transported to axons for axonal growth (60). However, studies in the early 1990s showed that the biosynthesis of PC and other phospholipids occurs in axons as well as in cell bodies (61, 62). In contrast, cholesterol is not made in axons but rather is supplied by the cell bodies or obtained from lipoproteins (62, 63).

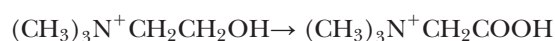
Although CTα is expressed in all tissues, CTβ2 and CTβ3 mRNAs are highly enriched in the brain compared with other tissues (22). Thus, the question arose why CTβ2 and CTβ3 are enriched in the brain. This question was addressed in rat pheochromocytoma (PC12) cells. When incubated with nerve growth factor, PC12 cells stop proliferating and differentiate into sympathetic neuron-like cells (64). This growth of neurites increases the demand for phospholipids, and the biosynthesis of PC is stimulated in response to nerve growth factor (65, 66). Indeed, during neurite outgrowth of PC12 cells, the amounts of cellular PC and CT activity double (66). CTβ2 mRNA increased within 1 day of nerve growth factor application and continued to increase during the growth of the neurites. Moreover, the translocation of CTβ2 to membranes was increased. In contrast, nerve growth factor did not alter CTα expression or translocation. In another model (Neuro2a cells), retinoic acid significantly increased both CT activity and CTβ2 protein without affecting CTα expression (66). Thus, it appears that the CTβ2 isoform is specifically activated during neuronal differentiation to increase the supply of PC for neurite growth.

When the expression of CTβ2 in PC12 cells was decreased by RNA silencing, a remarkable phenotype was observed. Neurite branching (i.e., numbers of primary and secondary neurites) was reduced dramatically, although the length of individual neurites was increased significantly (67). As a result, the total amount of neuronal membrane was unchanged (67). Thus, CTβ2 appears to play an unexpected role in the promotion of neurite branching. At this juncture, it is unclear what the requirement is for CTβ2. However, the phenomenon does not appear to be a cell line artifact, because primary sympathetic neurons from mice that lack CTβ2 also dis-

play decreased branching of neurites (L. Demizieux and J. E. Vance, unpublished results).

CHOLINE HOMEOSTASIS

Figure 1 shows the known pathways for the metabolism of choline in mammals. As noted above, choline can be derived from the diet or can be generated de novo from the PEMT reaction coupled with phospholipase catabolism of PC. Choline is also released during the biosynthesis of phosphatidylserine from PC catalyzed by phosphatidylserine synthase-1 (19). The catabolism of sphingomyelin or acetylcholine can also generate choline. The choline can be used to make PC, converted to betaine, or acetylated to acetylcholine. Betaine is used as a source of methyl groups in the conversion of AdoHcy to AdoMet (68). The oxidation of choline to betaine



occurs primarily in liver and kidney (68). The conversion of choline to acetylcholine is catalyzed by choline acetyltransferase (69). Acetylcholine was the first neurotransmitter to be described and plays an important role in learning, memory, and sleep. The action of acetylcholine is terminated by acetylcholine esterase, which hydrolyzes acetylcholine to choline and acetate (70). Choline is also incorporated into plasmalogen, choline plasmalogen, and platelet-activating factor (71).

By far, the major fate of choline is conversion to PC, and this occurs in all mammalian cells that have a nucleus. PC functions as a primary lipid in cellular membranes and is a precursor of signaling molecules. PC can be converted to phosphatidylserine or sphingomyelin or be degraded by phospholipases. In the liver, the major fates of PC are secretion into bile, secretion into VLDL, and use for the formation of high density lipoproteins in the plasma. Approximately 95% of biliary PC is reabsorbed by the intestine, but only ~40% of this PC is returned to the liver (72, 73). Thus, for each 100 molecules of PC secreted into bile, only 40 molecules of PC return to the liver, 5 are excreted, and 55 are used in other tissues. PC is also presumed to be important in the secretion of chylomicrons (which contain PC) from the intestine, but this function has not been demonstrated directly.

In summary, choline in mammals is derived either from the diet or from de novo synthesis via PEMT. The two major depletion pathways are choline oxidation and excretion of biliary PC.

CHOLINE IS AN ESSENTIAL NUTRIENT

Although choline is an important nutrient for animals (2, 8, 74–78), choline deprivation is not lethal in rodents and humans. Consequently, it was not clear that choline is an essential nutrient. Development of the *Pemt*^{-/-}/*Mdr2*^{-/-} mouse model fed a CD diet has now demonstrated that choline is indeed an essential nutrient. A

CHOLINE BALANCE AND ADAPTATION TO CHOLINE IMBALANCE

minimum threshold of total choline-containing metabolites in liver appears to be ~ 110 nmol/mg protein (79). When *Pemt*^{-/-} mice were fed the CD diet for 3 days, the levels of total choline metabolites diminished and the mice did not survive, whereas *Pemt*^{-/-}/*Mdr2*^{-/-} mice fed the CD diet were able to maintain ~ 110 nmol choline metabolites/mg protein in the liver for at least 90 days (79). Thus, the data strongly support the essential requirement of choline for at least rodent life. Choline may also be essential for humans, as was concluded by Zeisel (77), when excess methionine and folate are not available in the diet. It is certainly clear that in humans, inadequate choline intake can result in fatty liver or muscle damage (80).

Whether or not choline can be replaced by related compounds has been addressed. In yeast, the requirement for choline can be met by either dimethylethanolamine or propanolamine (81, 82). However, dimethylethanolamine did not substitute for choline in *Pemt*^{-/-} mice under complete choline deprivation (defined as *Pemt*^{-/-} mice fed a CD diet) (83). Moreover, choline could not be replaced by either methionine or a series of propanolamine derivatives, including 2-amino-propanol, 2-amino-isopropanol, or 3-amino-propanol, in CD-*Pemt*^{-/-} mice (84). Thus, the requirement of choline for mammalian life is very specific for choline, with its three *N*-methyl groups, and choline cannot even be replaced by dimethylethanolamine that has one less *N*-methyl group. This specificity is unexpected, because the physical properties of PC in membranes are very similar to those of phosphatidyl dimethylethanolamine (85).

Choline insufficiency is considered to be rare in humans and is manifest only during pregnancy, lactation, or starvation/fasting, because normal diets contain sufficient choline (75, 77, 86). Recently, patients with non-alcoholic steatohepatitis and brain disorders also showed signs of choline deficiency, despite what would be considered to be normal choline content in their diet (87–89). Betaine supplementation was found to be a potential therapy for alcoholic and nonalcoholic steatohepatitis, probably because betaine appears to stimulate PC biosynthesis via the PEMT pathway (88, 89). Moreover, gut microbes in insulin-resistant mice were found to catabolize choline into methylamines, which led to choline deficiency and nonalcoholic fatty liver disease (90). These observations support the view that choline absorption is important in maintaining normal choline homeostasis. Furthermore, specific organs, such as the brain, might be relatively deficient in choline. CDP-choline (citicoline) is used in several European countries and Asia to treat cognitive and memory impairment from Alzheimer's disease, stroke, or traumatic coma (91). CDP-choline, an intermediate in PC biosynthesis, is thought to protect cell membranes from damage by stimulating PC production (91–93).

Thus, choline deficiency is now thought to have an impact on human diseases such as liver disease, atherosclerosis (via lipoprotein secretion), and possibly neurological disorders.

The concentration of choline and its metabolites is balanced by two choline acquisition pathways (dietary intake of choline and the PEMT pathway) and by two choline depletion pathways (choline oxidation and biliary PC secretion) (Fig. 2). If either the acquisition of choline or its disposal is perturbed, choline imbalance occurs. It is now becoming apparent that compensatory mechanisms exist to restore choline balance in this situation. For example, the expression of PEMT is enhanced by ~ 2 -fold in livers of rats fed a CD diet (94, 95). In recent years, other mechanisms for restoring choline balance have been discovered.

Unlike the storage of the fatty acids in triacylglycerol and glucose in glycogen, there is no significant storage pool of choline in mammalian cells. PC might be considered to be a long-term storage form of choline, but this was not the case under the conditions of complete choline deprivation achieved in *Pemt*^{-/-} mice fed the CD diet; these mice died after 3 days (79). Interestingly, the response to complete choline deprivation was gender-dependent (96). Female *Pemt*^{-/-} mice maintained normal levels of hepatic PC/total choline during the first day of choline deprivation and escaped liver damage, whereas male *Pemt*^{-/-} mice did not (96). Additional data suggest that in female mice, the efflux of PC from extrahepatic tissues to HDL particles in the circulation was increased, thereby providing sufficient PC/choline for liver during the initial stages of choline deficiency. This sparing of the CD effect in females lasted for only 1 day. Why female mice can implement this particular adaptation is

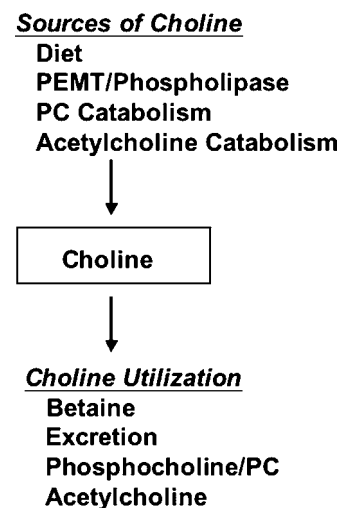


Fig. 2. Choline balance is determined by choline supply and choline utilization. The steady-state level of choline is adjusted according to the amounts generated from sources of choline less the amount that is used. Total body choline is determined by diet and phosphatidylethanolamine *N*-methyltransferase (PEMT), whereas total utilization in the body is governed by oxidation to betaine and excretion. PC and acetylcholine catabolism are involved in the recycling/redistribution of choline. Phosphocholine/PC and acetylcholine are biosynthetic products of choline.

unknown, but it might provide an evolutionary advantage attributable to the choline deficiency that occurs during pregnancy and lactation. Thus, mobilization of choline from extrahepatic tissues appears to be an acute response to severe choline deprivation.

Choline imbalance can also be reversed by modifications of hepatic choline metabolism. The liver is probably the most active organ for choline metabolism. In dealing with the stress of complete choline deprivation, choline recycling can be enhanced in the liver (79). Elimination of the loss of PC into bile facilitated the survival of *Mdr2*^{-/-}/*Pemt*^{-/-} mice fed the CD diet and enabled these mice to implement significant changes in the metabolism of PC and choline. Notably, the oxidation of choline to betaine was curtailed markedly, and the activities of CK and CT were increased to ensure reutilization of choline for PC biosynthesis (79). Increased phospholipase A₂ activity and PC catabolism also enhanced choline recycling. Moreover, as indicated above, the de novo synthesis of PC is required for normal lipoprotein secretion from the liver (49, 53, 54). An enigma that remains is that CD-*Pemt*^{-/-} mice continue to secrete PC into bile despite the onset of liver failure (79), even though, as shown in the *Mdr2*^{-/-} mice, PC secretion is not required for the secretion of bile acids into bile (46). Although choline recycling was discovered in the extreme model of CD-*Pemt*^{-/-} mice (79), it seems likely that this adaptation also occurs in less extreme situations, such as in choline deficiency in animals.

Choline recycling involves the reuse of choline in a particular tissue, for example, after the catabolism of PC and sphingomyelin in the liver. Choline redistribution also can occur in response to choline deprivation. In this case, choline from one tissue is transported to another tissue. These data suggest that mice attempt to maintain choline homeostasis in the brain and liver at the expense of other organs (97). When *Mdr2*^{-/-}/*Pemt*^{-/-} mice were fed the CD diet, the brain and liver maintained a low level of activity of choline oxidase but the level of total choline-containing metabolites was unchanged after 21 days. In studies with [³H]choline in intact mice, choline redistribution was found not only in CD-*Mdr2*^{-/-}/*Pemt*^{-/-} mice but also in CD-*Pemt*^{-/-} mice (97). Tissues that were important donors of choline were identified as kidney, lung, and intestine. Although choline redistribution was discovered in these extreme models, it most likely occurs during choline deprivation in wild-type models.

CHOLINE BALANCE THEORY

As stated, animals attempt to maintain a balance between gain and loss of choline that in extreme examples can lead to death. The ratio of PC to PE, choline recycling, choline redistribution, choline acquisition, and choline depletion all contribute to choline and PC homeostasis in mice. When choline balance is altered, mice attempt to restore the normal levels of choline and metabolites, par-

ticularly in the liver and brain. When *Mdr2*^{-/-}/*Pemt*^{-/-} mice and *Pemt*^{-/-} mice were fed the CD diet, there was no choline input. The mice minimized their output of choline by decreasing choline oxidation in liver and brain (79). If PC continued to be secreted from the liver into bile, a lethal reduction of choline occurred in CD-*Pemt*^{-/-} mice. However, when biliary PC secretion was ablated by elimination of the *Mdr2* gene in *Pemt*^{-/-} mice, a new level of choline metabolites was reached (79). In this example, choline balance was achieved even though there was no choline input and minimal choline output (oxidation). Unlike choline deprivation, choline excess is rarely reported (98).

Excess choline intake is toxic for humans (99). The tolerable upper limit for choline intake from dietary sources and supplements is 3.5 g/day. Symptoms of choline toxicity include fishy body odor, vomiting, increased salivation, increased sweating, and low blood pressure.

Figure 2 illustrates the choline balance theory, which states that there is a balance between the source of choline and its utilization that determines the survival of choline-containing organisms. The lives of these organisms are critically dependent on the maintenance of a minimal total choline level. The ratio of PC to PE, choline recycling, choline redistribution, and choline acquisition and depletion pathways might be adjusted in adaptations to choline imbalance. The studies with CD-*Mdr2*^{-/-}/*Pemt*^{-/-} mice and CD-*Pemt*^{-/-} mice suggest that the critical level of choline and its metabolites in liver is ~110 nmol/mg protein. The minimum level in other tissues is unknown. Much is now understood about how mice compensate for a decrease in choline input using the mechanisms discussed above: decreased choline oxidation, choline recycling, particularly in the liver, and choline redistribution (reverse choline transport) from other tissues to the liver. The regulatory mechanisms that govern these adaptations are unknown. ■■

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