Choline is an important nutrient for mammals. Choline can also be generated by the catabolism of phosphatidylcholine synthesized in the liver by the methylation of phosphatidylethanolamine by phosphatidylethanolamine N-methyltransferase (Pemt). Complete choline deprivation is achieved by feeding Pemt−/− mice a choline-deficient diet and is lethal due to liver failure. Mice that lack both Pemt and Mdr2 (multiple drug-resistant protein 2) successfully adapt to choline deprivation via hepatic choline recycling. We now report another mechanism involved in this adaptation, choline redistribution. Normal levels of choline-containing metabolites were maintained in the brains of choline-deficient Mdr2−/−/Pemt−/− mice for 90 days despite continued choline consumption via oxidation. Choline oxidase activity had not been previously detected in the brain. Plasma levels of choline were also maintained for 90 days, whereas plasma phosphatidylcholine levels decreased by >60%. The injection of [3H]choline into Mdr2−/−/Pemt−/− mice revealed a redistribution of choline among tissues. Although CD-Pemt−/− mice failed to adapt to choline deprivation, choline redistribution was also initiated in these mice. The data suggest that adaptation to choline deprivation is not restricted to liver via choline recycling but also occurs in the whole animal via choline redistribution.

In most mammalian cells, PC is used for the maintenance of membranes and as a precursor of signaling molecules. Hepatic PC is also required for very low density lipoprotein secretion (7, 8) and is an important component of bile. PC is transferred into the lumen of bile canaliculi via the action of MDR2/ABCB4 (9). We reasoned that, if biliary secretion of PC were blocked in Pemt−/− mice, the severe consequences of choline deprivation might be attenuated. Indeed, Mdr2−/−/Pemt−/− mice showed an adaptive response to choline deprivation, choline recycling in the liver, that permitted the mice to survive for at least 90 days (3). Although the level of total choline-containing metabolites in the liver decreased by ~50% after 21 days in Mdr2−/−/Pemt−/− mice fed a CD diet compared with choline-supplemented (CS) mice, this level of total choline-containing metabolites was maintained for at least 3 months aided by choline recycling and by decreased choline oxidation (3). Choline oxidation (the only catabolic pathway of choline) in the liver was not completely eliminated but continued at a low rate in CD-Mdr2−/−/Pemt−/− mice during prolonged choline deprivation (3). We, therefore, considered the possibility that choline is redistributed from non-hepatic tissues to the liver as another mechanism for adaptation to choline deprivation in Mdr2−/−/Pemt−/− mice.

During choline deprivation, no obvious phenotype was observed in organs other than liver in CD-Pemt−/− mice (6). The brain is an important organ for choline metabolism, because choline is required for biosynthesis of acetylcholine for neurotransmission as well as for the biosynthesis of PC (10). Interestingly, the brains in both CD-Pemt−/− and Mdr2−/−/Pemt−/− mice did not show any obvious damage during choline deprivation. Because the brain also appears to adapt to choline deprivation, we hypothesized that the brain obtains choline from peripheral tissues during choline deprivation.

We, therefore, tested the hypothesis that, during severe choline deprivation, a mechanism is triggered whereby a redistribution of choline from other tissues to liver and brain allows these organs to maintain sufficient levels of choline/PC. Our results demonstrate that adaptation to choline deprivation is not restricted to the liver via choline recycling but also involves the whole body via choline redistribution among tissues.

EXPERIMENTAL PROCEDURES

Animals, Lipid Analysis, and Choline Oxidase Assay—As in previous studies (3, 11), Mdr2−/−/Pemt−/− mice were produced by breeding Pemt−/− mice (C57BL/6; 129/J background) (12) with Mdr2−/− mice (FVB; 129/J background) (9). The mice
were fed a CD diet (ICN, catalog number 0290138710) or a CS diet (the CD diet supplemented with 0.4% (w/w) choline chloride). All mice were fed the chow diet for 10–12 weeks, after which the Pemt<sup>−/−</sup> and Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice were fed the CS diet for 24 h and then fed the CD diet for 3–90 days. The level of choline-containing metabolites was measured with the Phospholipids B kit (Wako). The amount of PC in the brain was measured by high pressure liquid chromatography after lipid extraction. A, choline-containing metabolites. B, the level of PC. Data are averages ± S.D. from 6–8 mice.

**Plasma S100B Protein Assay**—S100B is a protein in brain and, when found in the plasma, is a marker of brain damage (17, 18). Sandwich enzyme-linked immunosorbent assay was performed for measurement of the level of S100B protein in 20 μl of plasma using rabbit polyclonal anti-S100B protein antibodies (Santa Cruz Biotechnology). The secondary antibody was anti-rabbit IgG-conjugated to horseradish peroxidase, and 3,3′,5,5′-tetramethylbenzidine (Sigma) was used for color development. Mouse brain homogenates were used as a positive control for the measurement of S100B. Plasma from wild type mice was used as a negative control.

**Measurement of Choline-containing Metabolites**—Liver and brain homogenates were assayed for choline-containing metabolites including PC, sphingomyelin, glycerophosphocholine, and free choline with a Phospholipids B kit (Wako) based on the release of free choline by phospholipase D and subsequent choline oxidation by choline oxidase (15, 16).

**Immunoblotting for Choline High Affinity Transporter (CHT)** and **Choline Oxidase**—Proteins (50 μg) from liver and brain homogenates were separated by electrophoresis on 10% polyacrylamide gels containing 0.1% SDS and immunoblotted with anti-CHT antibodies (Santa Cruz Biotechnology) or anti-choline oxidase antibodies (a gift from Dr. Timothy Garrow, University of Illinois). S100B protein was immunoblotted as a loading control for brain analyses. Protein disulfide isomerase was blotted as a loading control for hepatic proteins.

**In Vivo Injection of [3H]Choline—Pemt<sup>−/−</sup> and Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice** were fed a CS diet or a CD diet for 3 (CS3/CD3) or 21 (CS21/CD21) days. [methyl-<sup>3</sup>H]Choline (100 μCi) (Amersham Biosciences) in 100 μl of saline was injected into mice via the tail vein. Mice were sacrificed 1 h or 24 h after injection. Blood was collected by cardiac puncture. Plasma was separated by centrifugation of whole blood at 2000 revolutions/min for 20 min in a refrigerated bench-top centrifuge. Brain, liver, intestine, kidney, heart, lung, and skeletal muscle were dissected and frozen in liquid N<sub>2</sub> and then at −70 °C before use. Tissues were homogenized with a Polytron in 5 volumes of 10 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1:100 protease inhibitor mixture (Sigma catalog number P8340). Total radioactivity in the whole tissues of brain, liver, intestine, kidney, heart, lung, and muscle, as well as plasma was measured. Total muscle weight was calculated according to the muscle/body weight index of mice (19). Total plasma volume was estimated as 2 ml in a 20-g mouse (20). Four mice were in each group, and [3H]choline incorporation was measured in duplicate. [3H]Betaine was isolated by thin-layer chromatography and then measured by a scintillation counter (15, 16). Data from individual mice were normalized to a mouse of 20 g body weight for comparison.

**RESULTS**

**Brain Damage Was Not Detected during Choline Deprivation**—The brain protein S100B, when found in the plasma, is a marker of brain damage (17, 18). S100B was not detected in the plasma of CS- and CD-Pemt<sup>−/−</sup> mice or in CS- and CD-Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice, indicating a lack of brain damage in both CD-Pemt<sup>−/−</sup> mice and CD-Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice (S100B level in 1:100 diluted brain homogenates: 0.84 color intensity as a positive control; no S100B was detected in plasma of wild type mice as a negative control). We also examined genomic DNA in the brains and did not observe any DNA laddering, suggesting that choline deprivation did not induce significant apoptosis in the brains of either mouse model.

**Brain Did Not Exhibit Choline Deficiency**—The level of choline-containing metabolites in brain homogenates was analyzed. Choline deprivation did not significantly change the total amount of choline-containing metabolites in the brains of either Pemt<sup>−/−</sup> mice or Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice (Fig. 1A). Furthermore, the amount of PC, the major choline-containing metabolite, was not significantly altered in the brains of either mouse model during choline deprivation (Fig. 1B). Interestingly, the brain maintained significant choline oxidation capacity in both mouse models during choline deprivation, according to both in vitro and in vivo assays (Figs. 2, A–C). Choline oxidase activity was measured by incubation of [3H]choline with mitochondria isolated from the brains of Pemt<sup>−/−</sup> and Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice fed the CS or CD diet (Fig. 2A). No detectable decrease in choline oxidase activity was observed in either model after 3 (Pemt<sup>−/−</sup> mice) or 21 (Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice) days of the CD diet. However, for the Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice, after 90 days on the CD diet, choline oxidase activity declined...
by >50% (Fig. 2A). As an alternative measurement of choline oxidation, we injected both strains of mice that had been fed the CS or CD diet with 100 μCi of [3H]choline. After 1 and 24 h, the mice were sacrificed and the formation of [3H]betaine was determined. No differences were observed between the Pemt−/− mice fed the CD versus CS diet (Fig. 2B). In contrast, in Mdr2−/−/Pemt−/− mice fed the CD diet for 21 days, the amount of labeled betaine in the brain was approximately double that in CS Mdr2−/−/Pemt−/− mice (Fig. 2C). After 24 h, approximately half of the labeled betaine had been depleted. Despite detecting choline oxidase activity in the brain, we were unable to detect the protein in the brains of the mice by immunoblotting (data not shown). However, the amount of choline oxidase protein in the livers appeared to be lower in CD-Pemt−/− mice than in CS-Pemt−/− mice (Fig. 2D), whereas hepatic protein levels of choline oxidase only decreased in Mdr2−/− mice at 21 days of choline deprivation compared with CS mice (Fig. 2D). Choline oxidation is the only known catabolic pathway for choline (3, 21). To our knowledge, this is the first report that mouse brain contains choline oxidase activity.

Active choline transport plays an important role in choline uptake and is also a critical step in maintaining choline homeostasis (22). The immunoblots in Fig. 3 show that the expression of CHT in brains was unaltered by choline deprivation (Fig. 3), whereas there was a small decrease in CHT expression in the livers of Mdr2−/−/Pemt−/− mice during choline deprivation. CHT is under feedback regulation by acetylcholine, the concentration of which is dependent on the pool size of total choline-containing metabolites in brain (23). Thus, unaltered CHT protein levels might result from reduced CHT transcription in brains of mice during choline deprivation.

FIGURE 3. Expression of the high affinity choline transporter in brain and liver. Pemt−/− and Mdr2−/−/Pemt−/− mice were fed the CS diet for 24 h and then fed the CD diet for 3, 21, or 90 days. Fifty μg of protein from liver or brain homogenates were separated by 10% SDS-PAGE and blotted with anti-CHT antibodies. Immunoblotting of S100β was used as a loading control for brain samples. Protein disulfide isomerase (PDI) was used as a loading control for the liver samples. Data are from one experiment and representative of three similar experiments. Intensities of the bands were normalized to proteins used as loading controls and also relative to values (1.0) from CS-Pemt−/− mice. Each band is from one mouse and representative of similar experiments for a total 6–8 mice in each group.
to another. Nevertheless, we obtained clues about the movement of choline within the body by injecting a bolus of \(^{3}H\)choline into the mice. To trace the distribution/redistribution of choline during choline deprivation, \(^{3}H\)choline was injected through the tail vein into Pemt\(^{-/-}\) mice or Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice that had been fed the CS or CD diet. Two time points (1 and 24 h) were used to assess choline labeling of tissues after injection. Total radioactivity in each tissue was calculated on the basis of a mouse with a body weight of 20 g.

The liver is the major organ for choline oxidation (24). We have shown previously that, during choline deprivation, the activity of hepatic choline oxidase is almost completely curtailed in both mouse models (3). At the same time, biliary PC secretion is dramatically reduced in CD-Pemt\(^{-/-}\) mice and then fed the CD diet for 3, 21, or 90 days. Choline was quantified with choline oxidase. Plasma PC was measured by high performance liquid chromatography. A, the amounts of choline in plasma. B, plasma PC content. Data are averages \(\pm\) S.D. from 6–8 mice.

FIGURE 4. Plasma choline levels were not decreased significantly by choline deprivation, whereas plasma PC decreased markedly. Plasma was collected from Pemt\(^{-/-}\) and Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice fed the CS diet for 24 h and then fed the CD diet for 3, 21, or 90 days. Choline was quantified with choline oxidase. Plasma PC was measured by high performance liquid chromatography. A, the amounts of choline in plasma. B, plasma PC content. Data are averages \(\pm\) S.D. from 6–8 mice.

Adaptation to Choline Deprivation

Redistribution of radiolabeled choline metabolites occurred in CS-Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice (Fig. 5A). Between 1 and 24 h after \(^{3}H\)choline injection, the liver, intestine, kidney, and lung lost 35, 36, 10 and 50%, respectively, of the radiolabel, whereas the radiolabeling of the brain, plasma, muscle, and heart increased by 100, 127, 90, and 100%, respectively. When the Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice were fed the CD diet, the radiolabeling of the intestine and kidney was decreased by 38 and 41%, respectively (Fig. 5B), during the same period of time. Conversely, labeling of the liver, brain, muscle, and lung increased by 36, 100, 65 and 50%, respectively (Fig. 5B).

The total amount of radioactivity in all tissues examined did not change significantly in CS- and CD-Pemt\(^{-/-}\) mice between 1 and 24 h after \(^{3}H\)choline injection (Fig. 5C). In contrast, in Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice, the total radioactivity in all selected tissues was lower in mice fed the CS diet than in mice fed the CD diet. However, the radioactivity decreased from 1 to 24 h after \(^{3}H\)choline injection in CS- but not in CD-Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice (Fig. 5C). These observations suggest that the total amount of choline labeling was maintained in the CD-Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice and was maintained to an even greater extent than in CD-Pemt\(^{-/-}\) mice, because total radioactivity in CD-Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice was approximately twice that in CD-Pemt\(^{-/-}\) mice (Fig. 5C).

These data support the conclusion that, between 1 and 24 h, choline or one of its metabolites was redistributed among tissues of the mouse. When CD and CS mice of both genotypes were compared, the liver appeared to be the donor of choline in CS mice but an acceptor of choline in CD mice. In all mice, the brain was a major acceptor of labeled choline during choline deprivation.

From Table 1, we found that body weight decreased, whereas liver weight increased in Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice fed the CD diet. This resulted in a 1.6-fold increase in liver-body weight ratio during choline deprivation. Furthermore, the weight of extrahepatic tissues decreased significantly after 21 or 90 days of choline deprivation. These results support the proposal that mice might sacrifice some tissues to provide choline to liver and brain. Such a redistribution of choline may be beneficial for mice during long-term choline deprivation.

DISCUSSION

Choline Redistribution—The well maintained level of total choline-containing metabolites in the liver and brain of CD-Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice and the continued consumption of choline by oxidation between 21 and 90 days of feeding the CD diet suggest that the liver and brain obtain choline from other tissues/sources. We traced choline redistribution in the mice by intravenous injection of \(^{3}H\)choline into CS- and CD-Pemt\(^{-/-}\) or Mdr2\(^{-/-}/Pemt\(^{-/-}\) mice. The change of labeling between 1 and 24 h after injection of \(^{3}H\)choline indicates which tissues participate in choline redistribution. The results do not exclude differential rates of choline uptake or elimination as concurrent mechanisms for the observed findings of
radioactivity within individual organs. Fig. 5, A and B, clearly show that the liver is a donor of choline in CS Pemt−/− mice but a receiver of choline in CD Pemt−/− mice. The data suggest that redistribution of choline from lung and kidney to liver occurs during choline deprivation (Fig. 6). In Mdr2−/−/Pemt−/− mice, choline was redistributed from intestine and kidney to liver, brain, lung, and muscle during choline deprivation. Interestingly, both CD mouse models showed that choline is redistributed from kidney to brain and liver. The major difference in choline redistribution between the two mouse models during choline deprivation is that the intestine is a donor of choline in Mdr2−/−/Pemt−/− mice but not in Pemt−/− mice.

Despite the attempted choline redistribution in the CD-Pemt−/− mice, these mice died of liver failure after 3 days. Thus, it would seem that the amount of choline mobilized under this circumstance was not sufficient to rescue these mice. We hypothesize that, as a mechanism of adaptation to choline deprivation, choline redistribution may also occur in starving animals in the wild.

We cannot exclude the possibility that tissues that we did not examine (e.g. skin, bones) served as either donors or receivers of...
Adaptation to Choline Deprivation

**TABLE 1**

| Body weight and liver weight of Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice |
|--------------------------------------------------|
| **Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice were fed the choline-supplemented (CS) diet for 24 h and then fed the choline-deficient (CD) diet for 3 (CD3), 21 (CD21), or 90 (CD90) days. Data are average ± S.D. from 6–8 mice. LW, liver weight; BW, body weight; BW–LW, body weight minus liver weight. | **CD3** | **CD21** | **CD90** |
|--------------------------------------------------|--------|--------|--------|
| Body Weight (g) | 19.6 ± 1.56 | 19.9 ± 1.40 | 18.3 ± 1.40 | 16.8 ± 1.19<sup>a</sup> |
| Liver Weight (g) | 1.07 ± 0.12 | 1.20 ± 0.04 | 1.75 ± 0.15<sup>b</sup> | 1.46 ± 0.12<sup>b</sup> |
| LW/BW Ratio | 0.056 ± 0.004 | 0.056 ± 0.004 | 0.086 ± 0.001<sup>a</sup> | 0.087 ± 0.002<sup>b</sup> |
| BW–LW (g) | 18.6 ± 1.17 | 18.7 ± 1.62 | 16.5 ± 0.87<sup>a</sup> | 15.4 ± 1.20 |

<sup>a</sup><sup>p</sup> < 0.05.

<sup>b</sup><sup>p</sup> < 0.01. *p* values were for CS versus CD mice.

choline during choline deprivation. Nevertheless, we consider this to be unlikely, because skin would probably lose choline as skin cells are replaced, and the turnover of bone metabolites is expected to be very slow.

The Maintenance of Choline Homeostasis via Choline Redistribution—The level of total choline-containing metabolites in the brain did not decline significantly during choline deprivation in either of the mouse models (Fig. 1). Nevertheless, as we previously reported, the level of total choline-containing metabolites in the liver decreased by ~50% (3) and the level of PC in the plasma decreased by 80–90% during choline deprivation in both mouse models (Fig. 4B) (3). Thus, a decreased level of total choline-containing metabolites in the liver, but not in other organs, during choline deprivation suggests that the liver is the organ that is the most responsive to the stress of choline deprivation. Moreover, the liver is the only organ that produces significant amounts of choline (via PEMT) that could be delivered to other organs via lipoprotein transport (12, 25).

The brain maintained normal activity of choline oxidase for 21 days (Fig. 2) whereas the liver decreased choline oxidase in both mouse models during choline deprivation (3). Our data suggest that the brain becomes an acceptor of choline during the whole period of choline deprivation. Whereas during the early phase of choline deprivation in CD-Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice liver acted as a donor of choline because the total choline level decreased before 21 days. In contrast, the liver becomes an acceptor of choline during the late phase of choline deprivation (21 days or later) in CD-Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice.

Thus, choline redistribution among tissues varied even during different stages of choline deprivation. As we found previously, the survival of CD-Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice for at least 90 days depended on the maintenance of the level of total choline-containing metabolites in liver (3). Thus, it was not surprising that extrahepatic choline was directed into the liver to maintain a minimal level of choline in these mice.

The Mechanism of Choline Redistribution to Brain—How does the brain maintain a stable level of total choline during choline deprivation? From in vivo labeling, we found that choline uptake in the brain accounted for only a minor part of total choline uptake in the whole body (Fig. 5, A and B). In addition, choline oxidase was still active in the brain of both mouse models during choline deprivation (Fig. 2). However, choline deprivation did not significantly decrease the total level of choline-containing metabolites in the brains of either strain of mice (Fig. 1A). These results suggest that choline deprivation does not significantly affect choline metabolism in the brain in either mouse model. Consequently, the brain must have obtained a continuous supply of choline from peripheral tissues during choline deprivation. Thus, during choline deprivation, choline redistribution was initiated, which guaranteed a steady supply of choline to the brain. Sufficient free choline in the plasma and unaltered levels of the high affinity choline transporter guaranteed a constant level of total choline-containing metabolites in the brain during choline redistribution (Figs. 3 and 4).

Previous studies have shown that apoptosis is induced in PC12 cells (brain tumor cells) cultured in a CD medium (26). This observation suggested that choline is an essential component for brain. However, in vivo, the brain did not exhibit choline deficiency in Pemt<sup>−/−</sup> mice or during dietary choline deprivation (Fig. 1), even though CD-Pemt<sup>−/−</sup> mice died of severe liver failure during complete choline deprivation (3, 6). These results indicate that, when mice experience the stress of choline deprivation, a fine regulatory mechanism is triggered to protect some important organs (such as brain and liver) from choline deprivation, whereas other tissues (such as kidney and intestine) sacrifice choline to the brain and liver via choline redistribution.
Choline Oxidase in the Brain—To our knowledge, we report for the first time the presence of choline oxidase activity in the brain. The specific activity of choline oxidase activity in brain mitochondria (Fig. 2A) was ~25% of that in the liver (3). Unlike liver, the brain consists of many different cell types and regions. Hence, it will be of interest to determine in which part of the brain choline oxidase is located.

Enzymatic activities of choline oxidase as measured in brains (Fig. 2A) and livers (3) were not consistent with the protein levels of choline oxidase as determined by immunoblot analysis (Fig. 2D). Hence, it is possible that the enzymatic activity of choline oxidase is regulated not only at the transcriptional/translational level but also post-translationally. In addition, we cannot exclude the possibility that other isoforms of choline oxidases were present and not recognized by the antibody we used. In the future, a knock-out mouse model of choline oxidase might provide insight with respect to possible isoforms in the brain.

It was surprising that choline oxidase activity in the brain was maintained up to 21 days of choline deficiency in the Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice, yet the labeling of betaine increased ~2-fold (Fig. 2, A and C). One possibility is that the amount of choline oxidase is not limiting in the formation of betaine. Alternatively, we cannot exclude that betaine transport across the blood-brain barrier might have changed.

Other Aspects of Choline Redistribution—We observed (Table 1) that with increasing the time of choline deprivation, the body weight of Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice decreased, whereas liver weight increased. Furthermore, the weight of extrahepatic tissues decreased significantly after 21 days of choline deprivation. These results support the idea that these mice might sacrifice some tissues to prevent choline depletion of the liver and brain. This redistribution of choline may be particularly beneficial for mice during long term choline deprivation.

Little is known about choline metabolism in the intestine. A considerable amount of choline labeling was observed in the intestines in both mouse models under both dietary conditions. This interesting finding suggests that choline metabolism plays a vital role in intestinal function. For example, mice absorb lipids from the intestinal lumen and form chyloplomers of which PC is an essential component.

The mechanism by which choline redistribution occurs is also an intriguing question. Fig. 4 shows that choline deprivation did not profoundly affect the amount of choline in plasma but dramatically reduced the amount of plasma PC in Pemt<sup>−/−</sup> mice after 3 days. Possibly, under acute choline deprivation, the liver depends more on PC than on choline. Plasma PC is mainly associated with high density lipoprotein, and the liver is the major site for clearance of high density lipoprotein (27). Thus, the majority of plasma PC taken up by the liver is from high density lipoprotein particles. Therefore, decreased high density lipoprotein-PC uptake by CD-Pemt<sup>−/−</sup> hepatocytes compared with CS-Pemt<sup>−/−</sup> hepatocytes (11) might cause failure to adapt to choline deprivation. Although plasma choline can be a substrate for the cytidine diphosphocholine pathway in liver, the reduction of choline kinase activity in the livers of CD-Pemt<sup>−/−</sup> mice might attenuate this possibility (3). However, in CD-Mdr2<sup>−/−</sup>/Pemt<sup>−/−</sup> mice, constant choline levels in plasma (Fig. 4) and increased activity of choline kinase (3) might guarantee that the choline enters the cytidine diphosphocholine pathway. Thus, plasma choline appears to have a critical role in choline redistribution.

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