The Concentration of Phosphatidylethanolamine in Mitochondria Can Modulate ATP Production and Glucose Metabolism in Mice

Phosphatidylethanolamine (PE) N-methyltransferase (PEMT) catalyzes the synthesis of phosphatidylcholine (PC) in the liver. Mice lacking PEMT are protected against diet-induced obesity and insulin resistance. We investigated the role of PEMT in hepatic carbohydrate metabolism in chow-fed mice. A pyruvate tolerance test revealed that PEMT deficiency greatly attenuated glucose production. The reduction in glucose production was specific for pyruvate; glucose production from glycerol was unaffected. Mitochondrial PC levels were lower and PE levels were higher in livers from Pemt<sup>−/−</sup> mice compared with Pemt<sup>+/+</sup> mice, resulting in a 33% reduction of the PC-to-PE ratio. Mitochondria from Pemt<sup>−/−</sup> mice were also smaller and more elongated. Activities of cytochrome c oxidase and succinate reductase were increased in mitochondria of Pemt<sup>−/−</sup> mice. Accordingly, ATP levels in hepatocytes from Pemt<sup>−/−</sup> mice were double that in Pemt<sup>+/+</sup> hepatocytes. We observed a strong correlation between mitochondrial PC-to-PE ratio and cellular ATP levels in hepatoma cells that expressed various amounts of PEMT. Moreover, mitochondrial respiration was increased in cells lacking PEMT. In the absence of PEMT, changes in mitochondrial phospholipids caused a shift of pyruvate toward decarboxylation and energy production away from the carboxylation pathway that leads to glucose production.

Obesity is often accompanied by hepatic steatosis and insulin resistance (IR) or type 2 diabetes. The prevalence of type 2 diabetes is increasing, and therefore understanding the underlying physiology is of major importance. Up to 75% of patients with obesity and diabetes also suffer from nonalcoholic fatty liver disease (NAFLD) (1,2), indicating that the liver plays an important role in the etiology of obesity-associated diabetes. Steatosis in the liver is often associated with hepatic IR; the exact mechanisms by which these conditions are related remain unclear. IR may cause accumulation of triacylglycerol in the liver (3,4). In an IR state, elevated plasma concentrations of both glucose and fatty acids can lead to increased hepatic de novo lipogenesis and steatosis (3,4). Intracellular accumulation of lipids, such as diacylglycerol or ceramide, can activate protein kinase C and subsequently impair insulin signaling (5,6). Hence, metabolism of fat and glucose in the liver is connected, particularly in the mitochondria, where fatty acid oxidation supplies acetyl-CoA to the tricarboxylic acid (TCA) cycle for ATP production. The TCA cycle also provides a source of carbons for gluconeogenesis. Therefore, abnormal lipid and glucose metabolism associated with IR and type 2 diabetes may be related to abnormalities in mitochondria.

Hepatic phosphatidylcholine (PC) synthesis also has a role in the development of IR (7). PC is synthesized via the cytidine diphosphate–choline pathway (8). Alternatively, hepatocytes can methylate phosphatidylethanolamine (PE) via PE N-methyltransferase (PEMT) (9). The PEMT pathway is responsible for ∼30% of hepatic PC.
synthesis; the remaining 70% is generated via the cytidine diphosphate–choline pathway (10). Unexpectedly, we reported that mice lacking PEMT are protected against high-fat diet (HFD)-induced obesity and IR (7). This protection was completely blunted when the diet was supplemented with choline, leading to the conclusion that PEMT provides an endogenous source of choline that supports normal weight gain (7). Although Pemt−/− mice were completely protected against IR, hepatomegaly and steatosis developed when the mice were fed an HFD. This protection against IR in the presence of hepatic steatosis led us to investigate the role of PEMT in hepatic carbohydrate metabolism, independent of body weight or fatty liver.

We report that in livers of Pemt−/− mice, the concentration of PE in mitochondria is increased, leading to increased flux of pyruvate into the TCA cycle for subsequent energy production. Consequently, hepatic glucose production from pyruvate was strongly diminished, suggesting that these changes may contribute to the protection against the development of IR in Pemt−/− mice.

RESEARCH DESIGN AND METHODS

Animals
Female CS7Bl/6 Pemt+/+ and Pemt−/− mice (backcrossed >7 generations) (7) were fed standard rodent chow (cat. no. 5001; LabDiet) ad libitum with free access to water. Experimental procedures were approved by the University of Alberta’s Institutional Animal Care Committee in accordance with the Canadian Council on Animal Care.

In Vivo Tolerance Tests
Mice (4–6 months old) were nonfasted before the glucose tolerance tests, fasted for 4 h before insulin tolerance tests and for 12 h before glucose, pyruvate, and glycerol tolerance tests. Subsequently, the mice were injected intraperitoneally with bovine insulin (1 units/kg body wt i.v.), glucagon (140 μg/kg body wt i.p.), sodium pyruvate (2 g/kg body wt i.p.), or glycerol (2 g/kg body wt i.p.) or alternatively, glucose (2 g/kg body wt i.p.) was administered by oral gavage. Blood glucose levels were measured using a glucometer (Accu-Chek).

Real-Time Quantitative PCR and Analysis of Mitochondrial DNA Content
RNA isolation, cDNA synthesis, and real-time quantitative PCR were performed as described (7). mRNA levels were normalized to cyclophilin mRNA. For determination of mitochondrial DNA copy number, total DNA was extracted from livers using a DNEasy kit (Qiagen). The mitochondrial DNA content was calculated using real-time quantitative PCR by measuring a mitochondrial gene (Nd1, forward: ACA CTT ATT ACA ACC CAA GAA CAC AT, reverse: TCA TAT TAT GGC TAT GGG TCA GG) versus a nuclear gene (Lpl, forward: GAA AGG TGT GGG GAG ACA AG, reverse: TCT GTC AAA GGC ACT GAA CG).

Isolation of Mitochondria
McArdle-RH7777 hepatoma cells were collected and homogenized in 1 mmol/L Tris-HCl, 0.13 mmol/L NaCl, 5 mmol/L KCl, and 7.5 mmol/L MgCl2 (pH 7.4). The homogenate was centrifuged at 1,000g for 10 min at 4°C. The pellet was resuspended in 10 mmol/L Tris-HCl, 10 mmol/L KCl, and 0.15 mmol/L MgCl2 (pH 6.7) and frozen at −80°C. Thawed cells were disrupted using a Dounce homogenizer, mixed with one-seventh the volume of 2 mol/L sucrose and centrifuged at 1,000g for 10 min. The supernatant was centrifuged at 12,000g for 15 min at 4°C. The pellet was resuspended in 10 mmol/L Tris-HCl, 0.15 mmol/L MgCl2, and 0.25 mol/L sucrose (pH 6.7). Mitochondria from livers of Pemt+/+ and Pemt−/− mice were isolated as previously described (11).

In Vivo 2-[3H]deoxy-o-Glucose Uptake
Nonfasted animals were injected with 0.1 units/kg insulin i.v., 2 g/kg glucose i.v., and 300 μCi/kg 2-[3H]deoxy-o-glucose i.v. (12). Fifteen minutes after injection, blood was collected by cardiac puncture into EDTA-containing tubes, plasma was prepared by centrifugation, and organs were harvested and snap-frozen in liquid nitrogen. Plasma proteins were precipitated with 0.3 N Ba(OH)2 and 0.3 N ZnSO4. Radioactivity in the supernatant was divided by the plasma glucose concentration to calculate the specific radioactivity of glucose. White adipose, skeletal muscle, and liver were homogenized in 0.5% perchloric acid, and the homogenate was centrifuged for 20 min at 2,000g. Half of the supernatant was treated with Ba(OH)2 and ZnSO4. Radioactivity was measured in both aliquots, and radioactivity in 2-[3H]deoxy-o-glucose was calculated as the difference between the supernatants divided by sample weight. This value was divided by the specific radioactivity of glucose to calculate glucose uptake per gram of tissue (micromoles per gram of tissue).

Phospholipid Labeling in Primary Hepatocytes
Hepatocytes were isolated from Pemt+/+ and Pemt−/− mice after perfusion of the liver with collagenase (13) and cultured in 60-mm collagen-coated dishes in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS for 18 h. Cells were washed twice with serum-free DMEM and pulsed with 10 μCi [3H]serine/dish for 1 h, followed by a chase of 0, 1, 2, or 4 h in the presence of 1 mmol/L hydroxyamine to inhibit phosphatidylserine decarboxylase (14). Mitochondria were isolated and lipids extracted with chloroform/methanol (2:1 v/v). Phospholipids were separated by thin-layer chromatography (15), followed by methanalysis with CH3OH/sulphuric acid at 80°C for 2 h. Since [3H]serine is also incorporated into fatty acids (16), radioactivity in only the phospholipid head groups was measured.

Analytical Procedures
Mitochondrial PC, PE, and phosphatidylserine were quantified by phosphorous assay (15) after separation by thin-layer chromatography. ATP in McArdle-RH7777 cells and primary hepatocytes was measured using a luciferase assay kit (Sigma-Aldrich) according to the manufacturer’s instructions. Hepatic glycogen content was measured as previously described (17). Plasma insulin concentrations were measured using a multiplex assay system (Meso Scale...
Discovery). Plasma glucagon levels were determined using Millipore RIA GL-32K. Samples were prepared for electron microscopy (7). Mitochondrial size and shape were quantified using ImageJ software. Pyruvate dehydrogenase (PDH) activity was measured using a microplate assay kit (Abcam) in the presence of phosphatase inhibitors. For measurement of PEMT activity, 50-µg cell or liver homogenate was incubated with phosphatidlymonomethylthanolamine and S-adenosyl[methyl-3H]methionine, and the incorporation of radiolabel was measured as described previously (18). Proteins in total liver homogenates or isolated mitochondria were resolved by SDS-PAGE. Immunoactive proteins were detected using the enhanced chemiluminescence system (GE Healthcare) according to the manufacturer’s instructions, and protein levels were quantified using ImageJ software.

**Activities of Oxidative Phosphorylation Complexes**

Enzyme activities of oxidative phosphorylation complexes were measured in mitochondria isolated from Pemt+/+ and Pemt−/− livers (11). Mitochondria were homogenized and sonicated before the assay. Succinate and NADH cytochrome c reductase were measured (19) in assay mixtures containing 0.5 mmol/L potassium cyanide (KCN), 0.1 mmol/L succinate (complex II) plus 2 mmol/L ADP. Respiratory substrates were 1.25 mmol/L rotenone (complex I inhibitor).

**Respirometry**

Oxygen consumption was measured at 30°C with a Clark-type oxygen electrode (Qubit Systems, Kingston, ON, Canada). Cells were diluted to 4 × 106 cells/mL in DMEM. Substrate-driven respiration was measured in cells permeabilized for 5 min with digitonin (20 µg/mL/2 × 106 cells) on ice and then resuspended in buffer (4 × 106 cells/mL) containing 125 mmol/L KCl, 20 mmol/L HEPES, 2 mmol/L MgCl2, 2.5 mmol/L KH2PO4, 0.1% BSA, and 2 mmol/L ADP. Respiratory substrates were 1.25 mmol/L pyruvate, 1.25 mmol/L malate (complex I), and 5 mmol/L succinate (complex II) plus 2 µmol/L rotenone (complex I inhibitor).

**Statistical Analysis**

Data were analyzed with GraphPad Prism software. All values are means ± SEM. Statistical analyses of the tolerance tests were performed using ANOVA, and correlations were determined by the Pearson correlation. For all other comparisons, a Student t test was performed. Level of significance of differences was P < 0.05.

**RESULTS**

**Glucose Production From Pyruvate Is Reduced in Chow-Fed Mice Lacking PEMT**

Under chow feeding, there was only a small difference in body weight between Pemt+/+ and Pemt−/− mice (21.1 ± 0.54 and 19.6 ± 0.45 g, respectively, P < 0.05), and the livers of Pemt−/− mice were not steatotic (205.3 ± 146.7 vs. 117 ± 64.5 nmol/mg protein for Pemt+/+). Fasting blood glucose levels were slightly reduced in Pemt−/− mice (Pemt+/+ mice, 4.86 ± 0.13 mmol/L; Pemt−/− mice, 4.31 ± 0.10 mmol/L, P < 0.05); fasting plasma insulin levels were unaltered by PEMT deficiency (Pemt+/+ mice, 420.5 ± 93.1 pg/mL; Pemt−/− mice, 424.2 ± 78.1 pg/mL). Next, we performed tolerance tests for glucose, insulin, pyruvate, and glycerol (Fig. 1). Pemt+/+ and Pemt−/− mice that were fed a chow diet were similarly sensitive to insulin (Fig. 1A). There was a slightly improved glucose tolerance in Pemt−/− mice compared with Pemt+/+ mice (Fig. 1B). This improvement appeared to be due to a lower peak in blood glucose reached after the oral gavage of glucose rather than enhanced glucose clearance. We investigated whether the improvement in glucose tolerance could be explained by a difference in gluconeogenesis. Sodium pyruvate was injected intraperitoneally, and the rise in blood glucose levels was assessed over 2 h. Glucose production from pyruvate was profoundly attenuated in Pemt−/− mice (Fig. 1C). This reduction was specific for pyruvate because the response to an intraperitoneal injection of glycerol, another precursor for glucose, was not different between Pemt+/+ and Pemt−/− mice (Fig. 1D).

Since this large difference in glucose production translated into only a minor decrease in fasting blood glucose, we measured hepatic glycogen levels. Under fed conditions, hepatic glycogen levels were not different between Pemt+/+ and Pemt−/− mice (29.0 ± 6.66 and 31.5 ± 3.71 mg/g liver, respectively). After an overnight fast, glycogen levels were 50% lower in Pemt−/− mice than in Pemt+/+ mice (Fig. 2A). Apparently, Pemt−/− mice use hepatic glycogen to maintain normal blood glucose as compensation for reduced gluconeogenesis. Uptake of glucose into the liver, measured in vivo by injection of 2-[^3H]deoxy-d-glucose, was 45% lower in Pemt−/− mice (Fig. 2B); uptake of glucose into muscle (Fig. 2C) and white adipose tissue (Fig. 2D) was unaffected. Thus, hepatic gluconeogenesis from pyruvate was reduced in mice lacking PEMT without hypoglycemia because of an increase in glycogenolysis and a decrease in hepatic glucose uptake.

**Hormonal and Transcriptional Regulation of Hepatic Gluconeogenesis Is Not Altered by PEMT Deficiency**

To determine why PEMT deficiency reduced gluconeogenesis in mice, we measured plasma glucagon that promotes gluconeogenesis and glycogenolysis. Plasma glucagon concentrations were not different in fasted mice (Pemt+/+ mice, 51.5 ± 4.08 pg/mL; Pemt−/− mice, 51.2 ± 3.30 pg/mL). Furthermore, blood glucose increased similarly in both mouse models after an intraperitoneal injection of glucagon (Fig. 3A), indicating equal sensitivity to glucagon. Hepatic mRNAs encoding pyruvate carboxylase (Pcx), phosphoenolpyruvate carboxykinase (Pepck), glucose-6-phosphatase (G6Pase), enolase 1 (Eno1), and fructose-1,6-bisphosphatase 1 (Fbp1), involved in hepatic glucose
production, were not affected by PEMT deficiency (Fig. 3B). Consistently, protein levels of Pepck were not different between Pemt+/+ and Pemt−/− mice (Fig. 3C and D). The protein levels of peroxisome proliferator–activated receptor γ coactivator (Pgc)1a, a transcription factor that influences hepatic glucose production (21), were also unaffected by PEMT deficiency (Fig. 3C and D). These data suggest that the reduction in glucose production from pyruvate in PEMT-deficient mice is not due to transcriptional regulation of these genes.

PEMT Deficiency Alters Mitochondrial Phospholipid Composition and Increases Oxidative Phosphorylation and ATP Levels

Since mRNAs and proteins involved in hepatic glucose production were not altered by PEMT deficiency (Fig. 3B and C), we investigated hepatic pyruvate metabolism. In addition to glucose production, pyruvate can be converted into acetyl-CoA for energy production. We, therefore, determined whether any changes occurred in hepatic mitochondria in response to PEMT deficiency. Since PEMT activity is present in mitochondrial associated membranes (MAM) (22), we analyzed the phospholipid composition of hepatic mitochondria. PC levels were lower in mitochondria of Pemt−/− mice compared with Pemt+/+ mice (Fig. 4A), whereas the amount of mitochondrial PE was increased by PEMT deficiency (Fig. 4B). Consequently, the molar ratio PC-to-PE in mitochondria of Pemt−/− mice was 33% lower than in Pemt+/+ mice (Fig. 4C). The number of mitochondria per cell, as assessed by mitochondrial DNA copy number, was not affected by PEMT deficiency (Fig. 4D). Electron microscopy revealed that mitochondria in livers from Pemt−/− mice were smaller than in Pemt+/+ mice (Fig. 4E and F). Moreover, mitochondria in PEMT-deficient livers were more elongated than in livers of Pemt+/+ mice (Fig. 4E and G). Hepatic protein levels of mitofusin (Mfn)1 and 2 and optic atrophy protein (Opa)1 were not different between Pemt+/+ and Pemt−/− mice (Fig. 4H and I), indicating that fusion and fission of mitochondria were not affected by the lack of PEMT.

We determined whether these changes in phospholipid composition and morphology affected mitochondrial function. Initially, we examined the hepatic expression of genes involved in the TCA cycle and oxidative phosphorylation (Fig. 5A). The levels of mRNAs encoding cytochrome synthase (CS-1), isocitrate dehydrogenase (Idh3a), ATP synthase (Atp5b), malate dehydrogenase (Mdh2), cytochrome c oxidase (Cox4i1), succinate dehydrogenase (Sdha), and PDH E1α1 (Pdha1) were not altered, whereas the expression of cytochrome c (Cytc) was slightly increased in Pemt−/− mice (Fig. 5A). Pdha1 is part of the PDH complex that converts pyruvate into acetyl-CoA that is used by the TCA cycle or for fatty acid synthesis. PDH activity was 25% lower in Pemt−/− mice.
than in Pemt\(^{+/+}\) mice (Fig. 5F). The activities of cytochrome c oxidase (complex IV) and succinate:cytochrome c reductase (complex II) were higher in mitochondria from PEMT-deficient livers, whereas the activity of NADH cytochrome c reductase (complex I) was not changed (Fig. 5C–E). The apparent increase in oxidative phosphorylation was underscored by increased amounts of proteins of complexes I–V in PEMT-deficient mitochondria (Fig. 5G and H). The amount of ATP was twofold higher in hepatocytes from Pemt\(^{-/-}\) compared with Pemt\(^{+/+}\) mice (Fig. 5B). The cells were cultured in DMEM (25 mmol/L glucose) without addition of fatty acids; thus, higher ATP levels are likely due to increased glucose/pyruvate oxidation rather than fatty acid oxidation. Thus, mitochondrial morphology and phospholipid composition are significantly modified by PEMT deficiency with increased activity of the mitochondrial electron transport chain.

**PEMT Deficiency Increases Mitochondrial Respiration**

For assessment of the effect of PEMT deficiency on O\(_2\) consumption, basal and substrate-driven respiration was measured in McArdle-RH7777 hepatoma cells with or without PEMT expression. Cells with PEMT activity comparable with liver (p38 cells) (Fig. 6A) were compared with control cells that did not express PEMT (pCI). Figure 6C and D show that, similar to what we observed in livers, cells that express PEMT have lower expression of oxidative phosphorylation complexes than cells that lack PEMT. Importantly, O\(_2\) consumption from endogenous substrates (basal respiration in intact cells) was 29% lower in cells that expressed PEMT (Fig. 6B). Furthermore, the rates of substrate-driven respiration through complexes I and II (from pyruvate/malate or succinate, respectively), were 31% and 32% lower in p38 cells (Fig. 6B). These results clearly show that PEMT expression directly reduces mitochondrial respiration in hepatocytes.

**Deficiency of PEMT Leads to PE Accumulation in Mitochondria**

To investigate the mechanism by which PEMT deficiency affected mitochondrial phospholipid composition, we used primary hepatocytes from Pemt\(^{+/+}\) and Pemt\(^{-/-}\) mice. Mitochondrial PE is made on mitochondrial inner membranes by phosphatidylserine decarboxylase (PSD) (23). We pulse-labeled hepatocytes with [\(^{3}\)H]serine to label the mitochondrial PE pool. During the 4-h chase period, PSD activity was inactivated by hydroxylamine to prevent formation of additional mitochondrial PE. During the chase period, radioactivity in mitochondrial PE decayed in Pemt\(^{+/+}\) hepatocytes—not in Pemt\(^{-/-}\) hepatocytes (Fig. 7A). In Pemt\(^{+/+}\) hepatocytes, radioactivity in PC increased, while no additional radiolabel was incorporated into PC in Pemt\(^{-/-}\) hepatocytes (Fig. 7B). These data indicate that in Pemt\(^{+/+}\) hepatocytes, mitochondrial PE is exported to the endoplasmic reticulum.
(ER)/MAM for methylation by PEMT. In contrast, in PEMT-deficient hepatocytes, PE is not methylated to PC, resulting in reduced PE export and thus accumulation of PE in mitochondria.

**PE Concentration Correlates With ATP**

To test whether mitochondrial phospholipid composition and ATP levels are directly correlated, we used McArdle-RH7777 hepatoma cells that stably express different amounts of PEMT. We analyzed phospholipid composition of mitochondria as well as ATP levels. As expected, increased PEMT activity was accompanied by an increased PC-to-PE ratio in mitochondria ($r^2 = 0.9717$). A striking correlation was observed between ATP levels and PEMT activity ($r^2 = 0.671$) (Fig. 8A) and between ATP and the PC-to-PE ratio in mitochondria ($r^2 = 0.632$) (Fig. 8B). Moreover, cellular ATP correlated with mitochondrial PE concentrations ($r^2 = 0.568$) (Fig. 8C) but not with mitochondrial PC (Fig. 8D). Thus, PEMT-mediated PC synthesis can regulate the phospholipid composition of mitochondria, and PE and/or the PC-to-PE ratio can affect ATP levels in hepatocytes.

**DISCUSSION**

Elevated gluconeogenesis contributes to the pathogenesis of IR and diabetes. Thus, inhibition of gluconeogenesis may be a pharmaceutical target for treatment of these disorders. We report that PEMT deficiency alters mitochondrial membrane phospholipid composition, which results in increased respiratory capacity. An increased utilization of pyruvate for ATP production leads to lower substrate availability for hepatic glucose production. The data indicate that elevated hepatic PE may have beneficial glucose-lowering effects in IR or diabetic patients.

PEMT-mediated PC synthesis contributes to the development of IR (7). When mice lacking PEMT were fed an HFD, the mice were protected against obesity and IR; however, they developed hepatic steatosis (7). Since steatosis is often associated with IR, we hypothesized that lack of PEMT may have direct beneficial effects on hepatic glucose metabolism, which may compensate for the detrimental effects of steatosis. Indeed, the data suggest that the profound reduction in hepatic glucose production induced by PEMT deficiency can contribute to the protection against diet-induced IR. Elevated gluconeogenesis is often a consequence of IR rather than a cause. Under the conditions used in this study, both animal models are normally sensitive to insulin. Therefore, it appears that PEMT deficiency has a direct effect on lowering hepatic glucose production, which could be beneficial for ameliorating hyperglycemia in IR or diabetic subjects.

Thus, the question remained how hepatic phospholipids might directly influence carbohydrate metabolism in the liver. Hepatic lipid and carbohydrate metabolism are
highly interconnected, particularly in mitochondria, where both fatty acids and carbohydrates can be used for energy production. PEMT activity is present in MAM (22). One function proposed for MAM is a membrane bridge between the ER and mitochondria, mediating lipid transfer between these two organelles (24). Mitochondrial PE is preferentially synthesized in mitochondria via PSD rather than being imported from the ER. Moreover, PSD-derived PE is exported from mitochondria to the MAM/ER for methylation to PC via PEMT. Therefore, it is not surprising that the lack of PEMT alters the phospholipid composition in hepatic mitochondria. The importance of phospholipids for mitochondrial function has been recognized. Cardiolipin, a mitochondrial-specific phospholipid, is important for mitochondrial function such as cellular respiration and ATP production (25). Moreover, modest depletion of mitochondrial PE in Chinese hamster ovary cells decreased respiratory capacity, ATP production, and electron transport chain complex activities and profoundly altered mitochondrial morphology (20). Our data show that an increase in mitochondrial PE positively correlates with electron transport chain complex activities, ATP levels, and respiration in hepatocytes.

How can increased mitochondrial energy production ultimately reduce gluconeogenesis? The TCA cycle and electron transport chain directly link the metabolism of lipids and glucose. Thus, defects in hepatic glucose production can be secondary to changes in fatty acid oxidation or flux through the TCA cycle. For example, both Pparaα−/− and Pgc-1α−/− mice have defects in mitochondrial metabolism and fatty acid oxidation (26,27) as well as defects in gluconeogenesis, despite normal expression of gluconeogenic enzymes (26–28). Consistent with our observations in Pemt−/− mice, both Pparaα−/− and Pgc-1α−/− mice are resistant to HFD-induced IR (28–31),
which can partly be explained by impairment of hepatic glucose production. PGC-1α deficiency directly decreased hepatic expression of enzymes of the TCA cycle and the electron transport chain but had no impact on the expression of genes involved in fatty acid oxidation or gluconeogenesis (27). Nevertheless, gluconeogenesis was diminished in Pgc-1α−/− mice, indicating that impaired hepatic energy production, as a result of the defects in the TCA cycle, inhibited hepatic glucose production. Cytosolic glucose production from glycerol was unaffected by PGC-1α deficiency (27). In contrast, instead of a decrease, Pemt−/− mice exhibited an increase in hepatic levels of proteins involved in the electron transport chain and an increase in ATP levels. Thus, decreased glucose production in PEMT-deficient mice is not caused by impaired hepatic energy production. Therefore, attenuated glucose production from pyruvate is caused by reduced substrate availability. The elevated flux through the TCA cycle in Pemt−/− mice shifts the utilization of pyruvate toward energy rather than glucose production. Surprisingly, PDH activity was slightly reduced by PEMT deficiency. Although seemingly counterintuitive, it could be a response to high levels of ATP, which can activate pyruvate dehydrogenase PDH kinase, which phosphorylates and inactivates PDH.

Mitochondrial dysfunction, as exemplified by impaired energy generation, is associated with IR in skeletal muscle (32–34). Muscle mitochondrial activity is reduced in diabetic patients (32,35) as well as in IR children of type 2 diabetic patients (34). Recent reports show that also in livers of diabetic patients, energy homeostasis is abnormal as indicated by lower rates of hepatic ATP synthesis (36). Importantly, reduced hepatic ATP concentrations related to IR independent of hepatic lipid content (37). Thus,
increasing hepatic energy production by PEMT inhibition could improve hepatic insulin sensitivity.

Brown adipose tissue is a highly energetic, mitochondria-dense organ responsible for diet- and cold-induced thermogenesis (38,39). Recently, a direct role for brown adipose tissue in the regulation of glucose homeostasis was reported (40). Transplantation of brown adipose tissue into mice improved metabolic parameters such as glucose tolerance and insulin sensitivity. Consequently, defects in mitochondrial function in brown adipose tissue could lead

![Figure 6](http://www.diabetesjournals.org/diabetes/article-pdf/63/8/2620/576385/2620.pdf)

**Figure 6**—Increased respiration in hepatoma cells lacking PEMT. **A**: PEMT activity in McArdle-RH7777 cells without (pCI) or with (p38) PEMT expression compared with normal mouse liver homogenate. **B**: Basal rate of O$_2$ consumption in intact cells from endogenous substrates (intact) and substrate-driven O$_2$ consumption in digitonin-permeabilized cells supplied with substrates: pyr/mal, pyruvate plus malate (complex I); Suc, succinate (complex II) plus rotenone (inhibitor of complex I). Values are means ± SEM of four independent experiments, each performed in duplicate. **C**: Protein expression of oxidative phosphorylation complexes I–IV in mitochondria from pCI and p38 McArdle-RH7777 cells. Expression was quantified using ImageJ software (D). *P* < 0.05.

![Figure 7](http://www.diabetesjournals.org/diabetes/article-pdf/63/8/2620/576385/2620.pdf)

**Figure 7**—Metabolism of mitochondrial PC and PE in hepatocytes. **A**: Decay of radiolabeled PE in mitochondria in primary hepatocytes from *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice. Cells were pulse labeled with [$^3$H]serine for 1 h, followed by a 4-h chase period. **B**: Incorporation of [$^3$H]serine into the choline head group of PC in *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ hepatocytes. Values are means ± SEM of three independent experiments.
to metabolic diseases including obesity and type 2 diabetes. Even in white adipose tissue, where mitochondria are less abundant, impaired mitochondrial activity appears to predispose mice to obesity and type 2 diabetes (41). Thus, elevation of PE or reduction of PC-to-PE ratio in mitochondria of other tissues could also have beneficial metabolic effects.

In conclusion, mitochondrial PE levels and/or PC-to-PE ratio dictate energy production in hepatocytes. We observed a strong correlation between cellular ATP levels and mitochondrial PE or PC-to-PE ratio. Moreover, respiratory capacity is increased when PEMT is lacking and the PC-to-PE ratio is low. This could cause an increased flux of pyruvate through the TCA cycle, reducing the availability of pyruvate for hepatic glucose production. Since elevated gluconeogenesis contributes to the pathogenesis of IR and type 1 and type 2 diabetes, understanding the role of mitochondrial phospholipids in respiratory capacity and hepatic glucose production may offer the prospect of more effective therapeutic approaches for treatment or prevention of these diseases.

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References
1. McCullough AJ. Update on nonalcoholic fatty liver disease. J Clin Gastroenterol 2002;34:255–262