

What Is the Metabolic Role of Phosphoenolpyruvate Carboxykinase?*

Published, JBC Papers in Press, July 27, 2009, DOI 10.1074/jbc.R109.040543

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The enzyme phosphoenolpyruvate carboxykinase (GTP; EC 4.1.1.32) (PEPCK)² has the unusual distinction of being very well studied but metabolically misunderstood. As we will document in this minireview, the enzyme has been almost exclusively linked to gluconeogenesis to the point that changes in the levels of PEPCK mRNA or its activity are associated with the control of hepatic glucose output and, more recently, with alterations in life span. That a tissue such as brown adipose tissue, which does not make glucose, has more PEPCK activity on a protein basis than is present in the liver is largely ignored. In addition, all eukaryotes have a gene for both a mitochondrial (PEPCK-M) and cytosolic (PEPCK-C) form of the enzyme. In the livers of most mammals studied to date (including humans), 50% of the total PEPCK activity is PEPCK-M. However, for reasons to be discussed, only PEPCK-C has been studied in any detail in mammals. Thus, the “strange case of PEPCK-M” deserves our attention. This minireview is an attempt to broaden our perspective on the metabolic role of this enzyme by reviewing the body of literature that has accumulated demonstrating that PEPCK plays a key role in a several metabolic processes associated with cataplerosis.

Some Facts about PEPCK

PEPCK catalyzes the following reaction.



Although the reaction is reversible physiologically, the $K_m(\text{oxalacetate})$ (12 μM) and $K_m(\text{GTP})$ (13 μM) are within the concentration range of these substrates in mammalian tissues, suggesting that PEPCK normally synthesizes P-enolpyruvate from oxalacetate. This is further supported by the fact that pyruvate carboxylase, a mitochondrial enzyme that can also synthesize oxalacetate, is present in many tissues and has a generally higher V_{max} (12 units/g in rat liver) than PEPCK (6 units/g) (1). PEPCK uses GTP or ITP, but not ATP, as a phosphate donor to form P-enolpyruvate. Most bacteria and yeast studied to date

contain an ATP-linked enzyme, which has little sequence similarity to mammalian PEPCK. However, Mukhopadhyay *et al.* (2) have purified a GTP-linked PEPCK from *Mycobacterium smegmatis*, which has a molecular mass of 72 kDa; this agrees well with the molecular mass of mammalian (3) and avian (4) PEPCK. In addition, the *M. smegmatis* enzyme has a 47–50% sequence identity to human and chicken PEPCK-C and a 46% similarity to chicken PEPCK-M (2), suggesting that the genes for the two isoforms of the enzyme diverged from a common bacterial enzyme, perhaps by duplication and gene rearrangement. The genes for human PEPCK-M and human and rat PEPCK-C contain the same organization: 10 exons and 9 introns (3, 5). The gene for human PEPCK-M is 9839 bp in length, whereas the genes for rat (6138 bp) and human (5345 bp) PEPCK-C are smaller; the size of the introns in PEPCK-M largely accounts for this difference.

PEPCK Is a Cataplerotic Enzyme and as Such Is a “Feeder Reaction” for Downstream Metabolic Processes

Our understanding of the metabolic role of PEPCK has greatly expanded in recent years. PEPCK should be viewed as a cataplerotic enzyme because it plays the important role of removing citric acid cycle anions for either the biosynthetic process or the subsequent complete oxidation of these compounds to carbon dioxide in the citric acid cycle. Thus, the downstream pathways in which PEPCK participates are linked to cataplerosis. There are four major pathways in which PEPCK plays a key role (Fig. 1). These are (a) gluconeogenesis, (b) glyceroneogenesis, (c) the synthesis of serine, and (d) the conversion of the carbon skeletons of amino acids, such as glutamine and glutamate, to P-enolpyruvate (via PEPCK) and then to pyruvate (via pyruvate kinase) for subsequent oxidation in the citric acid cycle as acetyl-CoA.

Gluconeogenesis

The role of PEPCK-C in hepatic and renal gluconeogenesis has been extensively reviewed (6), as have the factors that control transcription of its gene in these tissues (7). By removing citric acid cycle anions (*i.e.* in the conversion of oxalacetate to P-enolpyruvate), this enzyme links cataplerosis with a major biosynthetic pathway: glucose synthesis. Deletion of the gene for PEPCK-C in mouse liver leads to ablated gluconeogenesis in that tissue (8, 9), whereas a total body deletion of the enzyme results in profound hypoglycemia and death. It is well established that changes in the rate of transcription of the gene for PEPCK-C is a critical step in establishing the overall activity of the enzyme in rodent liver and kidney cortex, tissues that synthesize glucose (7). After years of study of PEPCK-C, the general consensus is that alterations in PEPCK-C gene transcription regulate the total activity of the enzyme in these tissues. It is unusual for an enzyme at a branch point in a metabolic pathway not to be subject to some type of allosteric regulation. Recently, Lin *et al.* (10) reported that PEPCK-C in yeast was acetylated at Lys¹⁹ and Lys⁵¹⁴ and that Sir2 deacetylated the enzyme both *in vitro* and *in vivo*. In addition, PEPCK-C in HepG23 cells, a

* This work was supported, in whole or in part, by National Institutes of Health Grant DK58620 (to R. W. H.). This work was also supported by the Cleveland Clinic (to S. C. K.). This is the second article of four in the Thematic Minireview Series on the Biology of Phosphoenolpyruvate Carboxykinase: 55th Anniversary. This minireview will be reprinted in the 2009 Minireview Compendium, which will be available in January, 2010.

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² The abbreviations used are: PEPCK, phosphoenolpyruvate carboxykinase; PEPCK-M, mitochondrial PEPCK; PEPCK-C, cytosolic PEPCK.

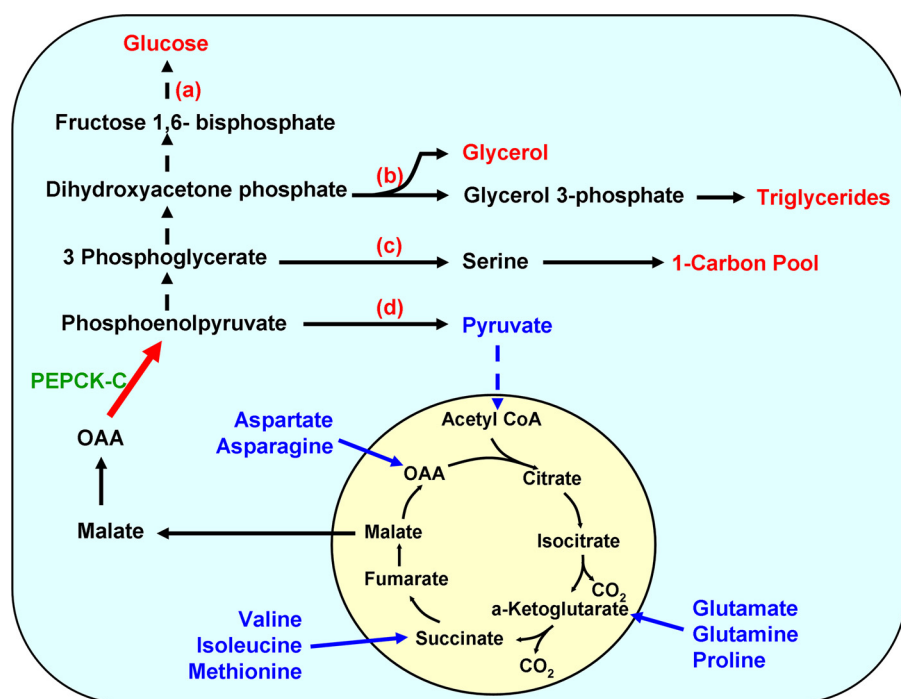


FIGURE 1. **Cataplerosis in action: PEPCK as a feeder reaction for carbon from the citric acid cycle to various biosynthetic and oxidative processes.** The entry of selected amino acids into the citric acid cycle and their subsequent disposal via PEPCK-C are shown. The biosynthetic pathways of gluconeogenesis (a), glyceroneogenesis (b), and serine synthesis (c) are outlined, as is the recycling/oxidation of the carbon skeletons of amino acids back into the citric acid cycle (d) as acetyl-CoA for subsequent oxidation or conversion to fatty acids. The metabolic basis for these various routes of disposal of amino acid carbon is discussed in detail in text. OAA, oxalacetate.

human hepatoma line, was inhibited by acetylation, and the amount of glucose produced by the cells was markedly decreased. The regulatory significance of acetylation of PEPCK-C in mammalian tissues where the enzyme is active remains to be established. In the absence of identified allosteric regulation or covalent modification, it has been assumed that flux through PEPCK-C is regulated by the concentration of key intermediates, such as oxalacetate and GTP. Rapid alterations in the activity of key regulatory steps in gluconeogenesis, such as fructose-1,6-bisphosphatase and pyruvate kinase, by allosteric activation or covalent modification are critical in setting the overall flux rate for glucose synthesis. PEPCK-C would thus serve as a feeder reaction for the overall process by removing carbon from the citric acid cycle for utilization by biosynthetic and oxidative pathways (Fig. 1). We thus consider that PEPCK-C provides substrates for a number of downstream processes. Although gluconeogenesis is quantitatively the major route of carbon flux in the liver and kidney cortex, it is only one of the metabolic pathways that depend on PEPCK for a source of carbon derived from citric acid cycle intermediates.

Glyceroneogenesis as a Source of Glyceride-Glycerol

Most textbooks of biochemistry teach that the glyceride-glycerol found in the triglyceride of mammals is derived from the triose phosphate that is generated by the metabolism of glucose via glycolysis. This is an attractive concept because it links the intake of glucose with the deposition of fat as triglyceride. Because insulin stimulates glucose transport into adipose tissue, the picture is complete; a diet high in carbohydrate will

stimulate fatty acid synthesis and deposition in adipose tissue, resulting in obesity. However, fatty acids are synthesized to a negligible extent in the adipose tissue of adult mammals, especially in humans (11); this is the job of the liver. Newly synthesized fatty acids are transported from the liver to the adipose tissue as triglycerides that are packaged in very low density lipoprotein; the triglycerides are subsequently degraded by lipoprotein lipase to their component fatty acids and used to resynthesize triglyceride. The carbon source of glycerol 3-phosphate required to support the synthesis of the triglyceride in the liver comes largely from glyceroneogenesis (12). In fact, two studies that determined the rates of glyceroneogenesis compared with glycolysis in overnight-fasted human subjects found that the majority (50–60%) of the glyceride-glycerol in triglyceride isolated from circulating very low density lipoprotein was derived from glyceroneogenesis, with a smaller

contribution from glucose (13, 14). The overall rate of glyceroneogenesis was measured in rats subjected to a variety of dietary conditions. Very high rates of glyceroneogenesis were noted in the liver, adipose tissue, and skeletal muscle, despite the fact that the rats were fed a diet high in carbohydrate and received an intravenous infusion of glucose during the experiment (12). A more detailed review of this subject can be found in Ref. 15.

Glyceroneogenesis as a Source of Glycerol in the Rainbow Smelt, Schistosomes, and Caenorhabditis elegans—The glycerol found in the blood of mammals is generated from the hydrolysis of triglyceride in adipose tissue by the action of lipases; the concentration of glycerol in the blood is often used as a measure of lipolysis. Glycerol becomes an important source of carbon for gluconeogenesis during prolonged starvation, when the breakdown of triglyceride is accelerated. However, the concentration of glycerol in human blood does not exceed 0.25 mM (16). In contrast, the blood of *Osmerus mordax*, the rainbow smelt, which lives in the waters of the North Atlantic, contains a concentration of glycerol that can reach 0.4 M in the winter, when the ambient sea temperatures approach 0 °C; the glycerol is acting as an “antifreeze” in these fish. The work of Driedzic, Ewart, and colleagues (17–22) provides a fascinating picture of the metabolic adaptations that are responsible for the synthesis of such high concentrations of glycerol in the blood of these fish. They noted that the levels of PEPCK-C mRNA in rainbow smelt liver rise as the sea temperature falls in the winter months; this parallels an increase both in glycerol in the blood and in the rate of glyceroneogenesis from amino acids (Fig. 2). Glyceroneogenesis is of special importance because the diet of

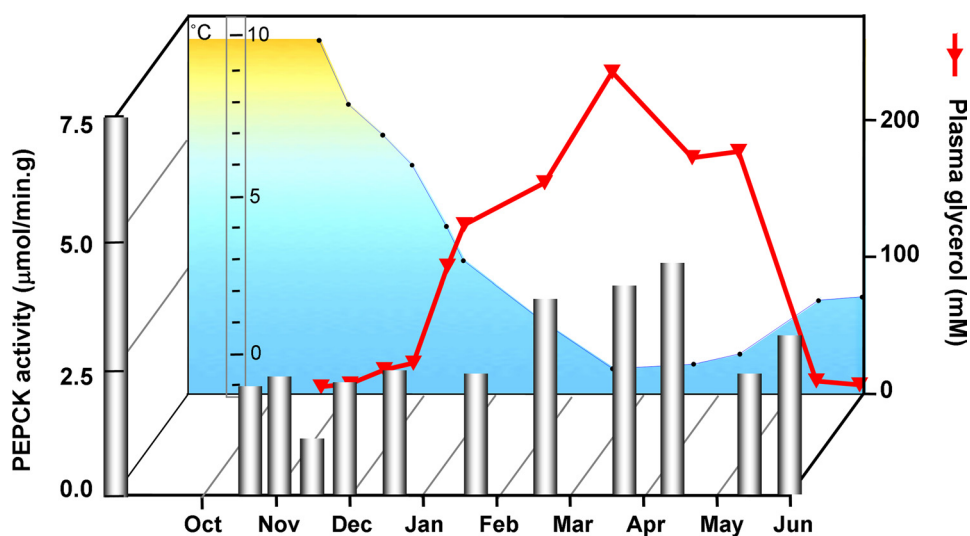


FIGURE 2. Changes in the activity of hepatic PEPCK and the concentration of glycerol in the blood of *O. mordax* (the rainbow smelt) in response to changes in ocean temperature. This figure has been redrawn from Fig. 4 in a review by Liebscher *et al.* (19), with the permission of the authors. Some of the data in this figure were derived from the published work of Lewis *et al.* (20). The activity of hepatic PEPCK is presented as a gray bar graph, the ocean temperature is shown in blue, and the concentration of glycerol in the blood of the rainbow smelt is presented as a red line.

the rainbow smelt consists largely of fat and protein (they eat small marine invertebrates), and the carbon skeletons of amino acids are thus the chief source of glycerol (17, 22). The direct synthesis of glycerol by a process that does not involve the lipolysis of triglyceride requires glycerol 3-phosphatase, an enzyme that has not been extensively studied in mammals. It seems likely, however, that the direct synthesis of glycerol also occurs in mammals. Jensen *et al.* (23) noted that ~15–20% of systemic glycerol in fasted humans was not formed from the lipolysis of adipose tissue triglycerides. As with the rainbow smelt, this would require glycerol 3-phosphatase to catalyze the direct dephosphorylation of the glycerol 3-phosphate.

Schistosoma mansoni is the parasite responsible for schistosomiasis, a chronic and debilitating disease that affects large numbers of individuals in tropical countries. The life cycle of the parasite includes propagation in the freshwater snail *Biomphalaria glabrata*, where it undergoes asexual multiplication of sporocysts and the generation of cercariae, nematode worms in the second stage of larval life that infect humans. Development of the parasite through its two larval stages requires considerable energy, which, in adult worms, is provided by glucose from the infected human host. However, glucose is limiting in the hemolymph of snails, and glutamine has been suggested as a major source of energy for the organism in this stage of development (24). PEPCK mRNA (at the present time, it is not clear which isoform) is present at 60 times the adult level in miracidia (the free-swimming larval stage) and 10 times the adult level in sporocysts. Assuming that changes in the level of PEPCK mRNA parallel its activity, these findings suggest that the organism can make glucose via either gluconeogenesis or glycerol, via glyceroneogenesis, presumably from glutamine as the source of carbon. Khayath *et al.* (24) reported that glutamine is converted mainly to free glycerol by sporocysts; this process does not involve the lipolysis of triglyceride in the organism. Treatment of the sporocysts with 3-mercaptopi-

colinate, an inhibitor of PEPCK (24), resulted in decreased synthesis of both glucose and glycerol, supporting the key role of the enzyme in this process. It thus seems likely that schistosomes, like the rainbow smelt, carry out glyceroneogenesis, which results in the direct generation of glycerol (*i.e.* the lipolysis of triglyceride is not required).

C. elegans embryos from mothers that were exposed to medium containing 0.3 M NaCl for 24 h were resistant to 0.5 M NaCl; embryos from mothers that were not preconditioned to hyperosmotic shock as larvae did not survive (25). Embryos from conditioned adults had 10 times the glycerol content found in control embryos (25). Worms with a mutation in the gene for PEPCK did not survive conditioning in 0.3 M NaCl, suggesting that

this enzyme is required for the formation of glycerol. The mobilization of glycogen in the worm was not sufficient to account for the levels of glycerol formed in response to hyperosmotic conditions. Perfusion of rat liver with a hyperosmotic medium markedly increased PEPCK-C mRNA, suggesting an effect on gene transcription (26), so that the role of this enzyme in response to hyperosmolarity is more general in organisms. As in the case of the rainbow smelt discussed above, *C. elegans* must have a glycerol 3-phosphatase to generate the glycerol formed from either glycogen mobilization or glyceroneogenesis. Although the rate of glyceroneogenesis has not been measured in *C. elegans*, it is likely that is an important process for glycerol formation.

Glyceroneogenesis, PEPCK, and Energy Metabolism in Developing Drosophila—The genes for both PEPCK-C and PEPCK-M are present in tandem on chromosome 2R of *Drosophila*. This contrasts with many mammalian species in which the genes are on separate chromosomes; for example, human PEPCK-C is on chromosome 20, and human PEPCK-M is on chromosome 14. Okamura *et al.* (27) noted that knockdown of the transcription factor ATF-2 by RNA interference resulted in a 70% decrease in the level of PEPCK mRNA in the fat bodies of flies compared with controls. This caused a 30% drop in the rate of glyceroneogenesis in the tissue as measured by the incorporation of [¹⁴C]pyruvate into glyceride-glycerol. This correlated directly with the level of triglyceride found in the fat bodies of the genetically modified flies as well as with a decreased body weight. Interestingly, transgenic flies with an extra copy of the gene for ATF-2 had 60% more triglyceride compared with controls.

PEPCK and the Synthesis of Serine

Rats fed a low protein diet (6% of calories) have a 30-fold increase in hepatic mRNA for 3-phosphoglycerate dehydro-

genase,³ an NAD-linked enzyme that converts 3-phosphoglycerate to 3-phosphohydroxypyruvate, the first step in the biosynthesis of serine from glycolytic intermediates. Although serine is not an essential amino acid, it is an important source of carbon for the one-carbon pool and for the synthesis of cysteine in the transsulfuration pathway. The induction of 3-phosphoglycerate dehydrogenase gene expression in the livers of rats fed a low protein diet suggests that the synthesis of serine is of special significance for the survival of the animals in situations in which dietary protein is limiting (Fig. 1). It is noteworthy that the pathway of glyceroneogenesis can also generate 3-phosphoglycerate, so it is possible that serine can be synthesized in the livers of starved animals using carbon derived from the P-enolpyruvate that was generated by PEPCK.

PEPCK-C and Recycling/Oxidation of the Carbon Skeletons of Amino Acids

A major cataplerotic function of PEPCK-C is the recycling of citric acid cycle anions back into the cycle to generate energy. This process involves the entry of amino acid carbon into the citric acid cycle, which will be further metabolized in the mitochondria to malate; malate exits the mitochondria and is oxidized to oxalacetate. PEPCK-C converts the oxalacetate to P-enolpyruvate, which is subsequently converted to pyruvate via pyruvate kinase and then to acetyl-CoA in the mitochondria by the pyruvate dehydrogenase complex; the acetyl-CoA is a substrate for complete oxidation in the citric acid cycle or can be used for fatty acid synthesis in the liver (Fig. 1). The same type of carbon cycling via PEPCK-C has been suggested as the fate of some of the glutamine carbon utilized by the kidney cortex (28). By this scenario, the conversion of citric acid cycle anions (*i.e.* via oxalacetate) to P-enolpyruvate (by PEPCK) provides a high degree of metabolic flexibility because tissues such as the small intestine or skeletal muscle can convert the P-enolpyruvate to pyruvate and derive energy by the subsequent oxidation of this compound as acetyl-CoA in the citric acid cycle. A tissue such as the liver makes glucose from citric acid cycle anions rather than recycling them back into the cycle for energy. However, some recycling of pyruvate into oxalacetate via pyruvate carboxylase and thus back into the citric acid cycle does occur in the liver. The rate of "futile cycling" was estimated to account for as much as 25% of the carbon flow during hepatic gluconeogenesis in fasted rats (29).

Overexpression of the Gene for PEPCK-C in Mouse Skeletal Muscle and the Interaction of Glyceroneogenesis and Cataplerosis

Skeletal muscle has a small but significant level of PEPCK-C activity (30). There have been several proposals regarding the metabolic role of this enzyme in muscle (30, 31). These include providing pyruvate (from P-enolpyruvate formed by PEPCK-C, as discussed above) for the synthesis of alanine by alanine aminotransferase. This is a potentially important process because alanine constitutes a considerable fraction of the amino acids released by skeletal muscle

during fasting, requiring the net synthesis of this amino acid in the muscle. Another possible metabolic role of PEPCK-C in skeletal muscle is glyceroneogenesis. Skeletal muscle contains triglyceride, and glyceroneogenesis is the major source of carbon for the glyceride-glycerol in the triglyceride in this tissue (12). PEPCK-C also has a cataplerotic function in skeletal muscle. The concentration of citric acid cycle anions greatly increases in the mitochondria during strenuous exercise and then falls when the muscle is at rest (32) and removed after exercise.

To determine the metabolic role of PEPCK-C in skeletal muscle, transgenic mice were generated using the cDNA for PEPCK-C, which was linked to the α -skeletal actin gene promoter (PEPCK-C^{mus} mice) and the 3'-end of the bovine growth hormone gene. By crossbreeding two founder lines, a line of mice was created that had ~100 times the activity of the enzyme in their skeletal muscle as was noted in control littermates (33). These mice were hyperactive; they ran up to 5 km at 20 m/min without stopping and were eight times more active than controls in their home cages. To support this high level of activity, the PEPCK-C^{mus} mice ate twice as much as control animals but weighed about half as much. During strenuous exercise, the PEPCK-C^{mus} mice used fatty acid as the primary fuel, and the concentration of lactate in the blood did not increase to the same extent as in control animals after the mice ran to exhaustion. The reason for this high level of aerobic activity in the PEPCK-C^{mus} mice is most likely the greatly increased number of mitochondria noted in their skeletal muscle. They also had markedly elevated levels of triglyceride in their skeletal muscle (33). The concentrations of various metabolic markers in the blood, such as glucose, free fatty acids, ketone bodies, and cholesterol, were not significantly different in fasted PEPCK-C^{mus} mice compared with controls. However, there was markedly less insulin and leptin in the blood of PEPCK-C^{mus} mice compared with controls.⁴ This is consistent with the effect of hyperactivity on glucose utilization by skeletal muscle (increased insulin sensitivity) and the reduced adipose tissue (reduced leptin formation) in the PEPCK-C^{mus} mice.

The mechanism by which overexpression of PEPCK-C in skeletal muscle causes such a marked increase in activity in the mice is not clear at present. We assume that the increased concentration of triglyceride in the skeletal muscle of the PEPCK-C^{mus} mice is caused by the elevated activity of PEPCK-C, which increases the rate of glyceroneogenesis. The fact that the concentration of triglyceride in the skeletal muscle of the PEPCK-C^{mus} mice was proportional to the activity of PEPCK-C in their muscle suggests that carbon flow over this pathway is an important determinant of the increased triglyceride levels in the muscle (33). The role of PEPCK in muscle metabolism is further supported by a recent study of rats bred for running endurance; these rats were also leaner, more active, and aggressive (34). There was a 2-fold higher PEPCK activity in the quadriceps of the more active rats. It was concluded that PEPCK may be an important factor in the enhanced running endurance noted in the lean rats (34).

³ S. C. Kalhan, unpublished data.

⁴ P. Hakimi, R. W. Hanson, N. A. Burger, and R. Tracy, unpublished data.

Why Are There Two Isoforms of PEPCK?

The current wisdom is that PEPCK-M is involved in gluconeogenesis from lactate (see Ref. 35 for a detail review). Because the oxidation of lactate to pyruvate via lactate dehydrogenase generates NADH in the cytosol, it is important to balance the cytosolic redox state by synthesizing P-enolpyruvate directly in the mitochondria. This obviates the need to transport malate, which must be oxidized to oxalacetate in the cytosol, generating NADH. Only one NADH is consumed per triose phosphate converted to glucose; the two molecules of NADH that could be potentially produced during gluconeogenesis from lactate would skew the cytosolic redox state. The synthesis of P-enolpyruvate directly in the mitochondria and its subsequent transport to the cytosol (36) bypass this problem. Support for this concept is based largely on studies with birds (they have PEPCK-M and no PEPCK-C activity in their livers), whose livers synthesize glucose largely from lactate (37, 38). However, lactate is derived from glucose metabolism in the large flight muscles of birds, so lactate carbon does not result in the *net* formation of glucose via gluconeogenesis; this is provided by gluconeogenesis from amino acids in the kidney. However, the metabolic role of PEPCK-M in other organisms has not been rigorously studied and is largely ignored.

In contrast to PEPCK-C, an extensive study of the tissue distribution of PEPCK-M activity in the rat and mouse has not been carried out. Recently, Stark *et al.* (39) reported the presence of PEPCK-M in mouse and rat pancreatic islets. They proposed that the enzyme is involved in cataplerosis by shunting excess oxalacetate as P-enolpyruvate from the mitochondria to the cytosol. The P-enolpyruvate would then be converted to pyruvate and recycled by β -cells. This process is critical for the complete metabolism of citric acid cycle intermediates formed from either pyruvate via pyruvate carboxylase or from the carbon skeletons of amino acids. Recycling from the mitochondria via PEPCK-M can contribute up to 40% of the cytosolic P-enolpyruvate pool. In addition, the GTP synthesized by succinyl-CoA synthetase is converted to GDP by PEPCK-M (39). These findings underline the importance of the direct delineation of the role of PEPCK-M in a wide variety of mammalian tissues.

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