Pyruvate carboxylase deficiency: Mechanisms, mimics and anaplerosis

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Abstract

Pyruvate carboxylase (PC) is a regulated mitochondrial enzyme that catalyzes the conversion of pyruvate to oxaloacetate, a critical transition that replenishes citric acid cycle intermediates and facilitates other biosynthetic reactions that drive anabolism. Its deficiency causes multiorgan metabolic imbalance that predominantly manifests with lactic acidemia and neurological dysfunction at an early age. Three clinical forms of PC deficiency have been identified: an infantile form (Type A), a severe neonatal form (Type B), and a benign form (Type C), all of which exhibit clinical or biochemical correlates of impaired anaplerosis. There is no effective treatment for these patients and most, except those affected by the benign form, die in early life. We review the physiology of this enzyme and dissect the major clinical, biochemical, and genetic aspects of its dysfunction, emphasizing features that distinguish PC deficiency from other causes of lactic acidemia that render PC deficiency potentially treatable using novel interventions capable of enhancing anaplerosis.

Introduction

Pyruvate carboxylase (PC; EC 6.4.1.1) is a biotin-containing nuclear genome-encoded mitochondrial enzyme discovered in 1959 by Utter and Keech [1]. The enzymatic activity facilitates flux through a key intermediary metabolism reaction; PC is responsible for the ATP-dependent carboxylation of pyruvate, yielding oxaloacetate. This reaction constitutes the best recognized interconversion required for the replenishment of pools of intermediates of the citric acid cycle (CAC), a process named anaplerosis that restores losses that CAC derivative products are subject to during normal metabolism. By extension, PC also participates in numerous metabolic pathways that depend on the availability of oxaloacetate such as gluconeogenesis, glycogen synthesis, lipogenesis, glycerogenesis, the synthesis of amino acids and neurotransmitters, and glucose-dependent insulin secretion. Because PC is essential for these interrelated aspects of anabolism, inherited deficiency of this enzyme can a priori be expected to cause metabolic disturbances in numerous tissues, with a predilection for organs whose metabolism depends upon high CAC flux such as liver and brain. PC deficiency (OMIM 266150) is an uncommon autosomal recessive disorder that predominantly causes lactic acidemia (serum lactic acid >5 mmol/L and bicarbonate <18 mmol/L), encephalopathy, and neurological dysfunction – the first manifestations in the neonatal or infantile period. Several dozen patients affected by the disorder have been described in detail, allowing for the formulation of overlapping disease-associated clinical and biochemical phenotypes. PC deficiency has been reported more often in particular ethnic groups such as Algonquian-speaking Amerindians and Arabs, but in most populations the general incidence of PC deficiency is relatively low (1:250,000) [2]. However, it is plausible that ascertainment biases limit the recognition of hypomorphic or altogether divergent clinical syndromes. In practice, biochemical abnormalities assist in the differential diagnosis of PC deficiency, while enzymatic assay is still often required to establish the definitive diagnosis. Current symptomatic or supportive treatments prove largely ineffective. Therefore, understanding alternative metabolic pathways that can be enhanced and associated metabolic dysregulation potentially amenable to compensatory intervention in light of recent biochemical information is a prerequisite for the development of new therapies.

PC as an anabolic carbon flux regulator

PC is localized within the mitochondrial matrix of the cells of many organs and tissues. Expression levels are highest in liver, adipose tissue, kidney, lactating mammary gland, and pancreatic islets; modest in heart, brain, and adrenal gland; lowest in white blood cells and skin fibroblasts [3–5]. PC normally fulfills an anaplerotic function by converting pyruvate into oxaloacetate in the presence of elevated acetyl-CoA levels, a reaction that places PC at the gateway of multiple synthetic mechanisms. It commits pyruvate to gluconeogenesis by providing oxaloacetate for subsequent
conversion into phosphoenolpyruvate, a precursor of glycerol (Fig. 1). Glycerol can also support the re-esterification of fatty acids (glycerogenesis). On the other hand, oxaloacetate can join acetyl-CoA in the mitochondrion to yield citrate by the action of citrate synthase (EC 2.3.3.1). Citrate can be exported from mitochondria to the cytoplasm and cleaved to form acetyl-CoA and oxaloacetate. Cytoplasmic acetyl-CoA serves a building block for the synthesis of fatty acids. 

**Fig. 1.** Schematic flux diagram illustrating metabolic abnormalities resulting from pyruvate carboxylase (PC) deficiency. CAC, citric acid cycle; OAA, oxaloacetate; α-KG, α-ketoglutarate; G-3-P, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate; PS-P, pyridoxal phosphate; AcAc, acetoacetate; 3HB, 3-hydroxybutyrate.

During catabolic states, PC also sustains intra-mitochondrial production of oxaloacetate. The integrity of both pyruvate dehydrogenase (which supplies acetyl-CoA derived from pyruvate) and PC is critical to maintain flux through citrate synthase aimed to fuel the CAC and leading to energy production via the respiratory chain. In fact, the most immediate role of the oxidation of glucose, branched-chain and other amino acids, as well as of the β-oxidation of fatty acids is to provide carbon to the CAC. Impairment of these catabolic pathways by disease-related enzymatic defects compromises the efficiency of the CAC in terms of ATP production and re-activation of synthetic pathways. Parallel covalent modification mediated by nutrient sensors can also suppress synthetic pathways [6]: AMP-mediated protein kinase (AMPK) is a major regulator of catabolic flux. AMPK is activated under conditions associated with elevated AMP/ATP ratio such as those that cause a decrease in energy. When activated, AMPK phosphorylates serine and threonine residues present in many cytosolic enzymes. The immediate result of these modifications is that sets of enzymes that participate in synthetic pathways become inactivated, while other enzymes that promote catabolism (by providing substrates for the CAC) undergo stimulation such that the net result is the enhancement of catabolism. These considerations are particularly relevant because new therapeutic approaches for PC deficiency discussed below aim to enhance ATP production, decreasing AMP/ATP ratio and inactivating AMPK, re-establishing flux via the synthetic pathways.

**Impact of PC dysfunction**

A salient feature of PC deficiency is the conjunction of central nervous system (CNS) maldevelopment (hypotrophy) and degeneration, which often dominate the phenotype. Neurological manifestations are prominent in PC deficiency as the result of primary glial dysfunction. In the CNS, PC activity is robust in glia but absent from neurons [7]. The two major glial cell types include astrocytes (abundant wherever neurons are located) and oligodendrocytes (present in white matter myelin). In astrocytes, the anaplerotic function of PC is required for gluconeogenesis and glycogen synthesis and to replenish α-ketoglutarate removed from the CAC for the synthesis of glutamine, the main neuronal precursor of both glutamate and γ-aminobutyric acid (GABA) [7]. PC is also involved in myelin lipid synthesis in oligodendrocytes, an underexplored process in the context of developmental disorders that may underlie the paucity of myelin and abundance of white matter lesions observed in these patients. Additionally, PC is indirectly involved in maintaining the highly active glutathione system by supplying oxaloacetate to counteract the loss of malate that may be required for the generation of NADPH both in microglia (a ubiquitous neural cell type) and oligodendrocytes [7]. Lastly, in ependymal cells, the anaplerotic function of PC may be required for the oxidation of substrates such as branched-chain amino acids used for energy generation and needed to sustain kineociliary activity [7,8]. Impairment of these critical roles of PC justifies the prominent brain lesions that PC deficiency patients manifest, such as spongiform degeneration, neuronal loss, gliosis, delayed myelination, ventricular enlargement, and hypodevelopment of the corpus callosum [9–12].
PC plays a critical role in gluconeogenesis in both liver and kidney. This phenomenon probably accounts for the hypoglycemia that patients can manifest during fasting, metabolic imbalance or, paradoxically, even postprandial states. Furthermore, in the liver, PC deficiency leads to decreased oxaloacetate availability, resulting in impaired acetyl-CoA oxidation which can then be diverted into ketone body and fatty acid synthesis (Fig. 1). This phenomenon can explain the lipid droplet accumulation often found in hepatocytes (steatosis) and associated hepatomegaly (enlarged liver) [10,12–14]. Additionally, it has been hypothesized that gluconeogenesis from oxaloacetate is directly linked to bicarbonate reabsorption in proximal renal tubular cells [15]. This mechanism may also explain the relationship between bicarbonate reabsorption impairment and renal tubular acidosis observed in the most severely affected PC deficient patients.

In pancreatic cells, PC participates in maintaining elevated ATP/ADP and NADPH/NADP ratios in the cytoplasm, which are required for the secretion of insulin in response to changes in plasma glucose levels. Interestingly, there is a genetic epidemiological association between PC and Type 2 diabetes: single nucleotide polymorphisms between PC and Type 2 diabetes: single nucleotide polymorphisms in the PC gene are associated with changes in acute insulin release, highlighting, together with the cited gluconeogenic function, the importance of PC in glucose homoeostasis [16].

### Phenotypes of PC deficiency

Three forms of presentation have been identified in the PC deficient patients described thus far:

- **Type A (infantile or North American form)**
- **Type B (neonatal or French form)**
- **Type C (benign form)**

These phenotypes can only be distinguished by their clinical presentation and probably constitute a continuum spanning from the most severe (Type B) to the less severe form (Type C). Biochemical studies can assist in the distinction among phenotypes, as the most severely affected patients exhibit typical, although no pathognomonic, findings such as elevated lactate/pyruvate (L/P) ratio, low hydroxybutyrate/acetocetate (H/A) ratio, hypercitrullinemia and hyperammonemia, parameters that often remain unaltered in Types A and C patients (Table 1). However, phenotypic inferences based on PC activity remain elusive, as there is no solid correlation between clinical phenotype and enzyme assay in fibroblasts, although it is generally postulated that PC abundance (protein or mRNA levels) and residual enzymatic activity influence the severity of each form of PC deficiency [2,17–19]. Disease severity has also been loosely correlated with the type of mutation that the PC gene harbors such that missense mutations are often associated with Type A, whereas truncating mutations are more prevalent in patients with Type B phenotype [20,21].

Type A PC deficiency is most common among North American Indians [14], particularly members of the Algonquian-speaking groups encompassing the Micmac, Cree, and Ojibwa tribes [2]. Moderate lactic acidemia with normal L/P ratio and ketoacidosis with normal H/A ratio are common biochemical findings (Table 1). These patients first manifest at the age of 2–5 months usually after a normal early development, presenting with failure to thrive, apathy, delayed mental and motor development, hypotonia, pyramidal tract dysfunction, ataxia, nystagmus, and seizures [10,22–25]. Neurodegeneration leading to cerebral atrophy and hypomyelination are common features. Early reports associated Type A PC deficiency with Leigh syndrome (subacute necrotizing encephalomyopathy) [26–30]. This association is well known but its frequency remains uncertain because only a minority of PC deficiency cases exhibited pathologically proven Leigh syndrome [26,28], because of a potential for the occurrence of suboptimal storage and assay conditions required to measure PC activity accurately and because a subsequent small case series study of Leigh syndrome patients failed to demonstrate PC deficiency [31]. Renal tubular acidosis has also been associated with the Type A phenotype [10,28,32]. The prognosis is poor and most Type A patients die during the first years of life.

Type B PC deficiency was first described in France [18] and is more common in patients of Arab descent, with cases reported in individuals of Algerian, Egyptian, and Saudi Arabian extraction.

### Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Lactic acid in blood</th>
<th>Lactic acid/pyruvate ratio in blood</th>
<th>Serum concentration of ammonia</th>
<th>Plasma concentration of amino acids</th>
<th>3-Hydroxybutyrate/acetocetate ratio in plasma</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>&gt;10 mmol/L [2]</td>
<td>Increased (&gt;25) [2]</td>
<td>Moderately increased (100–150 μmol/L) [33]</td>
<td>Increased citrulline, proline, lysine, and alanine [2], low glutamine [33]. Some reports document decrease of alanine [33]</td>
<td>Low (&lt;0.8) [2,11]</td>
<td>Hypoglycemia [53]. Ketonuria [2]. Both the PC protein and mRNA are absent in most patients [2].</td>
</tr>
</tbody>
</table>
Molecular genetics of PC deficiency

PC (located in 11q13.4–q13.5) is the only gene known to be associated with PC deficiency [46]. PC includes 20 coding exons and four non-coding exons as part of the five untranslated region (5’UTR), spanning 105.9 kb [18,47]. All four non-coding exons are involved in alternative splicing, resulting in three tissue specific PC transcripts carrying the same coding region: variant 1 (NM_000920) (expressed in brain and liver), variant 2 (NM_022172) (present in liver and kidney), and variant 3 (BC011617) (abundant in brain and liver) [3,17,19,48]. The protein consists of a homotetramer of several polypeptides, namely, biotin carboxylase, carboxyltransferase, pyruvate carboxylase tetramerization, and biotin carboxyl carrier protein. Biotin is covalently linked to a specific lysine residue located close to the C-terminus [4]. The lack of mutations at this biotin-binding region has been associated with response to biotin administration, presumably by allowing biotin-mediated catalytic enhancement [49].

Molecular genetic testing has been performed on a research basis. PC deficiency patients are usually homozygote and manifest the disorder with complete penetrance. Heterozygote carriers typically do not manifest clinical symptoms. Mosaicism has been invoked in some patients to explain the discordance between tissue enzyme abundance and clinical phenotypes identified in select cases [17].

Studies with skin fibroblasts of Type A patients typically demonstrate the presence of low levels of a mature biotin-containing PC protein of correct molecular weight [17,50]. Patients reported with this type of PC deficiency consistently harbor two missense mutations in homozygous or compound heterozygous states [19,21,25], which are mostly located in the biotin carboxylase or carboxyltransferase domains. Potential somatic mosaicism was reported in one individual afflicted by Type A PC deficiency and genotype R62C +/−, R631Q ++/− (mosaic mutation), A847V ++/− (mosaic mutation) [17], and who exhibited prolonged sur-

In Type C patients, both PC protein and mRNA are present at a higher level than in other types of PC deficiency [17]. The benign course characteristic of these patients may be due to the presence of different transcripts encoding PC forms that differ in their two exons [51]. A mutation affecting the first two exons of the liver isoform still allows the brain isoform to be expressed normally, while the liver manifests PC deficiency [51]. Two individuals have been reported with the following mutations: S266A +/+ (heterozygous mutation), S705X +/− (another potentially mosaic mutation), and T569A/L1137VfsX1170 (compound heterozygosity) [17].

Indicators of PC deficiency

Several analytical observations complemented with structural brain considerations assist in the diagnosis of PC deficiency.

Biochemical profile

Blood gasometry

Constant or intermittent metabolic acidosis caused by elevated lactate levels is typical of PC deficiency. Ketaoacidosis is also present and contributes to the metabolic acidosis. Type A and B patients usually manifest chronic metabolic acidosis with elevated lactate and ketosis, and Type C patients tend to manifest these features only intermittently during metabolic stress [46].

Blood glucose

Because PC is directly involved in the synthesis of glucose, hypoglycemia is commonly found in the course of the disease, although it may not be the dominant biochemical abnormality at presentation. Hypoglycemia is more prominent in Type A and B patients after fasting, metabolic decompensation or in the postprandium [52], whereas in Type C patients glucose levels may be normal [18], low [44], or elevated [43,45].

Blood lactate and pyruvate

Elevated levels of pyruvate and lactate are characteristically found in this disorder. PC deficiency causes an increase in pyruvate levels, which is subsequently converted to lactate resulting in lactic acidemia (Fig. 1), a reaction that can be enhanced by the administration of carbohydrates. The L/P ratio is a useful indicator of the underlying cause of lactic acidemia. In PC deficiency, only Type B patients exhibit an elevated L/P ratio (>25), reflecting a reduced redox state (high NADH/NAD ratio) in cytoplasm. The NADH/NAD ratio may also be elevated due to impairment of gluconeogenesis associated with low levels of aspartate, malate, and oxaloacetate. These compounds are reducing equivalent vehicles that transit from the cytosol into the mitochondria, resulting in increased NADH levels [2,33]. The elevated NADH contributes to the conversion of pyruvate to lactate. The L/P ratio, however, is usually normal in Type A and C patients.
Blood 3-hydroxybutyrate and acetoacetate

Ketonemia, detectable even in the fed state, is common in PC deficiency. Decreased oxaloacetate availability leads to failure of hepatic acetyl-CoA oxidation, which is then diverted into ketone body formation (Fig. 1), a phenomenon potentiated by high-fat diets [5,13,40,41]. The H/A ratio is also diagnostically helpful. Only Type B patients manifest a low H/A ratio (<0.8), reflecting the mitochondrial oxidized redox state (low NADH/NAD ratio). This finding probably also signifies impairment of the CAC not only leading to reduced NADH, but also to CAC intermediate abundance, which is responsible for decreased transport of reducing equivalents from the cytosol to the mitochondria. Therefore, low levels of mitochondrial NADH impair the conversion of acetoacetate into 3-hydroxybutyrate, resulting in a decreased H/A ratio. The H/A ratio is often normal in Type A and C patients.

Plasma amino acids

The deficit of oxaloacetate and, consequently, of aspartate, impairs the urea cycle (Fig. 1). Aspartate is required for the synthesis of arginosuccinic acid from citrulline and its deficit causes an increase in citrulline levels and a decrease of arginosuccinate and arginine, all of which are frequently detected in Type B patients. High levels of lysine may be detected in Type B and C patients [2]. A central step in lysine catabolism requires the transfer of the α-amino group to α-ketoglutarate through the intermediate saccharopine [53–55]. Low levels of α-ketoglutarate cause an impairment of lysine degradation throughout impeded production of succinyl-CoA, in turn increasing in lysine levels. High concentrations of ammonia due to impairment of the urea cycle can also cause hyperammonemia, as ammonia competes with the conversion of lysine into α-ketoglutarate and glutamate, the latter of which is then converted into glutamine [56]. Therefore, low levels of α-ketoglutarate can also explain the low concentrations of glutamate and glutamine typical of these individuals, especially in Type B patients. As with other states of lactic acidemia, the concentration of proline is elevated in PC deficiency [57], possibly because lactate can inhibit proline oxidase (EC 1.5.99.8) [57–59]. Alanine is also typically elevated in this disorder [2,18,43], probably because pyruvate can be reversibly converted into alanine through alanine aminotransferase (EC 2.6.1.2), a frequent correlate of lactic acidemia states.

Ammonia

Moderate hyperammonemia (100–150 μmol/L) is commonly observed in Type B patients, resulting from secondary impairment of ureagenesis [33,60]. Increased levels of lysine may also impair urea cycle flux by inhibiting arginase (EC 3.5.3.1), ornithine transcarbamylase (EC 2.1.3.3), and mitochondrial ornithine uptake and by competing with ammonia for α-ketoglutarate [60].

Urine analysis

The most constant finding is an elevated alanine level. Increased levels of proline, lysine, cystine, glycine, citrulline, ornithine or even branched-chain amino acid metabolites have been reported, mostly in Type B patients [35,41,42,45]. Renal tubular acidosis (RTA) has been reported in Type A and Type B patients, who manifest generalized amino aciduria and high levels of bicarbonate, and when RTA is associated with multiple defects of the proximal tubule high urinary levels of urate, phosphate, lactate, and sodium can also be detected [10,14,28,32,40–42].

Urine organic acids

Increased levels of lactate, pyruvate, 2-hydroxybutyrate, 3-hydroxybutyrate, and acetoacetate, in addition to low levels of CAC intermediates such as 2-oxoglutarate, fumarate, succinate, and malate have been documented [18,33]. These abnormalities are typical of Type A and B patients and are less often found in Type C patients [33,45].

Cerebrospinal fluid (CSF) analysis

Elevated levels of CSF lactate, pyruvate, alanine, proline, and low levels of glutamine have been reported, mainly in Type B patients [35,41,61,62].

Additional analytical features

Cholesterol

An elevation of total cholesterol or its precursors (mevalonic acid) may occur in Type A and B forms of PC deficiency [13,41]. The excess of ketone body production can serve as precursor of acetyl-CoA and acetoacetyl-CoA synthesis in the cytoplasm, which can be converted into β-hydroxy-β-methyl-glutaryl-CoA (HMG-CoA) and lead to enhanced cholesterol biosynthesis.

Glucose tolerance test (GTT)

GTT can yield variable results depending on the phenotype and the route of glucose administration. Persistently elevated levels of lactate after oral or intravenous (iv) glucose load in a Type B patient was reported [40]. However, the ketone body response was quite different, showing a marked decrease of ketogenesis after IV glucose administration, which returned to high levels when glucose was administered orally. In a Type A patient, the GTT induced a biphasic pattern in the L/P ratio, manifested as an initial decrease of the ratio, followed by a progressive increase after 60 min after the glucose load [49]. In another Type A patient, an iv glucose load caused a significant increase in lactate and pyruvate levels associated with a transient rise in ketone body levels [13]. In a Type C patient, this test did not result in significant changes in lactate or pyruvate concentrations [18].

PC enzyme assay

Assay of PC activity in fibroblasts, lymphocytes, and other tissues except muscle is definitive for the diagnosis of patients with suspected PC deficiency. However, residual PC enzymatic activity is of limited value for the distinction among the three phenotypes [2,17] because enzymatic analysis often yields activities below 5% of normal regardless of PC deficiency type [2,10,13,17,63]. An explanation of this phenomenon is that differences between enzyme activity in vitro and in vivo may be due to the rapid loss of PC activity when tissues are not immediately preserved (particularly liver tissue) or are improperly prepared [18]. On the other hand, measurement of PC activity in fibroblasts can be useful to identify carriers within the family of a proband [10,23]. The assay, however, is unreliable for carrier determination in the general population due to a significant overlap in residual enzyme activity between obligate carriers and non-carriers.

Neuroimaging

Brain structural abnormalities are frequently detected in Type A and B patients by magnetic resonance imaging. The neuroradiological findings reported in these patients include ischemic-like lesions [34], ventricular dilatation, periventricular cysts (identified almost invariably in Type B patients), reduced myelination [35,64], and subcortical leuкоencephalopathy [19,65,66]. These findings are usually detected in symptomatic neonates or infants, although ischemic-like lesions can be detected prenatally in Type B PC deficiency [34].
Diagnostic challenges

Several inborn errors of metabolism share features of PC deficiency by causing downstream or remote impairment of metabolic processes for which PC serves as an entryway, or by inducing secondary PC deficiency due to failure of cofactor action (Fig. 2).

Biotin disorders

Because PC is one of the five biotin-dependent carboxylases that use biotin as a cofactor to transfer carboxyl groups, disorders of biotin metabolism can cause impairment of PC activity. The two principal genetic disorders of biotin metabolism include biotinidase (BTD; EC 3.5.1.12) deficiency (OMIM 253260) and holocarboxylase synthetase (HCS; EC 6.3.4.10) deficiency (OMIM 253270). BTD deficiency typically causes late-onset multiple carboxylase deficiency (first few months or years of life) [67]. Profound enzymatic activity deficiency (<10% of normal serum enzymatic activity) and clinical manifestations can be detected at 3–6 months of age (similarly to Type A phenotype) in patients afflicted by hypotonia, lethargy, seizures, rash, and laryngeal stridor. Biochemical findings include raised lactate and pyruvate concentrations in blood or CSF, ketosis, mild hyperammonemia, and abnormal urinary organic acids with a characteristic increased 3-hydroxyisovaleric acid excretion [67]. HCS deficiency leads to early-onset multiple carboxylase deficiency. Similarly to PC deficiency Type B, it manifests during the first days of life with acute episodes of metabolic acidosis accompanied by lethargy, tachypnea, vomiting, hypotonia, and occasionally seizures and skin rash, leading to severe neurodevelopmental delay and early death if left untreated [68,69]. Urinary organic acid analysis can assist with the differential diagnosis with Type B phenotype, manifesting typically high levels of lactate, 3-methylcrotonylglycine, 3-hydroxyisovalerate, 3-hydroxypropionate and methylcitrate [70].

Disorders of pyruvate metabolism and the CAC

Pyruvate dehydrogenase complex (PDHC) deficiency: The majority of patients manifests severe, often fatal, neonatal or infantile lactic acidosis or exhibits a chronic neurodegenerative course including demyelination, cystic degeneration, ectopic olivary nuclei, hydrocephalus and agenesis of the corpus callosum. Most cases are caused by a deficiency of PDH-E1 α-subunit (OMIM 300502) [51]. Biochemical analysis is characterized by increased lactate in plasma, urine, and CSF [65] with preserved L/P ratio, elevation of alanine in blood and, occasionally, of proline and glutamic acid. In PDH E3 subunit deficiency, moderate increases in lactate, pyruvate, and of the CAC intermediates 2-oxoglutarate and 2-hydroxyglutarate, especially in E3 deficiency. Ketosis is a significant feature of E3 deficiency [73,74].

Of note, in patients with intractable lactic acidemia, normal L/P ratio and normal PDHC activity, a mitochondrial pyruvate carrier defect may be suspected [75]. Disorders of CAC are characterized by abnormal urinary organic acids with elevations of the specific metabolite metabolized by the impaired enzyme.

Respiratory chain disorders

In these disorders, the excess of NADH and lack of NAD arising from respiratory chain dysfunction result in an increase of both H/A (>2) and L/P (≥25) ratios, a typical feature of these patients in addition to hyperlactacidemia and hyperketonemia, particularly in the postabsorptive period [76]. Plasma amino acid analysis reveals only an increase of alanine and proline as the consequence
of hyperlactacidemia and, occasionally, hypermethioninemia and hypocitrullinemia [76,77].

Mitochondrial fatty acid oxidation disorders

These defects often lead to hypoketotic hypoglycemia associated with mild lactic acidemia, affecting predominantly liver, heart or muscle [78]. Other characteristic biochemical abnormalities include increased creatine kinase levels, increased or decreased levels of free carnitine and acylcarnitines in plasma and elevated levels of dicarboxylic acids in urine.

Gluconeogenic defects

Disorders of gluconeogenesis, such as fructose-1,6-biphosphatase (FBPase) deficiency (OMIM 229700), present with lactic acidemia and a more prominent hypoglycemia typically during fasting states [79]. Blood lactate, pyruvate, alanine, and ketone bodies levels are usually elevated.

Citrin (mitochondrial aspartate-glutamate carrier) deficiency

The neonatal form, similarly to PC deficiency Type B, manifests high levels of citrulline along with normal to mild elevation of ammonia, but usually without acidemia or ketonemia [80]. Distinctive features of this disorder are raised levels of threonine and methionine, galactosemia, and cholestatic jaundice. The adult form is characterized by hypercitrullinemia and a more prominent hyperammonemia than the neonatal form, increased serum arginine, and a significantly increased urinary excretion of argininosuccinate [80].

A framework for future therapies

The foremost goal of therapies for PC deficiency as well those devised for other inherited catabolic defects is the restoration of substrate flux into the CAC with the objective of suppressing unmitigated catabolism and to activate synthetic pathways. Interventions that enhance ATP production lead to decreased AMP/ATP ratio, thus inactivating AMPK, while reactivating the mammalian target of rapamycin (mTOR) [81] and re-establishing flux via synthetic pathways.

In the context of cancer therapeutic strategies, compounds that activate AMPK while reactivating mTOR offer potential to diminish state [79]. Blood lactate, pyruvate, alanine, and ketone bodies levels are usually elevated.

In a case of Type B PC deficiency, an anaplerotic diet therapy was formulated to contain 130 cal/kg/day with 30% of glucose, 30% of long-chain fatty acids, 5% of protein, and 35% of triheptanoin (4 g/kg/day) [82]. This diet was administered to a neonate with PC deficiency Type B [62], manifesting clinical and biochemical improvement with decrease of lactic acidosis, L/P and H/A ratios, progressive decrease of ammonia and citrulline levels in plasma, rapid normalization of liver function, and increase of urinary excretion of urinary CAC intermediates. After 4 months of treatment, the patient manifested a progressive decrease of CAC intermediates and bicarbonate associated with increased lactate and ketone bodies, which were reversed by the addition of citrate (7.5 mmol/kg/day), 2-chloropropionate (50 mg/kg/day), restriction of glucose intake (30% of total calories), and normal protein intake. The patient died at 6 months of age after a severe infection resulting in a fatal acute metabolic decompensation.

Conclusion

PC is a crucial flux facilitator for all synthetic pathways that rely upon the formation of oxaloacetate. Inherited deficiency of this enzyme causes broad disturbances that mostly reflect deranged liver and brain metabolism. The three identified clinical phenotypes display varying degrees of clinical severity in the setting of lactic acidemia and neurological disturbances. The diagnosis still relies upon analysis of amino acids in blood and urine and of urinary organic acids and upon enzyme assay of PC because molecular genetic testing is not readily available. At the present time, most interventions have met with little benefit in these patients, but future therapeutic efforts will focus on the replenishment of CAC intermediates to interrupt the hyperactive catabolic cascade associated with the disorder and to enhance ATP production. The use of
an exogenous substrate such as the anaplerotic compound trihep-
tetanoïn may provide the necessary source of both oxaloacetate and
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