12 Disorders of Pyruvate Metabolism and the Tricarboxylic Acid Cycle

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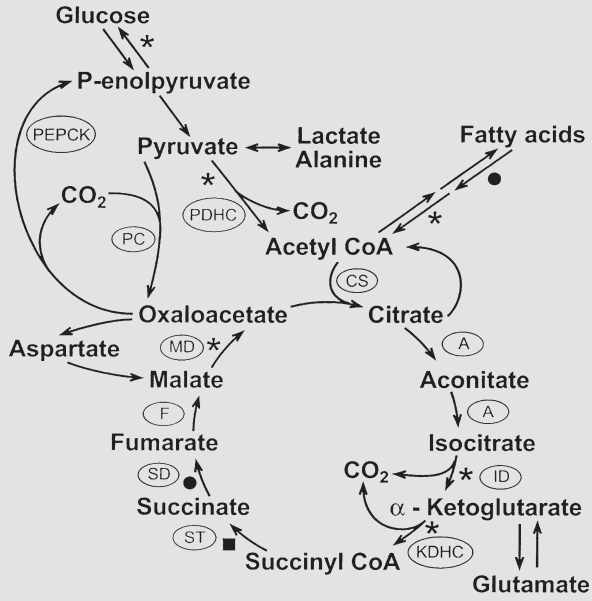
III

Pyruvate Metabolism and the Tricarboxylic Acid Cycle

Pyruvate is formed from glucose and other monosaccharides, from lactate, and from the gluconeogenic amino acid alanine (◘ Fig 12.1). After entering the mitochondrion, pyruvate can be converted into acetylcoenzyme A (acetyl-CoA) by the pyruvate dehydrogenase complex, followed by further oxidation in the TCA cycle. Pyruvate can also enter the gluconeogenic pathway by sequential conversion into oxaloacetate by pyruvate carboxylase, followed by conversion into phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. Acetyl-CoA can also be formed by fatty acid oxidation or used for lipogenesis. Other amino acids enter the TCA cycle at several points. One of the primary functions of the TCA cycle is to generate reducing equivalents in the form of reduced nicotinamide adenine dinucleotide and reduced flavin adenine dinucleotide, which are utilized to produce energy under the form of ATP in the electron transport chain.



◘ **Fig. 12.1.** Overview of glucose, pyruvate/lactate, fatty acid and amino acid oxidation by the tricarboxylic acid cycle. *A,* aconitase; *CS,* citrate synthase; *F,* fumarase; *ID,* isocitrate dehydrogenase; *KDHC,* α-or 2-ketoglutarate dehydrogenase complex; *MD,* malate dehydrogenase; *PC,* pyruvate carboxylase; *PDHC,* pyruvate dehydrogenase complex; *PEPCK,* phosphoenolpyruvate carboxykinase; *SD,* succinate dehydrogenase; *ST,* succinyl coenzyme A transferase. Sites where reducing equivalents and intermediates for energy production intervene are in dicated by following symbols: *, reduced nicotinamide adenine dinucleotide; ●, reduced flavin adenine dinucleotide; ■, guanosine triphosphate

Owing to the role of pyruvate and the tricarboxylic acid (TCA) cycle in energy metabolism, as well as in gluconeogenesis, lipogenesis and amino acid synthesis, defects in pyruvate metabolism and in the TCA cycle almost invariably affect the central nervous system. The severity and the different clinical phenotypes vary widely among patients and are not always specific, with the range of manifestations extending from overwhelming neonatal lactic acidosis and early death to relatively normal adult life and variable effects on systemic functions. The same clinical manifestations may be caused by other defects of energy metabolism, especially defects of the respiratory chain (Chap. 15). Diagnosis depends primarily on biochemical analyses of metabolites in body fluids, followed by definitive enzymatic assays in cells or tissues, and DNA analysis. The deficiencies of *pyruvate carboxylase* (PC) and *phosphoenolpyruvate carboxykinase* (PEPCK) constitute defects in gluconeogenesis, and therefore fasting results in hypoglycemia with worsening lactic acidosis. Deficiency of the *pyruvate dehydrogenase complex* (PDHC) impedes glucose oxidation and aerobic energy production, and ingestion of carbohydrate aggravates lactic acidosis. Treatment of disorders of pyruvate metabolism comprises avoidance of fasting (PC and PEPCK) or minimizing dietary carbohydrate intake (PDHC) and enhancing anaplerosis. In some cases, vitamin or drug therapy may be helpful. *Dihydrolipoamide dehydrogenase* (E3) deficiency affects PDHC as well as KDHC and the branched-chain 2-ketoacid dehydrogenase (BCKD) complex (Chap. 19), with biochemical manifestations of all three disorders. The deficiencies of the TCA cycle enzymes, the *2-ketoglutarate dehydrogenase complex* (KDHC) and *fumarase*, interrupt the cycle, resulting in accumulation of the corresponding substrates. *Succinate dehydrogenase* deficiency represents a unique disorder affecting both the TCA cycle and the respiratory chain. Recently, defects of *mitochondrial transport of pyruvate* and *glutamate* (► Chap. 29) have been identified. Treatment strategies for the TCA cycle defects are limited.

12.1 Pyruvate Carboxylase Deficiency

12.1.1 Clinical Presentation

Three phenotypes are associated with pyruvate carboxylase deficiency. The patients with French phenotype (type B) become acutely ill three to forty eight hours after birth with hypothermia, hypotonia, lethargy and vomiting [1–5, 5a]. Most die in the neonatal period. Some survive but remain unresponsive and severely hypotonic, and finally succumb from respiratory infection before the age of 5 months.

The patients with North American phenotype (type A) become severely ill between two and five months of age [2, 6–8]. They develop progressive hypotonia and are unable to smile. Numerous episodes of acute vomiting, dehydration, tachypnea, facial pallor, cold cyanotic extremities and metabolic acidosis, characteristically precipitated by metabolic or infectious stress are a constant finding. Clinical examination reveals pyramidal tract signs, ataxia and nystagmus. All patients are severely mentally retarded and most have convulsions. Neuroradiological findings include subdural effusions, severe antenatal ischemia-like brain lesions and periventricular hemorrhagic cysts, followed by progressive cerebral atrophy and delay in myelination [4]. The course of the disease is generally downhill, with death in infancy.

A third form, more benign, is rare and has only been reported in a few patients [9]. The clinical course is dominated by the occurrence of acute episodes of lactic acidosis and ketoacidosis, responding rapidly to glucose 10 %, hydration and bicarbonate therapy. Despite the important enzymatic deficiency, the patients have a nearly normal cognitive and neuromotor development.

12.1.2 Metabolic Derangement

PC is a biotinylated mitochondrial matrix enzyme that converts pyruvate and CO_2 to oxaloacetate (◘ Fig. 12.1). It plays an important role in gluconeogenesis, anaplerosis, and lipogenesis. For gluconeogenesis, pyruvate must first be carboxylated into oxaloacetate because the last step of glycolysis, conversion of phosphoenolpyruvate to pyruvate, is irreversible. Oxaloacetate, which cannot diffuse freely out of the mitochondrion, is translocated into the cytoplasm via the malate/aspartate shuttle. Once in the cytoplasm, oxaloacetate is converted into phosphoenol-pyruvate by phosphoenol-pyruvate carboxykinase (PEPCK), which catalyzes the first committed step of gluconeogenesis.

The anaplerotic role of PC, i.e. the generation of Krebs cycle intermediates from oxaloacetate, is even more important. In severe PC deficiency, the lack of Krebs cycle intermediates lowers reducing equivalents in the mitochondrial matrix. This drives the redox equilibrium between 3-OH-butyrate and acetoacetate into the direction of acetoacetate, thereby lowering the 3-OH-butyrate/acetoacetate ratio [6]. Aspartate, formed in the mitochondrial matrix from oxaloacetate by transamination, also decreases. As a consequence, the translocation of reducing equivalents between cytoplasm and mitochondrial matrix by the malate/aspartate shuttle is impaired. This drives the cytoplasmic redox equilibrium between lactate and pyruvate into the direction of lactate, and the lactate/pyruvate ratio increases. Reduced Krebs cycle activity also plays a role in the increase of lactate and pyruvate. Since aspartate is required for the urea cycle, plasma ammonia can also go up. The energy deprivation induced by PC deficiency has been postulated to impair

astrocytic buffering capacity against excitotoxic insults and to compromise microvascular morphogenesis and autoregulation, leading to degeneration of white matter [4].

The importance of PC for lipogenesis derives from the condensation of oxaloacetate with intramitochondrially produced acetyl-CoA into citrate, which can be translocated into the cytoplasm where it is cleaved to oxaloacetate and acetyl-CoA, used for the synthesis of fatty acids. Deficient lipogenesis explains the widespread demyelination of the cerebral and cerebellar white matter and symmetrical paraventricular cavities around the frontal and temporal horns of the lateral ventricles, the most striking abnormalities reported in the few detailed neuropathological descriptions of PC deficiency [1, 4].

PC requires biotin as a cofactor. Metabolic derangements of PC deficiency are thus also observed in biotin-responsive multiple carboxylase deficiency (► Chap. 27).

12.1.3 Genetics

PC deficiency is an autosomal recessive disorder. More than half of the patients with French phenotype have absence of PC protein, a tetramer formed by 4 identical subunits with MW of 130 kD, and of the corresponding mRNA. The patients with North American phenotype generally have cross-reacting material (CRM-positive) [2], as does the patient with the benign variant of PC deficiency [9]. Mutations have been detected in patients of both types A and B. In Canadian Indian populations with type A disease, 11 Ojibwa and 2 Cree patients were homozygous for a missense mutation A610T; two brothers of Micmac origin were homozygous for a transversion M743I [8]. In other families, various mutations were found.

12.1.4 Diagnostic Tests

The possibility of PC deficiency should be considered in any child presenting with lactic acidosis and neurological abnormalities, especially if associated with hypoglycemia, hyperammonemia, or ketosis. In neonates, a high lactate/pyruvate ratio associated with a low 3-OH-butyrate/acetoacetate ratio and hypercitrullinemia is nearly pathognomonic [5a]. Discovery of cystic periventricular leucomalacia at birth associated with lactic acidosis is also highly suggestive. Typically, blood lactate increases in the fasting state and decreases after ingestion of carbohydrate.

In patients with the French phenotype, blood lactate concentrations reach 10–20 mM (normal <2.2 mM) with lactate/pyruvate ratios between 50 and 100 (normal <28). In patients with the North American phenotype, blood lactate is 2–10 mM with normal or only moderately increased lactate/pyruvate ratios (<50). In the patients with the benign type, lactate can be normal, and only increase (usually above 10 mM) during acute episodes. Overnight blood glucose concentrations are usually normal but decrease after a 24 h fast. Hypoglycemia can occur during acute episodes of metabolic acidosis. Blood 3-OH-butyrate is increased (0.5–2.7 mM, normal <0.1) and 3-OH-butyrate/acetoacetate ratio is decreased (<2, normal 2.5–3).

Hyperammonemia (100–600 μM, normal <60) and an increase of blood citrulline (100–400 μM, normal <40), lysine and proline, contrasting with low glutamine, are constant findings in patients with the French phenotype [5a]. Plasma alanine is usually normal in the French phenotype, but increased (0.5–1.4 mM, normal <0.455) in all reported patients with the North-American phenotype. During acute episodes, aspartate can be undetectably low [9].

In cerebrospinal fluid (CSF), lactate, the lactate/pyruvate ratio and alanine are increased and glutamine is decreased. Urine organic acid profile shows, besides large amounts of lactate, pyruvate and 3-OH-butyrate, an increase of α-ketoglutarate.

Measurement of the activity of PC is preferentially performed on cultured skin fibroblasts [6]. Assays can also be performed in postmortem liver, in which the activity of PC is 10-fold higher than in fibroblasts, but must be interpreted with caution because of rapid postmortem degradation of the enzyme. PC has low activity in skeletal muscle, which makes this tissue not useful for assay. PC activity in fibroblasts is severely decreased, to less than 5% of normal, in all patients with the French phenotype, varies from 5 to 23% of controls in patients with the North American phenotype, and is less than 10% of controls in patients with the benign variant.

Prenatal diagnosis of PC deficiency is possible by measurement of PC activity in cultured amniotic fluid cells [10], direct measurement in chorionic villi biopsy specimens [3], or DNA analysis when the familial mutations are known.

12.1.5 Treatment and Prognosis

Since acute metabolic crises can be detrimental both physically and mentally, patients should be promptly treated with intravenous 10% glucose. Thereafter, they should be instructed to avoid fasting. Some patients with persistent lactic acidosis may require bicarbonate to correct acidosis. One patient with French phenotype was treated with high doses of citrate and aspartate [5]. Lactate and ketones diminished and plasma aminoacids normalized, except for arginine. In the CSF, glutamine remained low and lysine elevated, precluding normalization of brain chemistry. An orthotopic hepatic transplantation completely reversed ketoacidosis and the renal tubular abnormalities, and decreased lactic acidemia in a patient with a severe phenotype, although concentrations of glutamine in CSF remained low [11]. Recently, one patient with French phenotype treated

early by triheptanoin in order to restore anaplerosis, improved dramatically [12]. Biotin [1,6], thiamine, dichloroacetate, and a high fat or high carbohydrate diet provide no clinical benefits.

The prognosis of patients with PC deficiency depends on the severity of the defect. Patients with minimal residual PC activity usually do not live beyond the neonatal period, but some children with very low PC activity have survived beyond the age of 5 years. Those with milder defects might survive and have neurological deficits of varying degrees.

12.2 Phosphoenolpyruvate Carboxykinase Deficiency

12.2.1 Clinical Presentation

Phosphoenolpyruvate carboxykinase (PEPCK) deficiency was first described by Fiser et al. [13]. Since then, only 5 additional patients have been reported in the literature [14]. This may be explained, as discussed below, by observations that have led to the conclusion that PEPCK deficiency might be a secondary finding, which should be interpreted with utmost caution.

Patients reported to be PEPCK deficient presented, as those with PC deficiency, with acute episodes of severe lactic acidosis associated with hypoglycemia. Onset of symptoms is neonatal or after a few months. Patients display mostly progressive multisystem damage with failure to thrive, muscular weakness and hypotonia, developmental delay with seizures, spasticity, lethargy, microcephaly, hepatomegaly with hepatocellular dysfunction, renal tubular acidosis and cardiomyopathy. The clinical picture may also mimic Reye syndrome [15, 16].

Routine laboratory investigations during acute episodes show lactic acidosis and hypoglycemia, acompanied by hyperalaninemia and, as documented in some patients, by absence of elevation of ketone bodies. Liver function and blood coagulation tests are disturbed, and combined hypertriglyceridemia and hypercholesterolemia have been reported. Analysis of urine shows increased lactate, alanine and generalized aminoaciduria.

12.2.2 Metabolic Derangement

PEPCK is located at a crucial metabolic crossroad of carbohydrate, amino acid, and lipid metabolism (◘ Fig. 12.1). This may explain the multiple organ damage which seems to be caused by its deficiency. Since, by converting oxaloacetate into phosphoenolpyruvate, PEPCK plays a major role in gluconeogenesis, its deficiency should impair conversion of pyruvate, lactate, alanine, and TCA intermediates into glucose, and hence provoke lactic acidosis, hyperalaninemia and hypoglycemia. PEPCK exists as two separate isoforms, mitochondrial and cytosolic, which are encoded by two distinct genes. The deficiency of mitochondrial PEPCK, which intervenes in gluconeogenesis from lactate, should have more severe consequences than that of cytosolic PEPCK, which is supposed to play a role in gluconeogenesis from alanine.

12.2.3 Genetics

The cDNA encoding the cytosolic isoform of PEPCK in humans has been sequenced and localized to human chromosome 20. However, in accordance with the findings discussed below, no mutations have been identified.

12.2.4 Diagnostic Tests

The diagnosis of PEPCK deficiency is complicated by the existence of separate mitochondrial and cytosolic isoforms of the enzyme. Optimally, both isoforms should be assayed in a fresh liver sample after fractionation of mitochondria and cytosol. In cultured fibroblasts, most of the PEPCK activity is located in the mitochondrial compartment, and low PEPCK activity in whole-cell homogenates indicates deficiency of the mitochondrial isoform.

Deficiency of cystosolic PEPCK has been questioned because synthesis of this isoform is repressed by hyperinsulinism, a condition which was also present in a patient with reported deficiency of cytosolic PEPCK [15]. Deficiency of mitochondrial PEPCK has been disputed because in a sibling of a PEPCK-deficient patient who developed a similar clinical picture, the activity of PEPCK was found normal [16]. Further studies showed a depletion of mitochondrial DNA in this patient [17] caused by defective DNA replication [18]. The existence of PEPCK deficiency thus remains to be firmly established.

12.2.5 Treatment and Prognosis

Patients with suspected PEPCK deficiency should be treated with intravenous glucose and sodium bicarbonate during acute episodes of hypoglycemia and lactic acidosis. Fasting should be avoided, and cornstarch or other forms of slow-release carbohydrates need to be provided before bedtime. The long-term prognosis of patients with reported PEPCK deficiency is usually poor, with most subjects dying of intractable hypoglycemia or neurodegenerative disease.

III

Structure and Activation/Deactivation System of the Pyruvate Dehydrogenase Complex

PDHC, and the two other mitochondrial α- or 2-ketoacid dehydrogenases, KDHC and the BCKD complex, are similar in structure and analogous or identical in their specific mechanisms. They are composed of three components: E1, α- or 2-ketoacid dehydrogenase; E2, dihydrolipoamide acyltransferase; and E3, dihydrolipoamide dehydrogenase. E1 is specific for each complex, utilizes thiamine pyrophosphate, and is composed of two different subunits, E1α and E1β. The E1 reaction results in decarboxylation of the specific α- or 2-ketoacid. For the PDHC, the E1 component is the rate-limiting step, and is regulated by phosphorylation/dephosphorylation catalyzed by two enzymes, E1 kinase (inactivation) and E1 phosphatase (activation). E2 is a transacetylase that utilizes covalently bound lipoic acid. E3 is a flavoprotein common to all three 2-ketoacid dehydrogenases. Another important structural component of the PDHC is E3BP, E3 binding protein, formerly protein X. This component has its role in attaching E3 subunits to the core of E2.

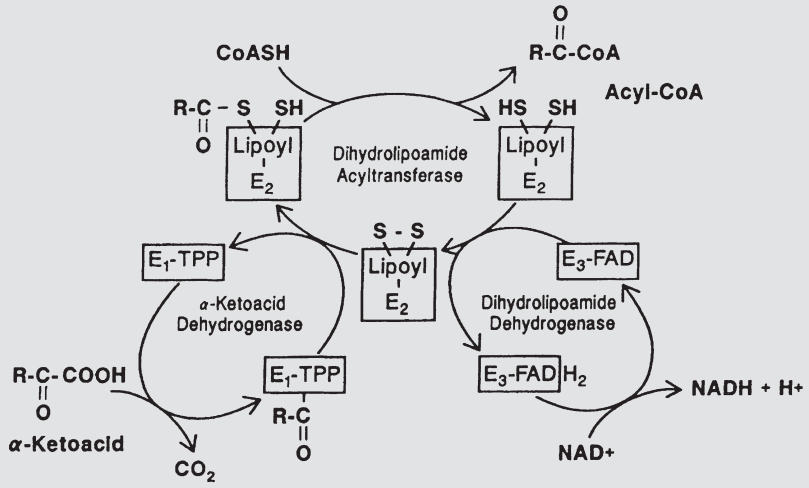


Fig. 12.2. Structure of the α- or 2-ketoacid dehydrogenase complexes, pyruvate dehydrogenase complex (PDHC), 2-ketoglutarate dehydrogenase complex (KDHC) and the branched-chain α-ketoacid dehydrogenase complex (BCKD). *CoA,* coenzyme A; *FAD,* flavin adenine dinucleotide; *NAD,* nicotinamide adenine dinucleotide; *R,* methyl group (for pyruvate, PDHC) and the corresponding moiety for KDHC and BCKD; *TPP,* thiamine pyrophosphate

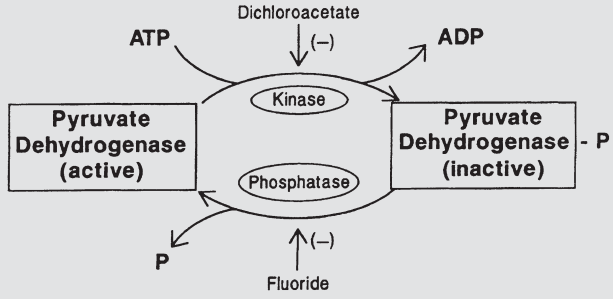


Fig. 12.3. Activation/deactivation of PDHE1 by dephosphorylation/phosphorylation. Dichloroacetate is an inhibitor of E1 kinase and fluoride inhibits E1 phosphatase. *ADP,* adenosine diphosphate; *P,* inorganic phosphate

12.3 Pyruvate Dehydrogenase Complex Deficiency

12.3.1 Clinical Presentation

More than 200 cases of pyruvate dehydrogenase complex (PDHC) deficiency have been reported [19–21], the majority of which involves the α subunit of the first, dehydrogenase component (E1) of the complex (◘ Fig. 12.2) which is X encoded. The most common features of PDHE1α deficiency are delayed development and hypotonia, seizures and ataxia. Female patients with PDHE1α deficiency tend to have a more homogeneous and more severe clinical phenotype than boys [22].

In hemizygous males, three presentations are encountered: neonatal lactic acidosis, Leigh's encephalopathy, and intermittent ataxia. These correlate with the severity of the biochemical deficiency and the location of the gene mutation. Severe neonatal lactic acidosis, associated with brain dysgenesis, such as corpus callosum agenesis, can evoke the diagnosis. In Leigh's encephalopathy, quantitatively the most important group, initial presentation, usually within the first five years of life, includes respiratory disturbances/apnoea or episodic weakness and ataxia with absence of tendon reflexes. Respiratory disturbances may lead to apnea, dependence on assisted ventilation, or sudden unexpected death. Intermittent dystonic posturing of the lower limbs occurs frequently. A moderate to severe developmental delay becomes evident within the next years. A very small subset of male patients is initially much less severely affected, with intermittent episodic ataxia after carbohydrate-rich meals, progressing slowly over years into mild Leigh's encephalopathy.

Females with PDHE1α deficiency tend to have a more uniform clinical presentation, although with variable severity, depending on variable lyonisation. This includes dysmorphic features, microcephaly, moderate to severe mental retardation, and spastic di- or quadriplegia, resembling non progressive encephalopathy. Dysmorphism comprises a narrow head with frontal bossing, wide nasal bridge, upturned nose, long philtrum and flared nostrils and may suggest fetal alcohol syndrome. Other features are low set ears, short fingers and short proximal limbs, simian creases, hypospadias and an anteriorly placed anus. Seizures are encountered in almost all female patients. These appear within the first six months of life and are diagnosed as infantile spasms (flexor and extensor) or severe myoclonic seizures. Brain MRI frequently reveals severe cortical/subcortical atrophy, dilated ventricles and partial to complete corpus callosum agenesis [23]. Severe neonatal lactic acidosis can be present. The difference in the presentation of PDHE1α deficiency in boys and girls is exemplified by observations in a brother and sister pair with the same mutation but completely different clinical features [22].

Neuroradiological abnormalities such as corpus callosum agenesis and dilated ventricles or in boys basal ganglia and midbrain abnormalities are often found. Neuropathology can reveal various degrees of dysgenesis of the corpus callosum. This is usually associated with other migration defects such as the absence of the medullary pyramids, ectopic olivary nuclei, abnormal Purkinje cells in the cerebellum, dysplasia of the dentate nuclei, subcortical heterotopias and pachygyria [24].

Only a few cases with PDHE1β deficiency have been reported [25]. These patients present with early onset lactic acidosis and severe developmental delay. Seven cases of E1-phosphatase deficiency (◘ Fig.12.3) have been identified [26], among which two brothers with hypotonia, feeding difficulties and delayed psychomotor development [27]. A few cases of PDHE2 (dihydrolipoamide transacetylase) deficiency have been reported recently [28]. The main clinical manifestations of E3BP (formerly protein X) deficiency are hypotonia, delayed psychomotor development and prolonged survival [29]. Often more slowly progressive, it also comprises early onset neonatal lactic acidosis associated with subependymal cysts and thin corpus callosum.

12.3.2 Metabolic Derangement

Defects of PDHC provoke conversion of pyruvate into lactate rather than in acetyl-CoA, the gateway for complete oxidation of carbohydrate via the TCA cycle (◘ Fig.12.1). The conversion of glucose to lactate yields less than one tenth of the ATP that would be derived from complete oxidation of glucose via the TCA cycle and the respiratory chain. Deficiency of PDHC thus specifically interferes with production of energy from carbohydrate oxidation, and lactic acidemia is aggravated by consumption of carbohydrate.

PDHC deficiency impairs production of reduced nicotinamide adenine dinucleotide (NADH) but, unlike respiratory chain defects, does not hamper oxidation of NADH. PDHC deficiency thus does not modify the $NADH/NAD^+$ ratio in the cell cytosol, which is reflected by a normal L/P ratio. In contrast, deficiencies of respiratory chain complexes I, III, and IV are generally characterized by a high L/P ratio because of impaired NADH oxidation.

12.3.3 Genetics

All components of PDHC are encoded by nuclear genes, and synthesized in the cytoplasm as precursor proteins that are imported into the mitochondria, where the mature proteins are assembled into the enzyme complex. Most of the genes that encode the various subunits are autosomal, except the E1α-subunit gene which is located on chromosome Xp22.3. Therefore, most cases of PDHC deficiency are

X-linked. To date, over 80 different mutations of the E1α subunit of PDHC have been characterized in some 130 unrelated families [30]. About half of these are small deletions, insertions, or frame-shift mutations, and the other half are missense mutations. While the consequences of most of the mutations on enzyme structure and function are not known, some affect highly conserved amino acids that are critical for mitochondrial import, subunit interaction, binding of thiamine pyrophosphate, dephosphorylation, or catalysis at the active site. No null E1α mutations have been identified in males, suggesting that such mutations are likely to be lethal. In males with recurrent E1α mutations disease there is still a variable phenotypic expression.

Only two defects of the E1β subunit have been identified [25]. The molecular basis of E3-binding protein (E3BP) deficiency has been characterized in 13 cases. Half of the patients have splicing errors, others have frameshift or nonsense mutations [31]. Recently mutations in E2 [28] and in the pyruvate dehydrogenase phosphatase gene (PDP1) [27] have been identified.

In about 25 % of cases the mother of a child with PDHE1α deficiency was a carrier of the mutation [30]. Therefore, since most cases of PDHC deficiency appear to be the consequence of new E1α mutations, the overall rate of recurrence in the same family is low. Based on measurement of PDHC activity in chorionic villus samples and/or cultured amniocytes obtained from some 30 pregnancies in families with a previously affected child, three cases of reduced activity were found. However, PDHC activities in affected females might overlap with normal controls. Therefore, prenatal testing of specific mutations determined in the proband is the most reliable method. Molecular analysis is also the preferred method for prenatal diagnosis in families at risk for E1β and E3BP deficiency.

12.3.4 Diagnostic Tests

The most important laboratory test for initial recognition of PDHC deficiency is measurement of blood and CSF lactate and pyruvate. Quantitative analysis of plasma amino acids and urinary organic acids may also be useful. Blood lactate, pyruvate and alanine can be intermittently normal, but, characteristically, an increase is observed after an oral carbohydrate load. While L/P ratio is as a rule normal, a high ratio can be found if the patient is acutely ill, if blood is very difficult to obtain, or if the measurement of pyruvate (which is unstable) is not done reliably. The practical solution to avoid these artifacts is to obtain several samples of blood, including samples collected under different dietary conditions (during an acute illness, after overnight fasting, and postprandially after a high-carbohydrate meal). Glucose-tolerance or carbohydrate-loading tests are not necessary for a definite diagnosis. In contrast to deficiencies of PC or PEPCK, fasting hypoglycaemia is not an expected feature of PDHC deficiency, and blood lactate and pyruvate usually decrease after fasting. CSF for measurement of lactate and pyruvate (and possibly organic acids) is certainly indicated, since there may be a normal blood lactate and pyruvate, and only elevation in CSF [32].

The most commonly used material for assay of PDHC is cultured skin fibroblasts. PDHC can also be assayed in fresh lymphocytes, but low normal values might make the diagnosis difficult. Molecular analysis of the PDHE1α gene in girls is often more efficient than measuring the enzyme activity. If available, skeletal muscle and/or other tissues are useful. When a patient with suspected but unproven PDHC deficiency dies, it is valuable to freeze samples of different origin such as skeletal muscle, heart muscle, liver, and/or brain, ideally within 4 h post-mortem [33]. A skin biopsy to be kept at 4°C in a physiological solution can be useful. PDHC is assayed by measuring the release of $^{14}CO2$ from [1-^{14}C]-pyruvate in cell homogenates and tissues [34]. PDHC activity should be measured at low and high TPP concentrations to detect thiamine-responsive PDHC deficiency [35]. PDHC must also be activated (dephosphorylated; ◘ Fig. 12.3) in part of the cells, which can be done by pre-incubation of whole cells or mitochondria with dichloroacetate (DCA, an inhibitor of the kinase; ◘ Fig.12.3). In E1-phosphatase deficiency there is a deficiency in native PDH activity, but on activation of the PDH complex with DCA, activity becomes normal [27]. The three catalytic components of PDHC can be assayed separately. Immunoblotting of the components of PDHC can help distinguish if a particular protein is missing. In females with PDHE1α deficiency, X inactivation can interfere with the biochemical analysis [32]. E3BP, which anchors E3 to the E2 core of the complex, can only be evaluated using immunoblotting, since it has no catalytic activity [29].

12.3.5 Treatment and Prognosis

The general prognosis for individuals with PDHC deficiency is poor, and treatment is not very effective. Experience with early prospective treatment to prevent irreversible brain injury is lacking. Perhaps the most rational strategy for treating PDHC deficiency is the use of a ketogenic diet [36]. Oxidation of fatty acids, 3-hydroxybutyrate, and acetoacetate are providers of alternative sources of acetyl-CoA. Wexler et al. compared the outcome of males with PDHC deficiency caused by identical E1 mutations and found that the earlier the ketogenic diet was started and the more severe the restriction of carbohydrates, the better the outcome of mental development and survival [37]. Sporadic cases of improvement under ketogenic diet have been published. Thiamine has been given in variable doses (500–2000 mg/day), with lowering of blood lactate and apparent clinical improvement in some patients [38].

DCA offers another potential treatment for PDHC deficiency. DCA, a structural analogue of pyruvate, inhibits E1 kinase, thereby keeping any residual E1 activity in its active (dephosphorylated) form (◘ Fig. 12.3). DCA can be administered without apparent toxicity (about 50 mg/kg/day). Over 40 cases of congenital lactic acidosis due to various defects (including PDHC deficiency) were treated with DCA in uncontrolled studies, and most of these cases appeared to have some limited short-term benefit [39]. Chronic DCA treatment was shown to be beneficial in some patients, improving the function of PDHC, and this has been related to specific DCA-sensitive mutations [40]. Sporadic reports have also shown beneficial effect of concomitant DCA and high dose thiamine (500 mg). A ketogenic diet and thiamine should thus be tried in each patient. DCA can be added if lactic acidosis is important, especially in acute situations.

12.4 Dihydrolipoamide Dehydrogenase Deficiency

12.4.1 Clinical Presentation

Approximately 20 cases of E3 deficiency have been reported [41–43]. Since this enzyme is common to all the 2-ketoacid dehydrogenases (◘ Fig. 12.2), E3 deficiency results in multiple 2-ketoacid-dehydrogenase deficiency and should be thought of as a combined PDHC and TCA cycle defect. E3 deficiency presents with severe and progressive hypotonia and failure to thrive, starting in the first months of life. Metabolic decompensations are triggered by infections. Progressively hypotonia, psychomotor retardation, microcephaly and spasticity occur. Some patients develop a typical picture of Leigh's encephalopathy. A Reye-like picture with liver involvement and myopathy with myoglobinuria without mental retardation is seen in the Ashkenazi Jewish population [44].

12.4.2 Metabolic Derangement

Dihydrolipoyl dehydrogenase (E3) is a flavoprotein common to all three mitochondrial α-ketoacid dehydrogenase complexes (PDHC, KDHC, and BCKD; ◘ Fig. 12.2). The predicted metabolic manifestations are the result of the deficiency state for each enzyme: increased blood lactate and pyruvate, elevated plasma alanine, glutamate, glutamine, and branched-chain amino acids (leucine, isoleucine, and valine), and increased urinary lactic, pyruvic, 2-ketoglutaric, and branched-chain 2-hydroxy- and 2-keto acids.

12.4.3 Genetics

The gene for E3 is located on chromosome 7q31-q32 [45] and the deficiency is inherited as an autosomal recessive trait. Mutation analysis in 13 unrelated patients has revealed eleven different mutations [46–50]. A G194C mutation is the major cause of E3 deficiency in Ashkenazi Jewish patients [51]. The most reliable method for prenatal diagnosis is through mutation analysis in DNA from chorionic villous samples (CVS) in previously identified families.

12.4.4 Diagnostic Tests

The initial diagnostic screening should include analyses of blood lactate and pyruvate, plasma amino acids, and urinary organic acids. However, the pattern of metabolic abnormalities is not seen in all patients or at all times in the same patient, making the diagnosis more difficult. In cultured skin fibroblasts, blood lymphocytes, or other tissues, the E3 component can be assayed using a spectrophotometric method.

12.4.5 Treatment and Prognosis

There is no dietary treatment for E3 deficiency, since the affected enzymes effect carbohydrate, fat, and protein metabolism. Restriction of dietary branched-chain amino acids was reportedly helpful in one case [52]. DL-lipoic acid has been tried but its effect remains controversial [51].

12.5 2-Ketoglutarate Dehydrogenase Complex Deficiency

12.5.1 Clinical Presentation

Isolated deficiency of the 2-ketoglutarate dehydrogenase complex (KDHC) has been reported in ten children in several unrelated families [53–55]. As in PDHC deficiency, the primary clinical manifestations included developmental delay, hypotonia, ataxia, opisthotonos and, less commonly, seizures and extrapyramidal dysfunction. On magnetic resonance imaging (MRI) bilateral striatal necrosis can be found [56]. All patients presented in the neonatal period and early childhood.

In one patient the clinical picture was milder [55]. This patient had suffered from mild perinatal asphyxia. During the first months of life, he developed opisthotonus and axial hypertonia, which improved with age. 2-Ketoglutaric acid (2-KGA) was intermittently increased in urine, but not in plasma and CSF. Diagnosis was confirmed in cultured skin fibroblasts. Surendam et al. [57] presented three families with the clinical features of DOOR syndrome

(onychoosteodystrophy, dystrophic thumbs, sensorineural deafness), increased urinary levels of 2-KGA, and decreased activity of the E1 component of KDHC.

12.5.2 Metabolic Derangement

KDHC is a 2-ketoacid dehydrogenase that is analogous to PDHC and BCKD (Fig. 12.2). It catalyzes the oxidation of 2-KGA to yield CoA and NADH. The E1 component, 2-ketoglutarate dehydrogenase, is a substrate-specific dehydrogenase that utilizes thiamine and is composed of two different subunits. In contrast to PDHC, the E1 component is not regulated by phosphorylation/dephosphorylation. The E2 component, dihydrolipoyl succinyl-transferase, is also specific to KDHC and includes covalently bound lipoic acid. The E3 component is the same as for PDHC. An E3-binding protein has not been identified for KDHC. Since KDHC is integral to the TCA cycle, its deficiency has consequences similar to that of other TCA enzyme deficiencies.

12.5.3 Genetics

KDHC deficiency is inherited as an autosomal recessive trait. The E1 gene has been mapped to chromosome 7p13-14 and the E2 gene to chromosome 14q24.3. The molecular basis of KDHC deficiencies has not yet been resolved. While prenatal diagnosis of KDHC should be possible by measurement of the enzyme activity in CVS or cultured amniocytes, this has not been reported.

12.5.4 Diagnostic Tests

The most useful test for recognizing KDHC deficiency is urine organic acid analysis, which can show increased excretion of 2-KGA with or without concomitantly increased excretion of other TCA cycle intermediates. However, mildly to moderately increased urinary 2-KGA is a common finding and not a specific marker of KDHC deficiency. Some patients with KDHC deficiency also have increased blood lactate with normal or increased L/P ratio. Plasma glutamate and glutamine may be increased. KDHC activity can be assayed through the release of $^{14}CO2$ from [1-^{14}C]-2-ketoglutarate in crude homogenates of cultured skin fibroblasts, muscle homogenates and other cells and tissues [53].

12.5.5 Treatment and Prognosis

There is no known selective dietary treatment that bypasses KDHC, since this enzyme is involved in the terminal steps of virtually all oxidative energy metabolism. Thiamine-responsive KDHC deficiency has not been described.

12.6 Fumarase Deficiency

12.6.1 Clinical Presentation

Approximately 26 patients with fumarase deficiency have been reported. The first case was described in 1986 [58]. Onset started at three weeks of age with vomiting and hypotonia, followed by development of microcephaly (associated with dilated lateral ventricles), severe axial hypertonia and absence of psychomotor progression.

Until the publication of Kerrigan [59] only 13 patients were described, all presenting in infancy with a severe encephalopathy and seizures, with poor neurological outcome. Kerrigan reported on 8 patients from a large consanguineous family. All patients had a profound mental retardation and presented as a static encephalopathy. Six out of 8 developed seizures. The seizures were of various types and of variable severity, but several patients experienced episodes of status epilepticus. All had a relative macrocephaly (in contrast to previous cases) and large ventricles. Dysmorphic features such as frontal bossing, hypertelorism and depressed nasal bridge were noted.

Neuropathological changes include agenesis of the corpus callosum with communicating hydrocephalus as well as cerebral and cerebellar heterotopias. Polymicrogyria, open operculum, colpocephaly, angulations of frontal horns, choroid plexus cysts, decreased white matter, and a small brainstem are considered characteristic [59].

12.6.2 Metabolic Derangement

Fumarase catalyzes the reversible interconversion of fumarate and malate (Fig. 12.1). Its deficiency, like other TCA cycle defects, causes: (i) impaired energy production caused by interrupting the flow of the TCA cycle and (ii) potential secondary enzyme inhibition associated with accumulation in various amounts of metabolites proximal to the enzyme deficiency such as fumarate, succinate, 2-KGA and citrate (Fig. 12.1).

12.6.3 Genetics

Fumarase deficiency is inherited as an autosomal recessive trait. A single gene, mapped to chromosome 1q42.1, and the same mRNA, encode alternately translated transcripts to generate a mitochondrial and a cytosolic isoform [60]. A variety of mutations have been identified in several unrelated families [60–63]. Prenatal diagnosis is possible by fumarase assay and/or mutational analysis in CVS or cul-

tured amniocytes [62]. Heterozygous mutations in the fumarase gene are associated with a predisposition to cutaneous and uterine leiomyomas and to kidney cancers [64].

12.6.4 Diagnostic Tests

The key finding is increased urinary fumaric acid, sometimes associated with increased excretion of succinic acid and 2-KGA. Mild lactic acidosis and mild hyperammonemia can be seen in infants with fumarase deficiency, but generally not in older children. Other diagnostic indicators are an increased lactate in CSF, a variable leucopenia and neutropenia.

Fumarase can be assayed in mononuclear blood leukocytes, cultured skin fibroblasts, skeletal muscle or liver, by monitoring the formation of fumarate from malate or, more sensitively, by coupling the reaction with malate dehydrogenase and monitoring the production of NADH [58].

12.6.5 Treatment and Prognosis

There is no specific treatment. While removal of certain amino acids that are precursors of fumarate could be beneficial, removal of exogenous aspartate might deplete a potential source of oxaloacetate. Conversely, supplementation with aspartate or citrate might lead to overproduction of toxic TCA cycle intermediates.

12.7 Succinate Dehydrogenase Deficiency

12.7.1 Clinical Presentation

Succinate dehydrogenase (SD) is part of both the TCA cycle and the respiratory chain. This explains why SD deficiency resembles more the phenotypes associated with defects of the respiratory chain. The clinical picture of this very rare disorder [65–69] can include: Kearns-Sayre syndrome, isolated hypertrophic cardiomyopathy, combined cardiac and skeletal myopathy, generalized muscle weakness with easy fatigability, and early onset Leigh encephalopathy. It can also present with cerebellar ataxia and optic atrophy and tumor formation in adulthood. Profound hypoglycemia was seen in one infant [70].

SD deficiency may also present as a compound deficiency state that involves aconitase and complexes I and III of the respiratory chain. This disorder, found only in Swedish patients, presents with life-long exercise intolerance, myoglobinuria, and lactic acidosis, with a normal or increased L/P ratio at rest and a paradoxically decreased L/P ratio during exercise [68].

12.7.2 Metabolic Derangement

SD is part of a larger enzyme unit, complex II (succinate-ubiquinone oxidoreductase) of the respiratory chain. Complex II is composed of four subunits. SD contains two of these subunits, a flavoprotein (Fp, SDA) and an iron-sulfur protein (Ip, SDB). SD is anchored to the membrane by two additional subunits, C and D. SD catalyzes the oxidation of succinate to fumarate (■ Fig. 12.1) and transfers electrons to the ubiquinone pool of the respiratory chain.

Theoretically, TCA-cycle defects should lead to a decreased L/P ratio, because of impaired production of NADH. However, too few cases of SD deficiency (or other TCA-cycle defects) have been evaluated to determine whether this is a consistent finding. Profound hypoglycemia, as reported once, might have resulted from the depletion of the gluconeogenesis substrate, oxaloacetate [70]. The combined SD/aconitase deficiency found only in Swedish patients, appears to be caused by a defect in the metabolism of the iron-sulfur clusters common to these enzymes [69].

12.7.3 Genetics

Complex II is unique among the respiratory chain complexes in that all four of its subunits are nuclear encoded. The flavoprotein and iron-sulfur-containing subunits of SD (A and B) have been mapped to chromosomes 5p15 and 1p35-p36, respectively, while the two integral membrane proteins (C and D) have been mapped to chromosomes 1q21 and 11q23. Homozygous and compound heterozygous mutations of SDA have been identified in several patients [67, 70–72]. In two sisters with partial SDA deficiency and late onset neurodegenerative disease with progressive optic atrophy, ataxia and myopathy, only one mutation was found, suggesting a dominant pattern of transmission [72].

Mutations in SDB, SDC or SDD cause susceptibility to familial pheochromocytoma and familial paraganglioma [73]. This suggests that SD genes may act as tumor suppression genes.

12.7.4 Diagnostic Tests

In contrast to the other TCA cycle disorders, SD deficiency does not always lead to a characteristic organic aciduria. Many patients, especially those whose clinical phenotypes resemble the patients with respiratory chain defects, do not exhibit the expected succinic aciduria and can excrete variable amounts of lactate, pyruvate, and the TCA cycle intermediates, fumarate and malate [70].

Diagnostic confirmation of a suspected SD deficiency requires analysis of SD activity itself, as well as complex-II (succinate-ubiquinone oxidoreductase) activity, which reflects the integrity of SD and the remaining two subunits

of this complex. These enzyme assays can be accomplished using standard spectrophotometric procedures. Magnetic resonance spectroscopy provides a characteristic pattern with accumulation of succinate [74].

12.7.5 Treatment and Prognosis

No effective treatment has been reported. Although SD is a flavoprotein, riboflavin-responsive defects have not been described.

12.8 Pyruvate Transporter Defect

12.8.1 Clinical Presentation

Only one patient has been completely documented [75]. Neonatal lactic acidosis in a female baby from consanguineous parents was associated with generalized hypotonia and facial dysmorphism. MRI of the brain revealed cortical atrophy, periventricular leukomalacia and calcifications. Progressive microcephaly, failure to thrive and neurological deterioration led to death at the age of 19 months. Selak et al. [76] described four patients with hypotonia, developmental delay, seizures and ophthalmological abnormalities and found decreased respiration rates in mitochondria with pyruvate, but not with other substrates, suggesting a decreased entry of pyruvate into the mitochondria.

12.8.2 Metabolic Derangement

The pyruvate carrier mediates the proton symport of pyruvate across the inner mitochondrial membrane. Consequently, the metabolic derangement should be the same as in pyruvate dehydrogenase deficiency.

12.8.3 Diagnostic Tests

As in PDHC deficiency, high lactate and pyruvate are found with normal lactate/pyruvate ratio. To evidence the transport defect, [2-^{14}C] pyruvate oxidation is measured in both intact and digitonin-permeabilized fibroblasts. Oxidation of [2-^{14}C] pyruvate is severely impaired in intact cells but not when digitonin allows pyruvate to bypass the transport step by disrupting the inner mitochondrial membrane.

12.8.4 Genetics

Chromosome localization and cDNA sequence of the pyruvate carrier is still unknown. Prenatal diagnosis on CVS can be done by the biochemical method [75].

12.8.5 Treatment and Prognosis

No treatment is known at this moment.

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