14 Disorders of Ketogenesis and Ketolysis

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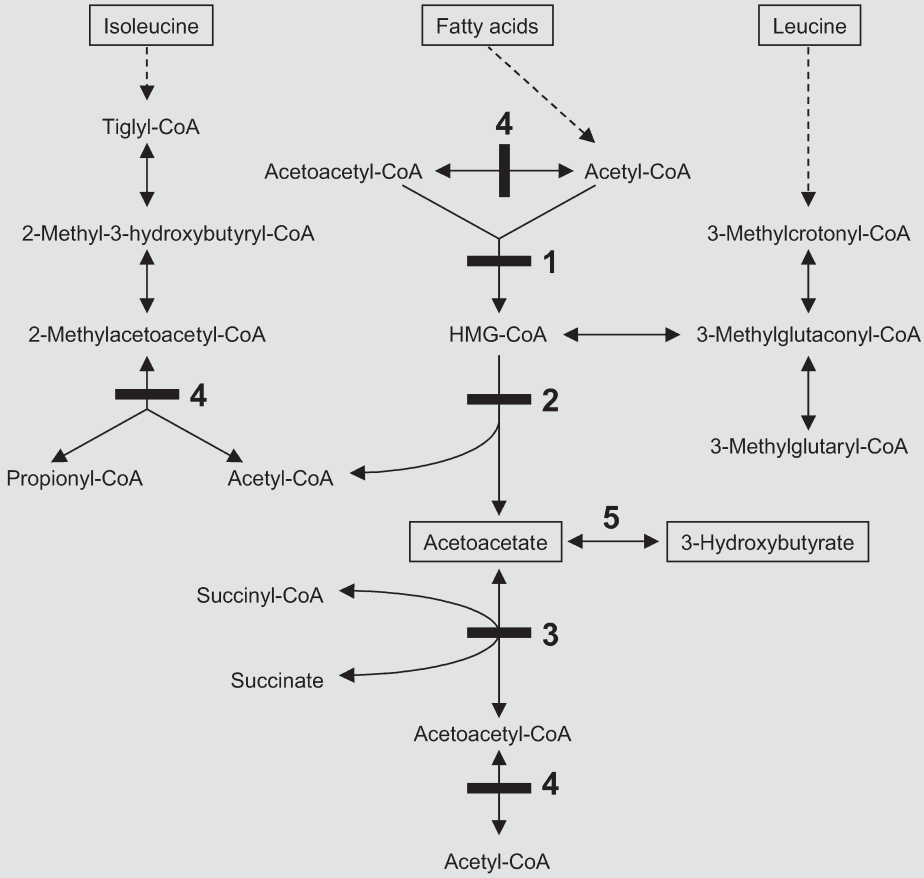
III

Ketogenesis and Ketolysis

During fasting, ketone bodies are an important fuel for many tissues, including cardiac and skeletal muscle. They are particularly important for the brain, which cannot oxidise fatty acids. The principal ketone bodies, acetoacetate and 3-hydroxybutyrate, are maintained in equilibrium by 3-hydroxybutyrate dehydrogenase; acetone is formed from acetoacetate non-enzymatically and eliminated in breath. Ketone bodies are formed in liver mitochondria, predominantly from fatty acids, but also from certain amino acids, such as leucine. For use as fuel, ketone bodies are converted to acetyl-CoA in the mitochondria of extrahepatic tissues. One of the ketolytic enzymes, mitochondrial acetoacetyl-CoA thiolase, is also involved in the breakdown of isoleucine (◘ Fig. 14.1).



◘ **Fig. 14.1.** Biochemical pathways involving enzymes of ketogenesis and ketolysis. *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A. **1,** Mitochondrial (m)HMG-CoA synthase; **2,** HMG-CoA lyase; **3,** succinyl-CoA 3-oxoacid CoA transferase (SCOT); **4,** mitochondrial acetoacetyl-CoA thiolase (T2); **5,** 3-hydroxybutyrate dehydrogenase. The enzyme defects discussed in this chapter are depicted by *solid bars* across the arrows

Disorders of ketone body metabolism present either in the first few days of life or later in childhood, during an infection or some other metabolic stress. In defects of ketogenesis, decompensation leads to encephalopathy, with vomiting and a reduced level of consciousness, often accompanied by hepatomegaly. The biochemical features – hypoketotic hypoglycaemia, with or without hyperammonaemia – resemble those seen in fatty acid oxidation disorders. In defects of ketolysis, the clinical picture is dominated by severe ketoacidosis. This is often accompanied by decreased consciousness and dehydration.

14.1 Clinical Presentation

14.1.1 Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency

Six patients with mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A (mHMG-CoA) synthase deficiency (enzyme 1 in ◘ Fig. 14.1) have been reported since 1997. All presented with hypoglycaemia following gastroenteritis, between the ages of 9 months and 6 years [1-3]. At presentation, most patients were comatose and had hepatomegaly, which subsequently resolved. Blood lactate and ammonia concentrations were normal and urine was negative for ketone bodies. All patients recovered promptly with intravenous glucose and none suffered long-term complications.

14.1.2 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency

Approximately 30% of patients with HMG-CoA lyase deficiency (enzyme 2 in ◘ Fig. 14.1) present by 5 days of age, after a short initial symptom-free period. Most of the other patients present later in the first year, when they are fasted or suffer infections [4]. A few patients remain asymptomatic for a number of years [5].

Clinical features at presentation include vomiting, hypotonia and a reduced level of consciousness. Investigations show hypoglycaemia and acidosis [4]. Ketone body levels are inappropriately low but blood lactate concentrations may be markedly elevated, particularly in neonatal onset cases [6]. Many patients have hyperammonaemia, hepatomegaly and abnormal liver function tests and, in the past, cases may have been misdiagnosed as Reye's syndrome [7]. Pancreatitis is a recognised complication [8], as in other branched-chain organic acidaemias. With appropriate treatment, most patients recover from their initial episode of metabolic decompensation. Unfortunately, a number suffer neurological sequelae, including epilepsy, intellectual handicap, hemiplegia or cerebral visual loss [4, 5, 7]. Imaging may show abnormalities in the basal ganglia and focal cerebral atrophy. Even in asymptomatic patients, magnetic resonance imaging (MRI) shows diffuse mild T_2 hyperintensity of the cerebral white matter with multiple foci of more severe signal abnormality [5]. The cause of these changes is unknown; myelination may be impaired because ketone bodies are a substrate for the synthesis of myelin cholesterol [9].

14.1.3 Succinyl-CoA 3-Oxoacid CoA Transferase Deficiency

The deficiency of succinyl-CoA 3-oxoacid CoA transferase (SCOT, also known as succinyl-CoA 3-ketoacid CoA transferase; enzyme 3 in ◘ Fig. 14.1) is characterised by recurrent episodes of severe ketoacidosis. Tachypnoea is often accompanied by hypotonia and lethargy. As for HMG-CoA lyase deficiency, 30 % of patients become symptomatic within a few days of birth [10] and most of the others present later in the first year. A few patients have had cardiomegaly at the time of presentation [11]. Blood glucose, lactate and ammonia concentrations are generally normal, though there have been reports of hypoglycaemia in neonates [10] and hyperglycaemia in older children [11]. Because ketosis and acidosis are common in sick children, SCOT deficiency enters the differential diagnosis for a large number of patients.

14.1.4 Mitochondrial Acetoacetyl-CoA Thiolase Deficiency

Recurrent episodes of ketoacidosis also occur in patients with a deficiency of mitochondrial acetoacetyl-CoA thiolase (also known as 2-methyl-acetoacetyl-CoA thiolase and as β-ketothiolase, abbreviated T2; enzyme 4 in ◘ Fig. 14.1) [12]. Neonatal onset is rare. Most patients present during the first two years but one asymptomatic adult has been diagnosed [13].

Episodes of decompensation generally start with tachypnoea and vomiting, which is followed by dehydration and a falling level of consciousness [12]. A few patients have seizures. Investigations show a severe metabolic acidosis with ketonuria. Blood glucose, lactate and ammonia concentrations are normal in most cases but hyper- and hypoglycaemia have been reported [11, 12]. The high acetoacetate levels in blood and urine may cause screening tests for salicylate to give false positive results [14]. Most patients make a full recovery from episodes of decompensation but some have mental retardation, ataxia or dystonia with abnormalities in the basal ganglia on MRI [15]. A few patients have developmental delay before their first episode of ketoacidosis [15].

14.2 Metabolic Derangement

Ketone bodies are synthesised in hepatic mitochondria, primarily using acetyl-CoA derived from fatty acid oxidation (◘ Fig. 14.1). mHMG-CoA synthase catalyses the condensation of acetoacetyl-CoA and acetyl-CoA to form HMG-CoA, which is cleaved by HMG-CoA lyase to release acetyl-CoA and acetoacetate. HMG-CoA can also be derived from the amino acid, leucine. Thus, mHMG-CoA synthase and HMG-CoA lyase deficiencies both impair ketogenesis but HMG-CoA lyase deficiency also causes the accumulation of intermediates of the leucine catabolic pathway. The hypoglycaemia seen in these defects may result from impaired gluconeogenesis or from excessive glucose consumption due to the lack of ketone bodies.

Ketone body utilisation occurs in extrahepatic mitochondria, starting with the transfer of coenzyme A from succinyl-CoA to acetoacetate, catalysed by SCOT. This forms acetoacetyl-CoA, which is converted to acetyl-CoA by T2. SCOT is not expressed in liver and has no role other than ketolysis; episodic ketoacidosis is, therefore, the only consistent biochemical abnormality in SCOT deficiency. In contrast, T2 is expressed in liver and has 3 different roles. Whereas T2 promotes ketolysis in extrahepatic tissues, in liver it participates in ketogenesis by converting acetyl-CoA to acetoacetyl-CoA. Finally, T2 cleaves 2-methylacetoacetyl-CoA in the degradation pathway for isoleucine. Patients with T2 deficiency present with ketoacidosis, implying that the enzyme is more crucial in ketolysis than in ketogenesis; they also excrete intermediates of isoleucine catabolism.

14.3 Genetics

All 4 disorders are inherited as autosomal recessive traits. Their prevalence is unknown but HMG-CoA lyase deficiency is relatively common in Saudi Arabia [6]. Mutations have been identified in patients with each of the 4 disorders [3, 12, 16]. Two common mutations have been found in HMG-CoA lyase deficiency, one in the Saudi population [17] and the other in Mediterranean patients [18]. Common mutations have not been identified in the other disorders.

Prenatal diagnosis is possible using molecular techniques in families where the mutations are known or there are informative polymorphisms [19, 20]. Alternatively, prenatal diagnosis can be performed by enzyme assay in chorionic villi or cultured amniocytes [19, 20]. This is not possible for mHMG-CoA synthase and some authorities prefer not to use chorionic villi for SCOT [21]. Organic acid analysis of amniotic fluid is a third method of prenatal diagnosis for HMG-CoA lyase deficiency [19].

14.4 Diagnostic Tests

HMG-CoA lyase and T2 deficiencies are diagnosed by detecting abnormal urinary organic acids. The abnormalities are sometimes hard to detect in T2 deficiency. The organic acid profile of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, another enzyme of the isoleucine degradation pathway (discusssed in ► Chap. 19), resembles that of T2 deficiency and cases have been misdiagnosed as such in the past. Recognition of the other defects is even more difficult and it is likely that many cases remain undiagnosed.

14.4.1 Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency

This diagnosis should be suspected when there is grossly impaired ketogenesis during fasting but there is normal fatty acid oxidation flux in vitro (► Chap. 3 and 13). Organic acid analysis typically shows massive dicarboxylic aciduria (saturated, unsaturated and hydroxy-compounds) but low levels of ketone bodies [2, 3]. Blood acylcarnitine analysis is normal. Measurement of enzyme activity requires a liver biopsy because it is only expressed at high levels in liver and testis. Moreover, enzyme assays are complicated by a cytoplasmic isoenzyme, involved in cholesterol synthesis, which normally accounts for approximately 10% of total activity. Enzymology has not been undertaken in recent patients and the diagnosis has been confirmed by mutation analysis [3].

14.4.2 3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency

Patients with this condition excrete increased quantities of 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids (◘ Fig. 14.1); 3-methylcrotonylglycine may also be present [4]. It is important to confirm the diagnosis enzymologically since a similar pattern of urinary organic acids has been found in patients with normal HMG-CoA lyase activity [22]. Assays can be undertaken on leukocytes or cultured fibroblasts as well as liver. Currently, the diagnosis is generally made following an acute presentation. There is, however, the potential to diagnose cases by neonatal screening, since 3-methylglutarylcarnitine accumulates in blood and can be detected by tandem mass spectrometry [23].

14.4.3 Succinyl-CoA 3-Oxoacid CoA Transferase Deficiency

Most patients have persistent ketonuria and a circulating concentration of ketone bodies (acetoacetate plus 3-hydroxybutyrate) above 0.2 mmol/l, even in the fed state [11]. A few patients do not have ketonuria when they are well. If a diagnostic fast is undertaken, there is an excessive rise in blood ketone body levels, sometimes to over 10 mmol/l, without hypoglycaemia [11]. Urinary organic acid analysis reveals high concentrations of 3-hydroxybutyrate, acetoacetate and sometimes 3-hydroxyisovalerate but no specific abnormalities. The diagnosis must, therefore, be confirmed by enzymology, which can be undertaken on lymphocytes or cultured fibroblasts. These assays generally show at least 20–35% apparent residual activity, due to the consumption of substrate by other enzymes [16].

14.4.4 Mitochondrial Acetoacetyl-CoA Thiolase Deficiency

Patients with T2 deficiency usually excrete increased amounts of 2-methylacetoacetate, 2-methyl-3-hydroxybutyric acid and tiglylglycine (◘ Fig. 14.1). Some patients, however, do not excrete tiglylglycine and 2-methylacetoacetate may not be detected because it is unstable. Indeed, the organic aciduria may be hard to detect even during episodes of ketoacidosis and an isoleucine load may be needed to demonstrate the abnormalities [24]. A similar organic acid picture can be seen in other disorders, such as 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, but 2-methyacetoacetate is not excreted in the latter disorder (Chap. 19). Enzymological confirmation in cultured fibroblasts is, therefore, essential. Assays are complicated by the presence of 3 other thiolases that act on acetoacetyl-CoA (cytosolic and peroxisomal acetoacetyl-CoA thiolases and mitochondrial 3-oxoacyl-CoA thiolase). 2-Methylacetoacetyl-CoA is a specific substrate for T2 but it is difficult to synthesise [25]. One solution is to measure acetoacetyl-CoA thiolysis in the presence and absence of potassium, which enhances the activity of T2 but not the other enzymes [14]. Blood acylcarnitine analysis in T2 deficiency often reveals tiglylcarnitine and other abnormalities but the sensitivity and specificity are not clear [23].

14.5 Treatment and Prognosis

All these patients can decompensate rapidly in early childhood. To prevent this, fasting must be avoided and a high carbohydrate intake must be maintained during any metabolic stress, such as surgery or infection (► Chap. 4). Drinks containing carbohydrate should be started at the first sign of illness; hospital admission is needed if these are not tolerated or if a patient with a ketolysis disorder develops moderate or heavy ketonuria. In hospital, patients require an intravenous infusion of glucose. Acidosis is common in HMG-CoA lyase deficiency and, particularly, in the ketolysis disorders. An intravenous infusion of bicarbonate is needed if the acidosis is severe (pH<7.1) and it may be given in milder acidosis but electrolytes must be monitored frequently: there is a risk of severe and potentially fatal hypernatraemia. Extra fluids may be needed to correct dehydration, which is common in the ketolysis disorders.

A moderate protein restriction is usually recommended in HMG-CoA lyase, SCOT and T2 deficiencies, because these enzymes are directly or indirectly involved in amino acid catabolism [4, 11, 12]. A low fat diet has also been recommended [26]. Protein and fat should certainly be avoided during illness. At other times, however, dietary restriction is unnecessary in some patients [13, 16]. Carnitine supplements are often given if serum levels are low, though their value is unproven.

Patients with these disorders can die or suffer irreversible neurological damage during episodes of metabolic decompensation. Outcomes have been least good for neonatal-onset cases of HMG-CoA lyase deficiency, such as those from Saudi Arabia [6]. Once the diagnosis has been made, the outlook is much improved. Patients become more stable with age, particularly those with ketolysis defects [12]. Late complications are rare. Fatal cardiomyopathy has been reported in one patient with HMG-CoA lyase deficiency [27] and two pedigrees with T2 deficiency, though the latter cases were not enzymologically proven [28]. One patient with T2 deficiency has had a healthy child following an uncomplicated pregnancy [29].

14.6 Cytosolic Acetoacetyl-CoA Thiolase Deficiency

Cytosolic acetoacetyl-CoA thiolase (CAT) is primarily involved in the synthesis of isoprenoid compounds, such as cholesterol, rather than ketone body metabolism. Two patients with CAT deficiency have been reported [30, 31]. Both presented with mental retardation after apparently normal early development. One patient developed severe ketoacidosis on a ketogenic diet, whilst the other had persistent ketonuria that resolved on a low fat diet. No treatment had any effect on the neurological problems. One patient had low acetoacetyl-CoA thiolase activity in the cytosolic fraction from a liver biopsy. Fibroblasts from the other patient showed reduced total acetoacetyl-CoA thiolase activity with normal T2 activity, and decreased cholesterol synthesis. The human CAT cDNA has been cloned but mutations have not been reported [32].

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