

Approach to the Child Suspected of Having a Disorder of Fatty Acid Oxidation

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William L. Nyhan, Stefan Kölker,
and Georg F. Hoffmann

Key Facts

- Hypoketotic hypoglycemia signifies a disorder of fatty acid oxidation. An absence of ketones in urine at the time of hypoglycemia is an important clue, but the presence of ketonuria may be misleading. Blood levels of free fatty acids and 3-hydroxybutyrate may be required to differentiate.
- The acylcarnitine (MS/MS) profile is a very helpful clue to the diagnosis.
- Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency resulting from the p.Ala985Gly mutation (ACADM gene) is so common that this test should be added to the initial work-up of a patient with hypoketotic hypoglycemia.

Initial presentation of disorders of fatty acid oxidation is usually with hypoketotic hypoglycemia. Hyperammonemia may suggest Reye syndrome but usually results from impaired synthesis of N-acetylglutamate due to the lack of acetyl-CoA. Sudden infant death syndrome (SIDS) is another acute presentation. Some patients present more chronically with myopathy or cardiomyopathy. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the only common disorder, and most patients have the same mutation. So a modern work-up may begin with assay of the DNA for the p.Ala985Gly mutation. Those not revealed in this way are now assayed by MS/MS for the acylcarnitine profile, and this may indicate the diagnosis and the appropriate enzymatic assay. Organic acid analysis should reveal the diagnosis in those with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. Those not elucidated by these measures are subjected to an algorithmic investigation, central to which is a prolonged monitored fast (Sect. 41.2.1) followed up by loading tests with specific lipids. Especially patients with 3-hydroxy-3-methylglutaryl-CoA synthase deficiency can be difficult to diagnose as in addition to hypoketotic hypoglycemia, there are no abnormal acylcarnitines and no specific elevations of organic acids.

Most patients with disorders of fatty acid oxidation present with fasting hypoglycemia which results from impaired ketogenesis (Chap. 15). The classic initial presentation is with hypoketotic hypoglycemia, usually at 6–12 months of age following a period of fasting of more than 12 h induced by vomiting or anorexia of an

W.L. Nyhan (✉)
Department of Pediatrics, University of California
San Diego, 9500 Gilman Drive, La Jolla, CA
92093-0830, USA
e-mail: wnyhan@ucsd.edu

S. Kölker • G.F. Hoffmann
Department of General Pediatrics,
University Children's Hospital,
Ruprecht-Karls-University Heidelberg,
Im Neuenheimer Feld 430, Heidelberg
69120, Germany
e-mail: stefan.koelker@med.uni-heidelberg.de;
georg.hoffmann@med.uni-heidelberg.de

intercurrent respiratory or gastrointestinal infection. The hypoglycemia may be manifest as lethargy or a seizure. It may progress rapidly to coma. There may be hyperammonemia, and this may lead to diagnosis of Reye syndrome. Liver biopsy in such a patient may reveal microvesicular fat, and this may seem to confirm the diagnosis of Reye syndrome. Actually most patients we see these days, in whom a diagnosis of Reye syndrome has been made, have a disorder of fatty acid oxidation. A few have a urea cycle defect. Orotic aciduria, a hallmark of ornithine transcarbamylase deficiency, has been seen acutely in disordered fatty acid oxidation.

The other major presentation is myopathic (see also Chap. 28). This may be acute with muscle pains and rhabdomyolysis, with or without hypoglycemia. It may be chronic with weakness and hypotonia. A major common presentation is with cardiomyopathy. The first manifestations in some patients are those of congestive failure. Arrhythmias are also common, especially in the acute episode of metabolic imbalance.

A third presentation is with the SIDS. One scenario has been to make a diagnosis of a disorder of fatty acid oxidation, most commonly MCAD deficiency, in an infant and to obtain a history of a previous infant dying of SIDS. We have obtained blood samples saved from newborn screening programs representing the previous infant and made a diagnosis of MCAD deficiency by DNA analysis. This type of posthumous diagnosis has also been made by assay for octanoylcarnitine. Disorders of fatty acid oxidation have also been detected in studies of sudden infant death by assay of postmortem liver for deficiency of carnitine and the presence of key organic acids by gas chromatography-mass spectrometry (GC-MS).

In the patient presenting with hypoglycemia, it is really helpful in suggesting the diagnosis if the test for ketones in the urine is negative. In this situation, hypoglycemia is clearly hypoketotic. However, these patients may be treated with parenteral glucose in the emergency room, and the first urine analysis done hours or days after the hypoglycemia has resolved, and so this clue to the diagnosis is not available. More commonly the diagnosis may be missed because ketones are found in the urine at the time of acute illness, in

particular in patients with deficient oxidation of short-chain fatty acids. These patients can readily be shown to be hypoketotic by analyzing the blood, but that does not exclude the possibility that the urine test for ketones may be positive. Documentation that hypoglycemia is hypoketotic is best done by quantification of the concentrations of free fatty acids, acetoacetate, and 3-hydroxybutyrate in the blood. This is most commonly done in the context of a controlled or monitored fast, because the stability of acetoacetate requires planning ahead for its analysis and is seldom considered at the time of the initial acute episode. Nevertheless, a good idea of the presence of hypoketosis can be obtained simply by the assay of 3-hydroxybutyrate and free fatty acids in the blood at the time of the acute hypoglycemic illness.

Remember

Do not be led away from a diagnosis of a disorder of fatty acid oxidation by the presence of ketones in the urine. Absence of ketones in a hypoglycemic patient is useful; their presence may be misleading.

Clues from the routine clinical chemistry laboratory that suggest the presence of a disorder of fatty acid oxidation are elevated levels of uric acid and creatine kinase (CK). Uric acid determinations are not regularly included in metabolic panels for pediatric patients, so it may have to be ordered separately and so may be the CK; levels over a 1000 U/L are commonly encountered on presentation. Alanine and aspartate aminotransferase levels may also be elevated. Analysis of organic acids in the urine may reveal dicarboxylic aciduria, and its pattern may provide direction as to the site of the enzymatic defect. During intervals between episodes of illness, these patients usually appear completely well. Furthermore abnormalities such as the dicarboxylic aciduria and elevations of uric acid and CK usually disappear completely. The patient is most often seen first in consultation after the initial hypoglycemia has been treated, and none of the abnormalities seen in the acute situation are present. Therefore, it has become important to develop a systematic algorithmic approach to the work-up (Fig. 16.1). In a

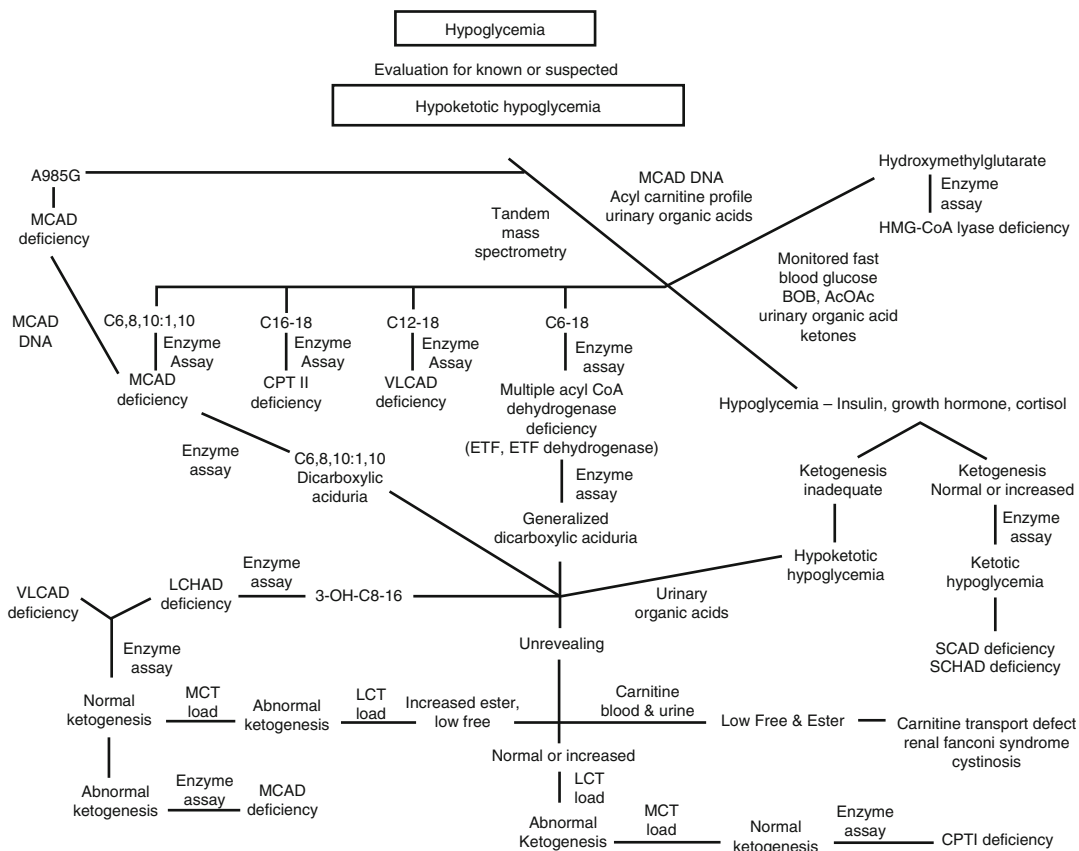


Fig. 16.1 An algorithmic approach to the work-up of a child with a possible disorder of fatty acid oxidation. *MCAD* medium-chain acyl-CoA dehydrogenase, *DNA* deoxyribonucleic acid, *CPT* carnitine palmitoyltransferase, *VLCAD* very long-chain acyl-CoA dehydrogenase, *ETF* electron transfer flavoprotein, *BOB* 3-hydroxybutyric

acid, *AcOAc* acetoacetic acid, *HMG* 3-hydroxy-3-methylglutaric acid, *SCAD* short-chain acyl-CoA dehydrogenase, *SCHAD* short-chain hydroxyacyl-CoA dehydrogenase, *MCT* medium-chain triglycerides, *LCT* long-chain triglycerides, *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase

patient suspected of having a disorder of fatty acid oxidation, the algorithm starts with an assay of the DNA from a blood sample for the common mutation in MCAD deficiency, assay of the blood for an acylcarnitine profile, and assay of the organic acids of the urine.

MCAD deficiency is the only common disorder of fatty acid oxidation. Its frequency has been estimated at 1 in 6–10,000 Caucasians. Most patients have the same mutation, an A985G change which makes for a protein containing glutamic acid where a lysine is found in the normal enzyme. So this simple DNA-diagnostic approach can be expected to yield a rapid diagnosis in a large number of the patients with this group of

disorders. The acylcarnitine profile obtained by MS/MS can also detect MCAD deficiency; in this instance, octanoylcarnitine is the key compound; hexanoylcarnitine may be present as well. In a patient negative for the p.Ala985Gly mutation and positive in the acylcarnitine assay, enzyme analysis will document MCAD deficiency. In that case, it might be useful to test for the 4 bp deletion, for which there is a rapid test, as there is for p.Ala985Gly and which with p.Ala985Gly accounts for 93% of the MCAD mutations seen in patients presenting with illness.

Patients detected by newborn screening also have another common mutation p.Thr199Cys, and these patients seldom develop clinical symptomatology.

Acylcarnitine assay may also point to enzyme assay for carnitine palmitoyltransferase (CPT) I and II deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, or multiple acyl-CoA dehydrogenase (MAD) deficiency. Acylcarnitine profiles are obtained by MS/MS, which can be done on as little as a drop of dry blood.

Organic acid analysis can be expected to reveal the presence of 3-hydroxy-3-methylglutarate (HMG) in the presence of HMG-CoA lyase deficiency. This compound is abundant in the urine of affected patients even after recovery from the acute hypoglycemic episode. It has already been indicated that in most of the other disorders organic acid analysis is more often normal than abnormal in intervals between episodes of acute illness.

In some patients, essentially all of those not elucidated by the tests of the last three paragraphs, a controlled prolonged fast is necessary to document that the hypoglycemia really is hypoketotic and to elucidate the nature of the defect. As fasting is not only unpleasant but can be very dangerous in disorders of fatty acid oxidation, it is mandatory that carnitine status and acylcarnitine profile are reliably negative before planning a fasting study. In response to this long fast, the body's first step is lipolysis which releases free fatty acids. In patients with disorders of fatty acid oxidation, concentrations of free fatty acids are higher than those of 3-hydroxybutyrate in the blood when hypoglycemia develops. In addition fatty acids that accumulate in the presence of defective oxidation undergo Ω -oxidation to dicarboxylic acids giving an elevated ratio of dicarboxylic acids to 3-hydroxybutyrate in the analysis of organic acids of the urine. The nature of the dicarboxylic aciduria at the time the hypoglycemia develops may indicate the site of the defect. Thus, C8- to C10-dicarboxylic aciduria is seen in MCAD deficiency and 3-hydroxy long-chain acids in LCHAD deficiency.

Patients during the long fast must be monitored closely so that symptomatic hypoglycemia is avoided. Testing is best done in units where the staff has experience with the protocol. An intravenous line is placed to ensure access for therapeutic glucose, and bedside monitoring of blood concentrations of glucose is done at regular inter-

vals. In abnormalities of fatty acid oxidation, fasting must be long enough to exhaust stores of glycogen and require the mobilization of fat and its oxidation.

Study of the concentrations of carnitine in the plasma and the urine and its esterification may point to the answer, particularly if a low level of free carnitine is documented in the blood and large amounts of esters are being excreted in the urine. Transport of long-chain fatty acids into the mitochondria, where β -oxidation takes place, requires carnitine, and the entry of carnitine into cells such as muscle requires a specific transporter which may be deficient as a cause of hypoketotic hypoglycemia. Assay of carnitine in the blood and urine reveals very low levels of free and esterified carnitine in these patients.

In patients in whom the blood and urine carnitine is normal or increased, a long-chain triglyceride (LCT) load may reveal abnormal ketogenesis, and MCT load normal ketogenesis. In such patients, MCT administration may even reverse fasting-induced hypoglycemia. In such patients, enzyme assay reveals the deficiency of carnitine palmitoyltransferase (CPT I). Esterification of carnitine with fatty acyl-CoA esters is catalyzed by acyltransferases, such as CPT I. The transport of acylcarnitines across the mitochondrial membrane is catalyzed by carnitine acylcarnitine translocase, and then hydrolysis, releasing free carnitine and the fatty acid acyl-CoA, is catalyzed by a second acyltransferase, CPT II. Inborn errors are known for each of these three enzymatic steps.

When the carnitine ester level of the urine is high and the free carnitine level of the blood is low, the basic problem is one in which the metabolic block causes the accumulation of acyl-CoA compounds which are esterified with carnitine and excreted in the urine, particularly in disorders of β -oxidation.

Low plasma-free carnitine may be seen in defective carnitine transporter or may be secondary to any condition in which acyl-CoA esters accumulate. These include the disorders of fatty acid oxidation and the organic acidurias (see Chap. 13). Elevation in the level of urinary esterified carnitine is also seen in both latter sets of conditions. Especially patients with 3-hydroxy-3-

methylglutaryl-CoA synthase deficiency can be difficult to diagnose as in addition to hypoketotic hypoglycemia there are no abnormal acylcarnitines and no specific elevations of organic acids. Molecular analysis then confirms the diagnosis.

Remember

In β -oxidation, the fatty acid is successively shortened by two carbons, releasing acetyl-CoA. Specific dehydrogenases with overlapping specificities for chain length include short-chain acyl-CoA dehydrogenase (SCAD), MCAD, and VLCAD. In addition, a trifunctional enzyme catalyzes long-chain 3-hydroxyacyl-CoA dehydrogenation (LCHAD), long- and medium-chain 2-enoyl-CoA hydration, and 3-oxoacyl-CoA thiolysis. Diseases involving defects in each of these steps have been defined. The last two known defects leading to hypoketotic hypoglycemia are HMG-CoA synthetase and HMG-CoA lyase deficiencies, the enzymes producing ketone bodies from acetyl-CoA. Whereas the latter usually constantly shows a characteristic organic aciduria and often elevated C5-OH-acylcarnitine, the first one is very difficult to spot as organic acid analysis is nonspecific and acylcarnitines normal. In this constellation, enzyme analysis of the liver or direct mutation analysis will provide the diagnosis. All of these patients would be expected to have abnormal ketogenesis following an LCT load. When these patients are tested with MCT, MCAD patients display abnormal ketogenesis, while those with LCAD, LCHAD and VLCAD deficiency have normal ketogenesis. The follow-up of this testing is via assay for the specific enzyme or enzymes or molecular testing as suggested in Fig. 16.1.

The specific enzyme assays for specific disorders are technically demanding and not gener-

ally available. A reasonable step following the fast, if a specific disease is not identified, is to pursue a more general study of metabolism in cultured cells in which oxidation of fatty acids of varying chain length is studied in vitro, and carnitine esters are separated and identified after incubation with ^{14}C - or ^{13}C -labeled long-chain fatty acids such as hexadecanoate. Impaired oxidation of long-chain fatty acids such as palmitate in vitro may also be seen in patients with mitochondrial disorders (see Chap. 14). Such patients may also have hypoketotic hypoglycemia with increased levels of 3-hydroxydicarboxylic acids because of failure to oxidize the NADH produced in the 3-hydroxyacyl-CoA dehydrogenase step via the electron transport chain. Such patients usually display lactic acidemia.

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