Acylcarnitines in fibroblasts of patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and other fatty acid oxidation disorders


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Summary: Mitochondrial fatty acid oxidation disorders cause hypoglycaemia, hepatic dysfunction, myopathy, cardiomyopathy and encephalopathy. Despite their recognition for more than 15 years, diagnosis and treatment remain difficult. To help design rational diagnostic and therapeutic strategies, we studied the pathophysiology of accumulating metabolites in a whole-cell system. Acylcarnitines were quantified in cells and media of cultured fibroblasts after incubation with l-carnitine and fatty acids. Following incubation with palmitate, long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD)-deficient fibroblasts compared with controls showed elevation of hydroxypalmitoyl- and palmitoyl-carnitine and reduction of and shorter acylcarnitines. Following incubation with linoleate an increase in and C\textsubscript{14}\textgreater\textsubscript{2}, C\textsubscript{18}\textgreater\textsubscript{2} hydroxy-C\textsubscript{18}\textgreater\textsubscript{2} and reduction in hydroxyacylcarnitines acylcarnitines C\textsubscript{10}\textsubscript{>1}. Incubation with decanoate and octanoate showed absence of hydroxylated acylcarnitines and correction of secondary metabolic disturbances, suggesting that optimal treatment should include medium-chain triglycerides of these chain lengths. Fibroblasts of patients with other fatty acid oxidation disorders showed distinct elevations of disease-specific acylcarnitines. This acylcarnitine analysis allows the diagnosis of LCHAD deficiency and its differentiation from other fatty acid oxidation disorders, which can pose difficulties in vivo. The strategy has allowed in-depth analysis with different substrates, providing suggestions for the rational design of treatment trials.
Mitochondrial fatty acid β-oxidation plays an important role in energy production, particularly in skeletal muscle and heart, and in hepatic ketone body formation. Disorders of fatty acid oxidation cause hypoglycaemia, hepatic dysfunction, encephalopathy, skeletal myopathy and cardiomyopathy (Roe and Coates 1995). More than a dozen disorders in the fatty acid oxidation pathway have been recognized, including medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (McKusick 201450), very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency (McKusick 201475), carnitine palmitoyltransferase II (CPT-II) deficiency (McKusick 255110), and multiple acyl-CoA dehydrogenase deficiency such as that caused by electron transfer flavoprotein (ETF) deficiency (McKusick 231680) and ETF dehydrogenase deficiency (McKusick 231675). Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (McKusick 143450) is recognized with increasing frequency, and is one of the most common disorders of mitochondrial fatty acid oxidation. This disorder is unique among the fatty acid oxidation disorders because, in addition to the above symptoms, patients have more pronounced and consistent lactic acidosis, and may develop cholestasis, pigmentary retinopathy and peripheral neuropathy (Pons et al 1996; Roe and Coates 1995; Tyni et al 1997). During pregnancy, mothers of affected infants can develop acute fatty liver of pregnancy or the hypertension, elevated liver enzymes and low platelets (HELLP) syndrome (Treem et al 1996).

The LCHAD enzyme is an inner mitochondrial membrane-associated heterooctamer of α₄β₄ configuration. The α-chain has 2-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, and the β-chain has 3-ketoacyl-CoA thiolase activity. Hence, the complex is a mitochondrial trifunctional enzyme. Most patients have a deficiency of 3-hydroxyacyl-CoA dehydrogenase activity, with mild reduction in the activities of the hydratase and the thiolase, and are classified as isolated LCHAD deficient. A single missense mutation 1528G > C in the α-chain is present in a large proportion of the alleles of patients with isolated LCHAD deficiency (Ijlst et al 1996). A few other patients have a severe deficiency of multiple enzyme activities, and are classified as mitochondrial trifunctional enzyme deficient.

Early and accurate diagnosis of these disorders provides information for dietary and medical treatment, thus avoiding severe clinical sequelae. Most fatty acid oxidation disorders have a rather similar clinical presentation, and apart from MCAD deficiency their biochemical diagnosis can be frustratingly difficult. Commonly used metabolite screens such as organic acids, acylcarnitines (Millington and Chace 1992; Van Hove et al 1997) and plasma fatty acids (Costa et al 1998) are influenced by dietary factors and the clinical status of patients, and often provide incomplete diagnostic information for disorders of long-chain fatty acid oxidation. Molecular assays for common mutations are limited by the frequent occurrence of compound heterozygotes with an uncommon private mutation that must be distinguished from unaffected heterozygotes.

Mitochondria have several enzymes of overlapping substrate activity for the oxidation of fatty acids (Eaton et al 1996; Roe and Coates 1995). The impact in the whole cell of a deficiency in a single enzyme on the metabolism of fatty acid substrates is incompletely predicted by knowledge of the substrate specificity of the