Mitochondrial very long chain acyl-CoA dehydrogenase deficiency – a new disorder of fatty acid oxidation

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Abstract
Very long chain acyl-CoA dehydrogenase is a newly characterised enzyme in mitochondrial fatty acid oxidation. A girl who presented on the second day of life with a sudden and severe illness due to deficiency of this enzyme is reported. There is evidence that some children (and perhaps all) originally diagnosed with a deficiency of long-chain acyl-CoA dehydrogenase, in fact, have a defect involving very long chain acyl-CoA dehydrogenase.

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To date 11 inherited defects of mitochondrial fatty acid oxidation have been described. Patients often have fasting hypoglycaemia and in some cases muscular disease or cardiomypathy. A new acyl-CoA dehydrogenase, located in the inner membrane of rat liver mitochondria, has been described by Izai et al.1 This enzyme was named ‘very long chain acyl-CoA dehydrogenase’ (VLCAD) because of substantial activity towards long chain and very long chain fatty acyl-CoA esters (C14–C22). VLCAD has also been found in human fibroblasts by Kelley.2 We report clinical and laboratory data on a patient with this enzyme deficiency; the enzymatic data have been published elsewhere.3

Biochemical investigation indicated metabolic acidosis (pH 7.19; bicarbonate 4 mmol/L), raised lactic acid of 16.7 mmol/L, and increased creatinine kinase of 3684 U/l (controls <100 U/l). Blood glucose was not measured before intravenous administration of glucose. Plasma total carnitine was normal (68 μmol/l with a moderately decreased free-total carnitine ratio at 0.44) (controls: 0.70–0.95). Urinary organic acid examination (GC/MS) showed a dramatic dicarboxylic aciduria, with only a modest amount of 3-hydroxybutyrate, suggesting a possible mitochondrial fatty acid oxidation defect.

After correction of metabolic acidosis with bicarbonate and treatment with intravenous L-carnitine (100 mg/kg/d), she improved and a low fat (10% of total caloric intake) diet was started. Hepatomegaly progressively disappeared and neurological status slowly returned to normal within four weeks. Echocardiography did not reveal any abnormality. At 1.5 months of age, she was taking five feeds a day and at 5 months of age could tolerate a normal 10 hour night fast, with normoglycaemia on waking. The patient presented no further episode of decompensation despite several febrile illnesses with consequent anorexia. At 3 years of age, she was well, with normal growth and psychomotor development while following a low fat diet (20% of total caloric intake) and oral L-carnitine supplementation (100 mg/kg bodyweight/day). Her heart was normal on clinical, electrocardiographic, and echographic examination. Ophthalmological investigations (fundus, visual evoked potentials, and electroretinograms) were performed to exclude the retinal abnormalities which have been associated with long chain 3-hydroxyacyl-CoA dehydrogenase deficiency. These yielded normal results as did muscle examination and measurement of blood creatine kinase concentrations.

Analysis of urinary organic acids was performed by combined gas chromatography-mass spectrometry after extraction with ethylacetate and trimethylsilyl derivatisation. 14C-labelled fatty acid oxidation studies using [1-14C]octanoate and [1-14C]palmitate, and acyl-CoA dehydrogenase activities (medium chain, long chain, and very long chain acyl-CoA dehydrogenases) were determined in skin fibroblasts, as described before.3

Case report
The patient, a girl, was the second child of healthy unrelated French parents. Their first child, also female, died suddenly at 2 days of age. At necropsy, massive hepatic steatosis was the only finding. The heart was normal on macroscopic and microscopic examination. No biochemical investigation was carried out.

The second child was born after an uneventful pregnancy and delivery. Birthweight was 3300 g, length 48 cm, and head circumference 35 cm. Breast feeding was begun three hours after birth. During the 46th hour of life, she became lethargic and refused to feed. Shortly thereafter, she experienced respiratory arrest and ventricular fibrillation. After resuscitation, substantial hepatomegaly was noted (palpable 6 cm below the right costal margin). In the next 12 hours, she had two generalised seizures, and neurological examination showed severe hypotonia with pyramidal tract signs.
3-hydroxydodecanedioic acid; 2-hydroxy n-butyric acid; (2) 2-hydroxy n-butyric acid; (3) 3-hydroxy n-butyric acid; (4) uric acid; (5) fumaric acid; (6) 2-phenylbutyric acid (internal standard) and 5-hydroxycaproic; (7) 3-methylfumaric acid; (8) adipic acid; (9) 5-hydroxymethyl-2-furan carboxylic acid; (10) 2-hydroxyglutaric acid; (11) pimelic acid; (12) 4-hydroxyphenylacetic acid; (13) cis-4-octene dicarboxylic acid; (14) suberic acid; (15) cis-aconitic acid and homoaconitic acid; (16) aspartic acid; (17) hippuric acid; (18) citric acid and cis-4-decenedic acid; (19) sebacic acid; (20) 4-hydroxyphenylacetic acid; (21) hydroxyhippuric acid; (22) 3-hydroxydecanedioic acid; (23) dodecanedioic acid and 3-hydroxydecanedioic acid; (24) 3-hydroxydodecanedioic acid.

determination in urine collected over six hours after the load. Investigations carried out at 2-5 years of age, after 15 days without oral L-carnitine, included fasting and long chain triglyceride loading tests (1.5 g/kg sunflower oil given after an overnight fast).

Results
At the time of neonatal decompensation, urinary organic acids (fig 1) showed a massive dicarboxylic aciduria with adipic acid (2276 mmol/mmol creatinine), suberic acid (562 mmol/mmol creatinine), sebacic acid (318 mmol/mmol creatinine) and dodecanedioic acid (140 mmol/mmol creatinine). Urinary 3-hydroxybutyric acid was 694 mmol/mmol creatinine. The chromatographic profile became normal after a few days. Repeated urinary organic acid profiles subsequently remained normal except during the fasting and the long chain fatty acid loading tests.

The phenylpropionate loading test did not lead to excretion of abnormal metabolites, thus excluding a diagnosis of medium chain acyl CoA dehydrogenase deficiency.

Stopping L-carnitine supplements before the fasting test led to a decrease in plasma carnitine (total 29 μmol/l, free 11 μmol/l). The fasting test (fig 2) was stopped after 20 hours because of drowsiness and acidosis. At that time, she was moderately hypoglycaemic (2.89 mmol/l). In spite of an increased concentration of free fatty acids (3.64 mmol/l), ketone bodies (3-hydroxybutyrate and acetocetate) did not rise as expected (0.349 mmol/l). Unexpectedly during the fast lactate increased to 8.48 mmol/l by 20 hours with a normal lactate:pyruvate ratio (16:6). Creatine kinase increased from 102 U/l to 1086 U/l by 20 hours.

A long chain triglyceride loading test showed no significant increase in plasma ketone bodies (from 0.268 to a maximum of 0.357 mmol/l at one hour).

[1-14C]-palmitate oxidation in intact skin fibroblasts was reduced to 56% of control values, whereas oxidation of medium chain fatty acids ([1-14C]-octanoate) was normal. Activity of matrix long chain acyl-CoA dehydrogenase (LCAD) was normal. Very long chain acyl-CoA dehydrogenase activity, performed on membrane preparation of skin fibroblasts, showed greatly decreased activity to 4% of the control value.

Discussion
We have described a child with a new disorder of mitochondrial fatty acid oxidation due to a deficiency of very long chain acyl CoA dehydrogenase activity. She presented with a typical neonatal clinical picture of fatty acid oxidation disorder, and dicarboxylic aciduria was compatible with this type of disease.

[1-14C]-palmitate oxidation analysis performed on skin fibroblasts and the results of the long chain triglyceride loading test suggested a diagnosis of long chain fatty acid oxidation defect. Unexpectedly, matrix LCAD activity was normal. The description of a new enzyme, located in the inner mitochondrial membrane and involved in long chain fatty acid oxidation, led us to perform the determination of the membrane-bound LCAD activity (VLCAD), which proved to be greatly reduced.

Metabolic investigations showed absence of severe hypoglycaemia during a prolonged fast. However, fasting led to an increase in plasma pyruvate, indicating subclinical muscular involvement. Lactic acidosis is not a common finding in patients with mitochondrial β-oxidation defects but has been reported in some fatty acid oxidation defects. The mechanism of this lactic acidosis is unknown: Jackson postulated inhibition of pyruvate dehydrogenase by metabolic intermediates of fatty acid oxidation or inhibition of the mitochondrial respiratory chain. In the latter case, the lactate:pyruvate ratio should be increased and this was not the case in our patient.

Other investigators have reported several cases of VLCAD deficiency. The patients presented with hypoketotic hypoglycaemia, hepatocellular dysfunction, cardiomyopathy or muscular problems. All of these clinical symptoms are commonly reported in other types of mitochondrial fatty acid oxidation. VLCAD deficiency is probably clinically indistinguishable from other types of β oxidation defects.
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particularly from LCAD. Patients with a fibroblast long chain fatty acid oxidation defect but without LCAD deficiency should be tested for very long chain acyl-CoA deficiency. Furthermore, the three patients with VLCAD reported by Yamaguchi et al. had been previously reported as having LCAD deficiency. Usually, the diagnosis of LCAD deficiency is carried out by measuring palmitoyl-CoA dehydrogenation in the supernatant fluid of sonicated fibroblasts. The assay is supposed to be specific for matrix soluble LCAD activity. According to Izai et al., the association between membrane-bound VLCAD and the membrane may not be very close. During the LCAD assay, possible contamination of the supernatant fluid by membrane bound palmitoyl-CoA dehydrogenase activity (VLCAD) could have explained the inaccurate diagnosis of LCAD deficiency in some patients.

Moreover, as reported by Aoyama et al., matrix LCAD activity in skin fibroblasts is responsible for only 0–4% of the total palmitoyl-CoA dehydrogenation. The study of labelled long chain fatty acid oxidation on intact skin fibroblasts is likely to show normal results in the truly LCAD deficient patients. Patients with previously diagnosed LCAD deficiency should be reinvestigated for VLCAD deficiency.

The respective role of matrix LCAD and membrane VLCAD in mitochondrial fatty acid oxidation remains to be determined. From studies performed in fibroblasts, LCAD seems to have a minor physiological role but further investigations are necessary in tissues with a high fatty acid oxidation requirement, such as liver or heart.

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