Medium chain acyl-CoA dehydrogenase deficiency

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Abstract
From 65 reported cases of medium chain acyl-CoA dehydrogenase deficiency, we found an average presenting age of 13.5 months and a mean age at death of 18.5 months, one quarter of patients died of a Reye-like syndrome and/or sudden infant death. In half the cases there had been at least one sibling death. Asymptomatic cases were not uncommon (12% of cases). The crises were generally induced by a prolonged fast and after a viral prodromal phase in three quarters of cases. The crises consisted of somnolence progressing to lethargy which could lead to coma. Vomiting was frequent (60% of cases). Seizures, which were found in 29% of cases, represented a bad prognosis. The physical examinations revealed frequently a variable and regressive anicteric hepatomegaly.

Blood and urine analysis revealed in most instances hypoglycaemia (96% of cases) with hypoketonuria and sometimes metabolic acidosis. Hepatic and muscular cytolytic enzymes were frequently raised, as were plasma ammonia, urea, and uric acid. Plasma total or free carnitine concentrations, especially non-fasting, were diminished in most cases. Plasma saturated medium chain fatty acids and particularly unsaturated cis-4-decenoye were on the other hand raised during the crises or during fasting. Urinary organic acid analysis revealed a characteristic profile of medium chain aciduria: C₆-C₁₀ dicarboxylic acids, hydroxy acids, glycin conjugates, and carnitine conjugates. Oral loading tests with carnitine or phenylpropanonate allow a precise diagnosis. The diagnosis is confirmed by specific assays in various tissues. Avoidance of prolonged fasting seems to be the mainstay of treatment.

Fatty acid oxidation plays an important part in maintaining energy homoeostasis during fasting. Deficiency of medium chain acyl-CoA dehydrogenase (M-CAD) had recently recognised as the most common hereditary disease of hepatic fatty acid oxidation and one of the commonest inborn errors of metabolism. The incidence is estimated at 1/20 000 newborn infants and more than 85 patients have now been reported, in 65 of whom the diagnosis has been confirmed enzymatically. This enzymatic deficit was first suspected in 1976 and was initially reported as 'hypoketotic hypoglycaemia with C₆-C₁₀ dicarboxylic aciduria' related to fasting. M-CAD deficiency has been reported in Reye-like syndromes. It is also recognised in familial and recurrent Reye's syndrome, particularly in the first two years of life. M-CAD deficiency has been demonstrated in some cases of sudden infant death syndrome (SIDS). Disease entities previously reported as systemic carnitine deficiency have recently been demonstrated to be cases of M-CAD deficiency.

The specific diagnosis of M-CAD deficiency was initially made in patients with an acute onset of the disease; although more difficult, the diagnosis is now made in asymptomatic children. The diagnosis has been mainly based on plasma and urinary organic acid analysis by means of gas chromatography and mass spectrometry (GC-MS). The outcome may be fatal in the absence of appropriate therapeutic management.

In this general survey of the published patients with M-CAD deficiency, we discuss the principal features of this recently recognised enzymatic defect.

Results and discussion

CLINICAL FEATURES (table 1)
The average age for the first presenting episode in M-CAD deficiency was 13.5 months and varied between 2 months and 4 years. There was an equal distribution between the sexes (27 males and 26 females). The mortality rate was one quarter of the studied cases. Roe et al found the mortality rate as high as 60% for a first episode between the ages of 15 and 26 months. The mean age at death was 18.5 months. The Reye-like syndrome and/or SIDS were the causes of death. In half the families studied, one or more previous sibling deaths had occurred.

Table 1. Clinical information in 65 M-CAD deficient patients

<table>
<thead>
<tr>
<th>Patient's history</th>
<th>Sex distribution (M/F)</th>
<th>Mean age of first episode (months)</th>
<th>Mortality rate</th>
<th>Presenting symptoms and signs</th>
<th>%</th>
<th>Range (months)</th>
</tr>
</thead>
</table>
Medium chain acyl-CoA dehydrogenase deficiency

The main presenting symptom of M-CAD deficiency was somnolence in 100% of symptomatic cases, progressing to lethargy which could lead to coma. This symptom was induced by fasting for longer than 12 hours. A viral prodromal episode, comprising mainly digestive and respiratory symptoms, was found in three quarters of the cases studied. Vomiting was frequent (60%). Seizures, which were less frequent (30%), indicated a poorer prognosis: six deaths and two cases of epilepsy were noted among the 14 patients with seizures. Frank hepatomegaly was noted in 38% of cases. Asymptomatic cases were not uncommon (12%).

It is important to note the absence of any delay in psychomotor development and growth, and the absence of clinical myopathic manifestations in the heart and skeletal muscles, such as frequently encountered in long chain acyl-CoA dehydrogenase deficiency.23 Previous acute crises and recurrences are frequent in M-CAD deficiency.20

PLASMA FEATURES (table 2)

During the crises, hypoglycaemia was constantly present (96%). Plasma β-hydroxybutyrate was disproportionately low given the hypoglycaemia, thus defining a hypoketotic hypoglycaemic state. M-CAD deficiency was characterised by a deficient ketogenesis, demonstrated by an abnormal (high) free fatty acids:ketone bodies ratio during fasting in all analysed cases. Moderate metabolic acidosis was found in 30% of the studied cases. Plasma transaminases concentrations were almost always raised. This increase has sometimes been progressive with the appearance of a secondary hepatomegaly.11 There was moderate hyperammonaemia in half the cases. Plasma uric acid, urea, lactate dehydrogenase, and creatine kinase were also frequently at high concentrations during the crises. The prothrombin time was sometimes diminished (3/7) and plasma bilirubin was normal.

M-CAD deficiency is generally characterised by secondary carnitine deficiency and abnormal high acylcarnitine:free carnitine ratios (table 3).26 In the fed state, free or total carnitine were almost always at low concentrations in the plasma, thus defining a permanent hypocarnitinaemic state. In acute crises, or in fasting tests, hypocarnitinæmia was found in 77% of cases, less frequently than in the fed remission state. In six untreated M-CAD deficient patients3 tissue carnitine concentrations in muscle and liver are about 25% of normal. The acylcarnitine:free carnitine ratio was abnormal in seven out of eight cases during the crises or fasting tests. This parameter seems to be the best indicator of symptomatic M-CAD deficiency. There was a constant high concentration of saturated octanoate and especially unsaturated cis-4-decanoate in plasma during crises or fasting tests. cis-4-Decanoate detection is considered to be pathognomonic of M-CAD deficiency and is not influenced by dietary supplementation with medium chain triglycerides.27

URINARY FEATURES (table 4)

Hypoketonuria was found in 85% of cases. The analysis of urinary organic acids by GC-MS during the acute episodes revealed a C₆-C₁₀ dicarboxylic aciduria (C₆ adipate, C₈ suberate, C₁₀ sebacate) and a monocarboxylic (omega-1) hydroxylated aciduria (5-hydroxyhexanoate). These metabolites are produced by the deviation of fatty acid oxidative metabolism towards alternative microsomal pathways. This urinary profile is characteristic of M-CAD deficiency.24

Specific metabolites of M-CAD deficiency (especially hexanoylglycine, suberylglycine, and phenylpropionylglycine) are found and quantified during the crises and remissions using recent sensitive assays, especially the stable

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Table 2 Plasma values at presentation in M-CAD deficient patients

<table>
<thead>
<tr>
<th>Investigation</th>
<th>No (%) with abnormal result</th>
<th>Range or value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>45/47 (96)</td>
<td>0-10-0.25</td>
</tr>
<tr>
<td>β-hydroxybutyrate × glucose (mmol/l)</td>
<td>5/5 (100)</td>
<td>0-17-3.70</td>
</tr>
<tr>
<td>Free fatty acid: β-hydroxybutyrate (fasting)</td>
<td>10/10 (100)</td>
<td>1-4-56</td>
</tr>
<tr>
<td>Carbon dioxide (mmol/l)</td>
<td>7/23 (30)</td>
<td>11-15</td>
</tr>
<tr>
<td>Mild acidosis</td>
<td>9/23 (39)</td>
<td>16-19</td>
</tr>
<tr>
<td>SGOT (U/I)</td>
<td>17/19 (90)</td>
<td>43-924</td>
</tr>
<tr>
<td>SGPT (U/I)</td>
<td>33/35 (100)</td>
<td>44-80</td>
</tr>
<tr>
<td>Ammonia (µmol/l)</td>
<td>26/26</td>
<td>&gt;145</td>
</tr>
<tr>
<td>Moderate hyperammonaemia (C₆-C₁₀)</td>
<td>9/18 (50)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Mild hyperammonaemia (C₆-C₁₀)</td>
<td>5/18 (28)</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Uric acid (µmol/l)</td>
<td>7/7 (100)</td>
<td>565-1428</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/I)</td>
<td>3/3 (100)</td>
<td>503-1260</td>
</tr>
</tbody>
</table>

*SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SGOT and/or SGPT were measured.

Table 3 Concentrations of plasma carnitine and medium chain fatty acids in M-CAD deficiency

<table>
<thead>
<tr>
<th>Investigation</th>
<th>No (%) with abnormal result</th>
<th>Range or value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission, fed state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carnitine (µmol/l)</td>
<td>21/22 (96)</td>
<td>7-36</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3-24</td>
</tr>
<tr>
<td>Free</td>
<td></td>
<td>40-60</td>
</tr>
<tr>
<td>Acute, fasting state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carnitine (µmol/l)</td>
<td>10/13 (77)</td>
<td>≥0-5</td>
</tr>
<tr>
<td>Acylcarnitine:free carnitine ratio</td>
<td>7/8 (88)</td>
<td>&lt;0-5</td>
</tr>
<tr>
<td>Octanone (C₈, µmol/l)</td>
<td>18/18 (100)</td>
<td>8-394</td>
</tr>
<tr>
<td>Decanone (C₁₀, µmol/l)</td>
<td>14/16 (88)</td>
<td>7-96</td>
</tr>
<tr>
<td>cis-4-decanoate (µmol/l)</td>
<td>15/15 (100)</td>
<td>3-168</td>
</tr>
</tbody>
</table>

*Reference values.1 23
†Other references indicate: total carnitine 5-23 µmol/l in nine patients,22 free carnitine 1-24 µmol/l in 15 patients.26
‡Including fasting tests.
ND, not detected.

Table 4 Main urinary metabolites in M-CAD deficiency

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>No (%) with abnormal result</th>
<th>Range or value</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketones (ketosticks)</td>
<td>18/21 (85)</td>
<td>Negative to + ≥ ++</td>
<td></td>
</tr>
<tr>
<td>1. Dicarboxylic acids (in acute state)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipate C₆</td>
<td>41/43 (95)</td>
<td>+ to +50</td>
<td></td>
</tr>
<tr>
<td>Suberate C₈</td>
<td>41/42 (98)</td>
<td>+ to +40</td>
<td></td>
</tr>
<tr>
<td>Sebacate C₁₀</td>
<td>37/39 (95)</td>
<td>+ to +30</td>
<td></td>
</tr>
<tr>
<td>2. Hydroxy acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Hydroxyhexanoate C₆</td>
<td>20/21 (95)</td>
<td>+ to +50</td>
<td></td>
</tr>
<tr>
<td>Hexanoylglycine</td>
<td>25/28 (90)</td>
<td>+ to +10</td>
<td></td>
</tr>
<tr>
<td>Suberylglycine</td>
<td>27/27 (100)</td>
<td>+ to +9</td>
<td></td>
</tr>
<tr>
<td>Phenylpropionylglycine</td>
<td>24/25 (96)</td>
<td>+ to +9</td>
<td></td>
</tr>
<tr>
<td>Carnitine conjugates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octanoylglycine</td>
<td>26/26 (100)</td>
<td>+ to +4</td>
<td></td>
</tr>
</tbody>
</table>

*Age: 1 day-15 years (n=70) receiving regular diet with no supplementary medium chain triglycerides.22
†‡1 Patients accurately identified with stable-isotope dilution assay.28
‡‡1 Patients exclusively identified by FAB-MS.24

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isotope dilution measurement.28 This assay allows the differential diagnosis between M-CAD deficient infants and normal infants receiving formulas supplemented with medium chain triglycerides.29 Urinary medium chain acylcarnitines, especially octanoylcarnitine, are also found in the analysed cases, mainly using fast atom bombardment mass spectrometry (FAB-MS).30

PATHOLOGICAL FEATURES
Panlobular microvesicular macrovesicular hepatic steatosis was generally present in M-CAD deficient patients during the acute crises. Ultrastructural analysis of hepatic mitochondria may differentiate M-CAD deficiency from idiopathic Reye’s syndrome, mainly by a condensed matrix appearance with widening of the intracristal spaces.21

IN VIVO DYNAMIC TESTS
Fasting tests revealed M-CAD deficiency by the detection of specific plasma and urinary metabolites. These tests are potentially dangerous, as shown by eight out of 16 cases where serious clinical manifestations were provoked, including lethargy, one case of coma after only 12 hours of fasting31 and one case of death.4 These clinical manifestations are not necessarily correlated with hypoglycaemia.20 32

Oral loading tests with lipid and medium chain triglycerides were also potentially dangerous, inducing neurological deterioration in two cases,8 11 and an aggravation of hepatomegaly in one case.10 Oral loading with carnitine (100 mg/kg) helped in establishing the diagnosis of M-CAD deficiency, with no risk to the patients, by the detection of urinary octanoylcarnitine.16 30

The phenylpropionic acid oral loading test is based on the fact that phenylpropionate is oxidised by the M-CAD enzyme to benzoic acid, which is then excreted in the urine as hippuric acid.33 After oral loading with phenylpropionate (25 mg/kg), control subjects and parents excreted only hippurate, whereas the urinary metabolites of M-CAD deficient patients consisted of approximately one third phenylpropionylglycine and two thirds hippurate.34 35

IN VITRO TESTS
Enzymatic diagnoses were first performed by a global assay of fatty acid oxidation in intact cultured fibroblasts.36 37 This procedure consists of measuring liberated carbon dioxide from 1-13C fatty acid substrates of varying chain length (C16 palmitate, C8 octanoyl, C4 butyrate) in order to determine the specific enzymatic activity for the three acyl-CoA dehydrogenases (long, medium, and short chain ACD) involved in mitochondrial β-oxidation. In M-CAD deficiency, the mean enzymatic activity with 1-13C octanoyl as substrate varies between 10% and 29% of normal (n=21).38

The specific enzymatic dosages3 have been obtained in hepatocytes, fibroblasts, peripheral mononuclear leucocytes, cardiac myocytes, skeletal myocytes, and amniocytes. In M-CAD deficiency, the specific enzyme activity measured with octanoyl-CoA as substrate is between 3% and 10% (n=40) with a mean of 6%.3

The molecular studies confirmed a single prevalent point mutation consisting of lysine to glutamate substitution on chromosome 1.39 This point mutation is present in more than 90% of the alleles40 and could represent a highly distinctive feature of M-CAD deficiency.

POSTMORTEM RECOGNITION
M-CAD deficiency should be suspected after unexpected death in infancy and death from Reye’s syndrome. Specific postmortem diagnosis may be made by the finding of raised concentrations of plasma cis-4-decenoate27 and the detection of urinary or hepatic octanoylcarnitine by FAB-MS.41 Specific enzymatic assays in various frozen tissues led to the diagnosis of M-CAD deficiency in SIDS.42

Retrospective studies estimate the frequency of M-CAD deficiency in the SIDS population at about 1-2% in England14 17 and possibly also in France (1/110) (P Divry, personal communication).

PRENATAL DIAGNOSIS
Prenatal diagnosis has already been achieved in a sibling of a SIDS case with M-CAD deficiency, and later confirmed in the neonatal period.43

TREATMENT
During the crises, symptomatic treatment is necessary to overcome the hypoglycaemia, cerebral oedema, seizures, or metabolic acidosis. The mainstay of treatment of M-CAD deficiency is the prevention of an acute episode by avoiding prolonged fasting periods and by providing glucose supplementation.2 32 A low fat diet is not necessarily recommended.12 L-Carnitine administration (100 mg/kg/day) is considered to be beneficial,30 31 but this is contested by some authors who did not find an improvement in fasting—induced ketogenesis after L-carnitine supplementation.33 44 Carnitine seems to be important in the elimination of potentially toxic metabolites accumulating after enzymatic block. This is recently confirmed by the finding of a greater excretion of medium chain acylcarnitines after L-carnitine administration.45

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33 Seakins JWT, Rumsby G. The use of phenylpropionic acid as a loading test for M-CAD deficiency. J Inherited Metab Dis 1988;i1:suppl 2;221-4.