J. Inher. Metab. Dis. 17 (1994) 283–286

Short Communication

Secondary 3-Hydroxydicarboxylic Aciduria Mimicking Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency

M. J. BENNETT[1], M. J. WEINBERGER[1], W. G. SHERWOOD[1] and A. B. BURLINA[2]

[1]*Kimberly H. Courtwright and Joseph W. Summers Metabolic Disease Center, Baylor University Medical Center, 3500 Gaston Avenue, Dallas, TX 75246, USA;*
[2]*Department of Pediatrics, University of Padua, Padua, Italy*

Long-chain L-3-hydroxyacyl-CoA dehydrogenase (L-CHAD, EC 1.1.1.35) deficiency is a recently described inborn error of mitochondrial fatty acid oxidation (McKusick 143450; Wanders et al 1989, 1990; Hale et al 1990). The enzyme activity in human liver resides in one subunit of a recently described inner-mitochondrial membrane-bound trifunctional protein (Carpenter et al 1992). Approximately 20 cases of L-CHAD deficiency have been reported to date. The clinical features include acute fasting intolerance with a Reye syndrome-like illness and a chronic course of skeletal and cardiac muscle myopathy, hepatic cirrhosis, peripheral neuropathy and in one case pigmentary retinopathy. Biochemically the disorder is characterized by a hypoketotic C_6–C_{14} 3-hydroxydicarboxylic aciduria in urine samples collected when ill. However, a significant number of patients with 3-hydroxydicarboxylic aciduria do not appear to have primary L-CHAD deficiency or other fatty acid oxidation defect expressed in cultured skin fibroblasts (Pollitt 1990; Olpin et al 1992).

Recently, Bergoffen et al 1993 reported 3-hydroxydicarboxylic aciduria in a poorly nourished patient with glycogen storage disease. We report here on three patients in whom secondary L-CHAD abnormalities and abnormalities in other mitochondrial NAD^+-requiring enzymes appear to be related to NAD^+ deficiency resulting from primary defects in the respiratory chain.

CASE HISTORIES

Patient 1 presented at the age of 6 months with a recent history of vomiting, poor weight gain and muscle weakness. Metabolic screening, including organic acids, was normal. She demonstrated a 3-hydroxydicarboxylic aciduria following an 18 h controlled fast. On the basis that she had a fatty acid oxidation defect she was treated with a high-carbohydrate/low-fat diet and initially stopped vomiting and gained weight. Subsequently she regressed and developed neurological abnormalities consistent with Leigh disease. Urine tiglylglycine, a marker associated with respiratory-chain

abnormalities, was elevated at 33.7 μmol/mmol creatinine (normal 0.2–3.8, $n = 24$; Bennett, Powell and Gibson, unpublished data). A muscle biopsy revealed a defect between respiratory chain complex I and III with less than 5% residual activity (John Shoffner, Emory Hospital).

Patient 2 presented at the age of 3 months with a history of vomiting, weight loss, muscle weakness and developmental delay. Abnormal blood lactate levels were recorded on two occasions (2.8 and 2.6 mmol/L, normal 0.3–1.3) although on each occasion he was poorly perfused. Unstressed urine collected on admission demonstrated a significant hypoketotic C_6–C_{14} 3-hydroxydicarboxylic aciduria. Additional metabolites of probable diagnostic significance included malate and isocitrate (Figure 1). Tiglylglycine was elevated at 5.6 μmol/mmol creatinine. The introduction of a high-carbohydrate/low-fat diet resulted in an initial clinical improvement, although hypotonia remains. There has been no clinical justification for a muscle biopsy.

Patient 3 presented at 13 days of age with vomiting, difficulty in swallowing and failure to thrive. She was given an initial diagnosis of gastro-oesophageal reflux. A urine collected when ill demonstrated a C_6–C_{14} 3-hydroxydicarboxylic aciduria. She had a blood lactate of 4.6 mmol/L and urinary tiglylglycine excretion was elevated at 5.7 and 9.7 μmol/mmol creatinine. A muscle biopsy revealed cytochrome *c* oxidase deficiency. She has mental delay.

Cultured skin fibroblasts were obtained from all three patients for the study of fatty acid oxidation.

METHODS AND RESULTS

The oxidation of [9,10-^{3}H]myristate and [9,10-^{3}H]palmitate was studied according to the method of Manning et al (1990). L-CHAD and short-chain 3-hydroxyacyl-CoA dehydrogenase activities were measured according to the method of Wanders et al (1990).

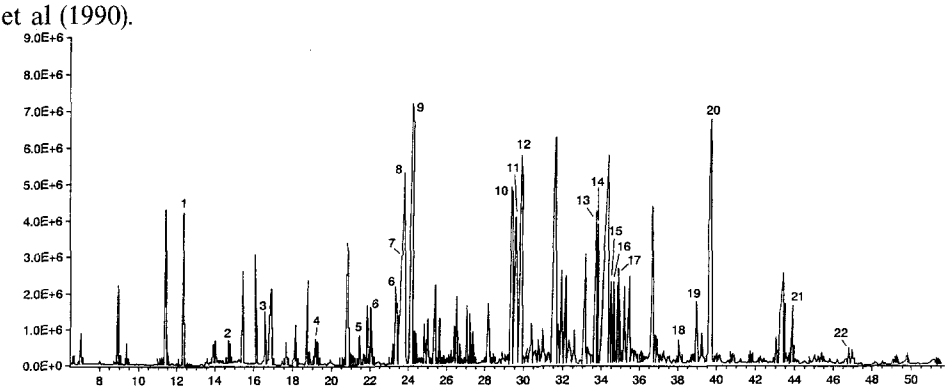

Figure 1 Urine organic acid profile in patient 2 in an unstressed sample collected on admission. Peaks: 1, 3-hydroxybutyrate/3-hydroxyisobutyrate; 2, 2-ethylhydracrylate; 3, ethylmalonate; 4, 5-hydroxyhexanoate; 5, 3-methylglutarate; 6, 3-methylglutaconate; 7, internal lactone of 3-hydroxyadipate; 8, malate; 9, adipate; 10, unsaturated suberate; 11, 3-hydroxyadipate; 12, suberate; 13, citrate; 14, isocitrate; 15, unsaturated sebacate; 16, 3-hydroxysuberate; 17, sebacate; 18, internal lactone of 3-hydroxysuberate (probably); 19, unsaturated 3-hydroxysebacate; 20, 3-hydroxysebacate; 21, 3-hydroxydedocanedioate; 22, diunsaturated 3-hydroxytetradecanedioate

Normal results for both fatty acid oxidation rates and enzyme activities were obtained from patients 1 and 2, indicating that neither patient has a primary fatty acid oxidation defect. The fibroblasts from patient 3 demonstrated reduced fatty acid oxidation (palmitate 8.0 pmol/min per mg protein, controls 34.0–39.0; myristate 12.5 pmol/min per mg protein, controls 15.6–27.9.

DISCUSSION

These results extend our earlier observations that primary defects in the respiratory chain impose a secondary effect on mitochondrial enzymes that require NAD^+ as a cofactor (Bennett et al 1993). Patient 1, in whom a severe deficiency of the respiratory chain was demonstrated, is following a clinical course consistent with Leigh disease. Interestingly, we have never been able to demonstrate an elevated blood lactate level in her. Patient 2 has not yet developed clinical severity to justify a muscle biopsy. He demonstrated urine abnormalities consistent with L-CHAD deficiency and also defects of both malate and isocitrate dehydrogenases. He also had a modest lactic acidaemia at a time when there was poor tissue perfusion. Patient 3 is less easy to define in terms of whether there is a primary respiratory-chain or fatty acid oxidation defect, even though she demonstrated an unequivocal lactic acidaemia. One possibility is that a primary defect in the respiratory chain is sufficiently severe to be reflected in secondary fatty acid oxidation abnormalities. This concept is supported by a number of cases reported recently in which patients with primary disorders of the respiratory chain have presented with organic acidaemia consistent with a fatty acid oxidation deffect (Christensen et al 1993; Lehnert and Ruitenbeek 1993) and abnormal fatty acid oxidation in cultured fibroblasts (Hagenfeldt et al 1992).

It is clear from our studies that the demonstration of hypoketotic 3-hydroxydicarboxylic aciduria is not sufficient grounds to provide a diagnosis of L-CHAD deficiency, and that further clinical and metabolic clues to the basic defect should be sought, particularly in patients with neurological abnormalities. The diagnosis should always be confirmed enzymatically.

ACKNOWLEDGEMENT

Supported in part by Telethon-Italy (grant 378).

REFERENCES

Bennett MJ, Sherwood WG, Gibson KM, Burlina AB (1993) Secondary inhibition of multiple NAD-requiring dehydrogenases in respiratory chain complex I deficiency: possible metabolic markers for the primary defect. *J Inher Metab Dis* **16**: 560–562.

Bergoffen J, Kaplan P, Hale DE, Bennett MJ, Berry GT (1993) Marked elevation of urinary 3-hydroxydecanedioic acid in a malnourished infant with glycogen storage disease, mimicking long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inher Metab Dis* **16**: 851–856.

Carpenter K, Pollitt RJ, Middleton B (1992) Human liver long-chain 3-hydroxyacyl-coenzyme-A dehydrogenase is a multifunctional membrane-bound beta oxidation enzyme of mitochondria. *Biochem Biophys Res Commun* **183**: 443–448.

Christensen E, Brandt NJ, Schmalbruch H, Kamieniecka Z, Hertz B, Ruitenbeek W (1993) Muscle cytochrome *c* oxidase deficiency accompanied by a urinary organic acid pattern mimicking multiple acyl-CoA dehydrogenase deficiency. *J Inher Metab Dis* **16**: 553–556.

Hagenfeldt L, Wibom R, Venizelos N, von Dobeln U (1992) Oxidation of fatty acids in fibroblasts from patients with respiratory chain defects. *Proc 30th Symposium SSIEM*, P.136 (Abstract).

Hale DE, Thorpe C, Braat K et al (1990) The L-3-hydroxyacyl-CoA dehydrogenase deficiency. *Prog Clin Biol Res* **321**; 503–510.

Lehnert W, Ruitenbeek W (1993) Ethylmalonic aciduria associated with progressive neurological disease and partial cytochrome *c* oxidase deficiency. *J Inher Metab Dis* **16**: 557–559.

Manning NJ, Olpin SE, Pollitt RJ, Webley J (1990) Comparison of [9,10-^{3}H]palmitic and [9,10-^{3}H]myristic acids for the detection of fatty acid oxidation defects in intact cultured fibroblasts. *J Inher Metab Dis* **13**: 58–68.

Olpin SE, Manning NJ, Carpenter K, Middleton B, Pollitt RJ (1992) Differential diagnosis of hydroxydicarboxylic aciduria based on release of ^3H_2O from [9,10-^{3}H]myristic and [9,10-^{3}H]palmitic acids by intact cultured fibroblasts. *J Inher Metab Dis* **15**: 883–890.

Pollitt RJ (1990) Clinical and biochemical presentation in 20 cases of hydroxydicarboxylic aciduria. *Prog Clin Biol Res* **321**: 495–502.

Wanders RJA, Duran I, IJlst L et al (1989) Sudden infant death and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Lancet* **1**: 52–53.

Wanders RJA, IJlst L, van Gennip et al (1990) Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of a new inborn error of mitochondrial fatty acid β-oxidation. *J Inher Metab Dis* **13**: 311–314.