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Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency

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ABSTRACT. We describe the clinical features and biochemical findings of two patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Both children presented with an acute metabolic crisis. Both had hypoglycemia and excreted even-chain unsubstituted dicarboxylic and 3-hydroxy-dicarboxylic acids in the urine. Measurement of the enzymes of fatty acid oxidation in cultured skin fibroblasts showed low activity of long-chain 3-hydroxyacyl-CoA dehydrogenase, but normal activity of short-chain 3-hydroxyacyl-CoA dehydrogenase. The defect was further characterized by immunoprecipitating the short-chain enzyme using monospecific antibodies. It is probably inherited as an autosomal recessive trait, inasmuch as intermediate enzyme activity was found in the fibroblasts from the parents of one child. (*Pediatr Res* 29: 406–411, 1991)

Abbreviations

NR, normal range
ETF, electron transfer flavoprotein
CTP, carnitine palmitoyltransferase

Fatty acids are an essential metabolic fuel, particularly at times of stress or prolonged fasting. They are predominantly metabolized in the mitochondria, and defects of mitochondrial fatty acid oxidation are increasingly recognized as an important group of inborn errors of metabolism that can cause sudden infant death syndrome ("cot death"), Reye's syndrome, hypoketotic hypoglycemic coma, and muscle weakness (1).

Mitochondrial β-oxidation requires activation of fatty acids by long-chain acyl-CoA synthetase to form the fatty acyl-CoA ester. The acyl groups are then transferred into the mitochondrial matrix by the concerted action of CPT I, carnitineacylcarnitine translocase, and CPT II. Acyl-CoA esters then undergo β-oxidation by a repeated sequence of flavoprotein-linked dehydrogenation, hydration, NAD^+-linked dehydrogenation, and thiolysis to generate acetyl-CoA (2). There are two or three enzymes with overlapping chain-length specificities for each of the reactions of β-oxidation: three acyl-CoA dehydrogenases, two enoyl-CoA hydratases, two 3-hydroxyacyl-CoA dehydrogenases, and two 3-oxoacyl-CoA thiolases. ETF and ETF:ubiquinone oxidoreductase transfer electrons from the acyl-CoA dehydrogenases to the respiratory chain. All these enzymes are required for the complete oxidation of long-chain fatty acids. Defects of several enzymes of β-oxidation have been described in detail, and these include abnormalities of short-chain (3), medium-chain (4), and long-chain (5) acyl-CoA dehydrogenases, ETF, and ETF dehydrogenase (6).

The 3-hydroxyacyl-CoA dehydrogenases catalyze the reversible dehydrogenation of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA. In mitochondria, there appear to be two enzymes that have different substrate specificities (7). The membrane-associated higher molecular weight enzyme has greater activity to the long-chain substrates, whereas the enzyme with maximum activity for the short-chain substrates appears to be in the mitochondrial matrix. There have only been two previous reports of defects of the long-chain 3-hydroxyacyl-CoA dehydrogenase (8–10). We describe the clinical and biochemical features of two children who had severe hypoglycemia, metabolic acidosis, and lipid storage due to a defect of long-chain 3-hydroxyacyl-CoA dehydrogenase.

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CASE REPORT

Patient 1. This girl was born 2 mo prematurely. At birth, the patient required ventilatory support for 2 d but otherwise the perinatal period was uneventful. Family history revealed that a brother had died suddenly at 7 mo of age. He had been born 9 wk prematurely and had developed infantile respiratory distress syndrome from which he had made a good recovery. Before his death he had developed a mild upper respiratory tract infection. At *post-mortem* the liver looked pale, suggesting fat accumulation although no histology was performed. Another brother had been stillborn at term, but yet another brother was alive and well, aged 3 y.

At 5 mo of age the patient presented with a 4-d history of vomiting and anorexia. On the day of admission, she became lethargic, floppy, and unresponsive, and had stopped crying on handling. She was cool, pale, and markedly hypotonic. Marked hepatomegaly was noted. She developed apneic episodes and seizures and required ventilatory support. Initial investigations showed that the child was hypoglycemic (blood glucose < 1 mM), acidemic secondary to a metabolic acidosis (pH 7.25, base excess −15 mM) with a lactic acidemia (7.4 mM), and had a mild hyperammonemia (115 μM). The Quick prothrombin ratio was prolonged at 1.7 but plasma albumin was maintained at 36 g/L. The urine contained no ketones or reducing substances. Hb was 8.4 g/dL, white cells 9.6×10^9/L, and platelets 18×10^9/L, although subsequent platelet counts were normal. An infection

screen including cerebrospinal fluid was negative, although C-reactive protein was raised at 1.8 mg/dL (normal <0.8).

The metabolic acidosis persisted and considerable amounts of glucose were required to maintain her blood glucose concentration. Initial urinary organic acid analysis showed a marked dicarboxylic aciduria (see Results and Fig. 1). Echocardiograph showed a moderately severe pericardial effusion and significant left ventricular hypertrophy.

Despite intensive support with strict maintenance of her blood glucose above 5 mM, she became increasingly ill. Cardiac function became impaired although there was no evidence of tamponade. The hepatomegaly increased and hepatic function deteriorated. She died peacefully on the 8th d of admission.

At *post-mortem*, the findings were an enlarged pale and fatty liver, slightly swollen and pale kidneys, and a heart of normal size. Histology revealed widespread fat deposition including the heart, liver, and kidneys.

Patient 2. This young girl presented at the age of 3½ mo with a 3-d history of vomiting and anorexia. She was the first child of apparently healthy parents, although the mother developed a swollen fatty liver during pregnancy. On the day of admission she became sleepy, floppy, and lethargic. She had two seizures followed by a respiratory arrest for which she was intubated and ventilated. She remained markedly hypotonic with hepatomegaly. Initial investigations showed that she was hypoglycemic (blood glucose 1 mM) and hyponatremic (plasma sodium 130 mM) with a metabolic acidosis (base deficit 15 mM). She had a persistent lactic acidosis (2.5–9.9 mM) and mild hyperammonemia (82–138 μM).

A liver biopsy was performed and there was gross macroglobular panlobular fatty change but normal amounts of glycogen. An ECG showed a sinus rhythm with an axis of +70° with the changes of left ventricular hypertrophy with strain. A two-dimensional echocardiograph showed mild left ventricular hypertrophy. The thickness of the intraventricular septum was 7 mm and the posterior wall 6 mm. The left ventricular contractility was normal.

A provisional diagnosis of fatty acid oxidation defect was made and at the age of 6 mo she was started on a low long-chain fat diet (1 g/d). Although it was intended to give her a high carbohydrate intake, she had a marked intolerance of all sugars. If the concentration of carbohydrate in her feed exceeded 9%, she developed diarrhea with glucose, galactose, and isomaltose in the stool. She was discharged on a diet of 1 g long-chain fat, 2% medium-chain triglycerides, and 9% carbohydrate (lactose and soluble glucon polymer) with protein (3 g/kg/d). This diet gave her a total daily intake of 110 kcal/kg. On this diet she improved, becoming more active, and after 3 mo she had good head control and learned to sit without support. On two-dimensional echocardiography, the thickness of both the intraventricular septum and the posterior wall of the left ventricle became normal (both 5 mm). The ECG was largely unchanged.

MATERIALS AND METHODS

Materials. All biochemicals were obtained from the Sigma Chemical Company (St. Louis, MO) or from Boehringer Mannheim (East Sussex, UK), apart from those synthesized as detailed below and 2-tetradecenoic acid, which was obtained from K and K Laboratories (supplied by Kodak, Liverpool, UK).

Organic acid analysis. Organic acids were extracted from acidified urine with diethyl ether and ethyl acetate and were converted to trimethylsilyl derivatives using bistrimethylsilyltrifluoroacetamide containing 1% chlorotrimethylsilane and pyridine (1:1 by volume). Derivated extracts were examined by gas chromatography-mass spectrometry using a VG Masslab 12-250 (VG Masslab, Ltd., Cheshire, UK) instrument with a nonpolar

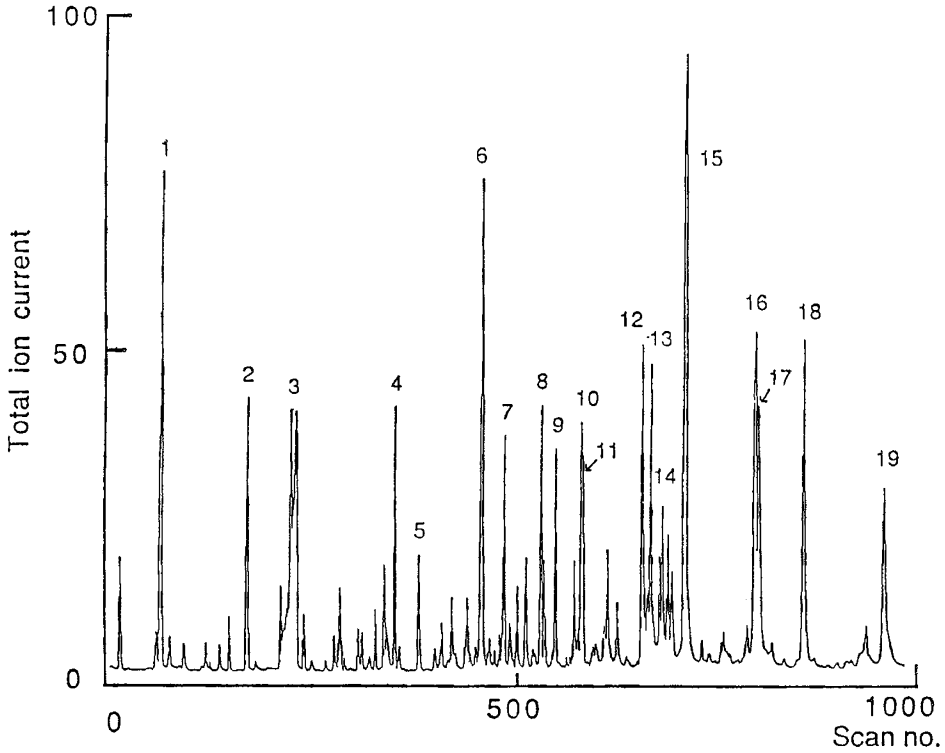

Fig. 1. Total ion current trace of derivatized extract of a urine sample collected 4 d after admission of patient 1. Peaks are due to the trimethylsilyl derivatives of: *1*, lactic acid; *2*, 3-hydroxy-n-butyric and 3-hydroxy-isobutyric acids; *3*, urea and 3-hydroxy-isovaleric acid; *4*, 3,4-dihydroxybutyric acid; *5*, glutaric acid; *6*, adipic acid; *7*, 2-hydroxymethyl-5-furoic acid; *8*, 2-oxoglutaric acid; *9*, p-hydroxyphenylacetic acid; *10*, 3-hydroxyadipic acid; *11*, suberic acid; *12*, hippuric acid; *13*, citric acid; *14*, 3-hydroxyoctendioic acid (*highest peak*) followed by 3-hydroxysuberic acid and sebacic acid; *15*, p-hydroxyphenylactic acid; *16*, p-hydroxyphenylpyruvic acid; *17*, 3-hydroxysebacic acid; *18*, margaric acid (internal standard 0.23 mmol/L); *19*, 3-hydroxydodecanedioic acid.

(OV-1 type) bonded-phase capillary column programmed from 80–260°C (11).

Preparation of 3-ketohexadecanoyl-CoA. Hexadecynoyl-CoA was prepared by the mixed anhydride method (12) starting with 500 μmol hexadecynoic acid and 60 mg of CoA. The impure product was then dissolved in 50 mM potassium phosphate, pH 5.3, and purified by adsorption onto a C_{18} octadecyl Bond Elute column (Analytichem, supplied by Jones Chromatography, Mid Glamorgan, UK) previously equilibrated with 50 mM potassium phosphate buffer, pH 5.3. The column was washed step-wise with 2-mL volumes of 25, 30, 32.5, 35, 37.5, and 40% vol/vol solutions of acetonitrile in 50 mM potassium phosphate, pH 5.3. The acetonitrile in each of the fractions was removed with a stream of nitrogen, the UV absorbance at 260 nm determined, and the relevant fractions analysed by HPLC (13). The 37.5% fractions yielded pure hexadecynoyl-CoA. The hexadecynoyl-CoA was diluted with water so that the final concentration of potassium phosphate was 10 mM, EDTA was added to a final concentration of 0.3 mM, and the pH was adjusted to 7 with KOH. The hexadecynoyl-CoA was then converted to 3-oxohexadecanoyl-CoA by addition of crotonase (14). This yields a 96–100% pure product. The pH was adjusted to 5 with HCl, and the reaction mix added to a Bond Elute column, which both removed the crotonase and concentrated the product. The column was washed with 20% acetonitrile in potassium phosphate, pH 5.3, and eluted with 37.5% acetonitrile in 50 mM potassium phosphate pH 5.3. Acetonitrile was removed under nitrogen.

Preparation of 2-tetradecenoyl-CoA. Tetradecenoyl-CoA was prepared by the mixed anhydride method from 2-tetradecenoic acid and CoA. The product was dissolved in 50 mM potassium phosphate, pH 5.3, and then purified by adsorption on a C_{18} octadecyl Bond Elute column by the same method used for the purification of 3-ketohexadecanoyl-CoA. Pure 2-tetradecenoyl-CoA was obtained in the 35% acetonitrile fraction.

Culture of fibroblasts. Fibroblasts were cultured in minimal essential medium supplemented with 10% FCS, 1% nonessential amino acids, 1% minimum essential medium vitamins, 0.1 mg/mL streptomycin, and 100 U/mL benzylpenicillin. Monolayers were harvested by trypsinization and washed twice with Dulbecco's PBS. Pelleted cells were frozen at −80°C and assayed within 3 wk.

Hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities. The fibroblast pellet was suspended in 25 mM potassium phosphate, 0.2 mM EDTA, pH 8, and then sonicated (30 W) on ice for 3 × 10 s with a 1-min interval between bursts. Triton X-100 was added to two thirds of the homogenate to give a Triton X-100 to protein ratio of 1:1 and to the remaining third to give a ratio of 0.5:1. This was followed by incubation on ice for 30 min and centrifugation at 11 600 × *g* for 10 min. Hydroxyacyl-CoA dehydrogenase activities (7) and short-chain enoyl-CoA hydratase (15) activity were then measured in the supernatant of the 1:1 Triton X-100 to protein fraction using acetoacetyl-CoA, 3-ketohexadecanoyl-CoA and crotonyl-CoA as substrates, respectively. Long-chain enoyl-CoA hydratase activity was determined in the 0.5:1 Triton X-100 to protein fraction using 2-tetradecenoyl-CoA as substrate (15). The lower concentration of Triton X-100 was used to decrease the nonspecific absorbance at 263 nm.

3-Oxoacyl-CoA thiolase activities. The fibroblast pellet was thawed in 100 mM Tris-HCl, pH 8.0, sonicated (30 W) 3 × 10 s with a 1-min interval between bursts. After the addition of Triton X-100 to give a Triton X-100 to protein ratio of 2:1, the sonicate was incubated on ice for 30 min and centrifuged at 11 600 × *g* for 10 min. Thiolase activities were determined in the supernatant using acetoacetyl-CoA and 3-ketohexadecanoyl-CoA as substrates (16). K^+ stimulation of the acetoacetyl-CoA-specific mitochondrial thiolase was also determined by adding 50 mM KCl to the incubation medium (17).

Acyl-CoA dehydrogenase activities. Tissue was thawed in 25 mM potassium phosphate buffer, pH 8.0, containing 0.2 mM EDTA and disrupted by sonication as for the other enzymes. After centrifugation at 11 600 × *g* for 10 min, acyl-CoA dehydrogenase activities were measured in the supernatant using butyryl CoA, octanoyl CoA, and hexadecanoyl CoA as substrates by a fluorometric ETF-reduction assay as previously described (18).

Immunoprecipitation of short-chain 3-hydroxyacyl-CoA dehydrogenase. We raised polyclonal antibodies in a rabbit to beef heart short-chain 3-hydroxyacyl-CoA dehydrogenase (obtained from Sigma Chemical Co.). The antibody, partially purified from serum by ammonium sulphate precipitation and dialysis, cross-reacted with a single major band on immunoblotting human and rat mitochondrial fractions. Antibody to short-chain 3-hydroxyacyl-CoA dehydrogenase was added to aliquots of sonicated, detergent solubilized, centrifuged fibroblasts and incubated on ice for 2 h. After centrifugation at 11 600 × *g* for 10 min, the activities of the short-chain and long-chain enzymes were assayed in the supernatant. The results were compared with those using ammonium sulphate-precipitated preimmune serum from rabbit.

RESULTS

Organic Acids. Patient 1. Two urine samples (collected 1 and 4 d after admission) were examined for organic acids with rather similar results (Fig. 1). There were moderate amounts of even-chain unsubstituted dicarboxylic acids and even-chain 3-hydroxy-dicarboxylic acids up to 12 carbon atoms length. p-Hydroxyphenyl-lactic and p-hydroxyphenylpyruvic acids were present in excess.

Patient 2. At time of admission, she had a marked dicarboxylic aciduria that included excretion of 3-hydroxydicarboxylic acids. On a medium-chain triglyceride diet, in addition to the excretion of suberate, sebacate, adipate, 7-hydroxyoctanoate, and 5-hydroxyhexadecanoate, she excreted large amounts of 3-hydroxysebacate.

Fasting Protocol on Patient 2. Patient 2 was fasted under carefully controlled conditions at the age of 6 mo. After 5½ h of the fast, the blood glucose concentration had fallen to 2.8 mM; simultaneously, the plasma FFA were 1.32 mM and blood 3-hydroxybutyrate concentration 0.2 mM. During the fast, the blood lactate was raised (up to 3.8 mM). Her plasma carnitine concentrations at the end of the fast were: free carnitine 7.9 μM (NR 22–50), short-chain acylcarnitine 2.3 μM (NR 3.5–10), long-chain acylcarnitine 5.4 μM (NR 0.6–2.0). This low free carnitine concentration with elevated long-chain acylcarnitine concentration is suggestive of a defect of long-chain fatty acid oxidation.

Activity of β-Oxidation enzymes. The activities of the acyl-CoA dehydrogenases were normal for both patients (Table 1). The values for patient 2 and a control performed at the same time were higher than for patient 1 and the other controls. This increased activity was associated with the use of a different catalase preparation. The activity of the short-chain enzymes was normal, but for both patients the activity of long-chain enoyl-CoA hydratase and thiolase were low. The most marked abnormality, however, was in the activity of long-chain 3-hydroxyacyl-CoA dehydrogenase, which was 35 and 30% of control values for the two patients (Table 1).

This activity to the long-chain substrate in our patients could be due to the short-chain enzyme and to confirm this we immunoprecipitated the short-chain enzyme from the fibroblast supernatants of both the patients and two controls (Fig. 2). In control subjects, there was a decrease of approximately 25% in total activity to the long-chain substrate, whereas in the patients there was a further 75% decrease of the original low value (Fig. 2*B*). The residual activity in the patients' fibroblasts with the long-chain substrate after immunoprecipitation of the short-chain enzyme was 8.5 and 10% of two control values.

We also measured the β-oxidation enzymes in the parents of patient 2. The activity of the long-chain 3-hydroxyacyl-CoA

Table 1. *Activities of mitochondrial fatty acid oxidation enzymes in cultured skin fibroblasts**

Enzyme	Patient 1	Controls (10)	Patient 2	Control
Acyl-CoA dehydrogenase				
Butyryl-CoA	0.24	0.22 ± 0.1	1.03	1.04
Octanoyl-CoA	0.94	0.95 ± 0.17	5.74	2.19
Hexadecanoyl-CoA	1.52	1.42 ± 0.21	3.68	3.96

		Patient 2			
	Patient 1	Patient	Mother	Father	Controls (6)
Enoyl-CoA hydratase					
Crotonyl-CoA	102.9	109.4	94.5	184.0	160 ± 42.9
2-Tetradecenoyl-CoA	36.8	35.7	34.5	45.3	61.7 ± 14.9
3-Hydroxyacyl-CoA dehydrogenase					
Acetoacetyl-CoA	36.2	39.9	21.5	40.3	42.9 ± 8.4
3-Ketohexadecanoyl-CoA	12.8	11.1	19.0	23.2	36.9 ± 6.8
3-Oxoacyl-CoA thiolase					
Acetoacetyl-CoA	8.8	9.6	8.5	7.7	8.8 ± 1.2
Acetoacetyl-CoA + KCl	12.1	14.0	10.3	12.3	15.7 ± 4.2
3-Ketohexadecanoyl-CoA	12.3	10.4	15.3	19.4	20.2 ± 3.5

* Results are nmols of ETF reduced·min^{-1}·mg $protein^{-1}$ (acyl-CoA dehydrogenases), nmols of enoyl-CoA hydrated·min^{-1}·mg $protein^{-1}$ (enoyl-CoA hydratases), nmols of NADH oxidized (3-hydroxyacyl-CoA dehydrogenases), and nmols of oxoacyl-CoA cleaved min^{-1}·mg $protein^{-1}$ (3-oxoacyl-CoA thiolases). The control values are the mean ± SD and the number of controls is shown in parentheses.

dehydrogenase was intermediate between controls and patients; this was best seen after immunoprecipitation of the short-chain enzyme by the monospecific antibody (Fig. 2*B*). The activity of the long-chain enoyl-CoA hydratase, but not the long-chain thiolase, was low in the parents. The mother also had low activity of the short-chain 3-hydroxyacyl-CoA dehydrogenase.

DISCUSSION

Most of the patients previously described with defects of mitochondrial fatty acid oxidation have had defects involving the acyl-CoA dehydrogenases (19). However, many patients who present with symptoms and investigations (*e.g.* hypoketotic hypoglycemia) suggestive of a defect of fatty acid oxidation do not have abnormalities of the acyl-CoA dehydrogenases (20). Both children that we described presented with a short history of lethargy and rapidly became seriously ill. On examination there was hepatomegaly with fat infiltration and both unsubstituted and 3-hydroxy dicarboxylic acids were present in the urine. These findings all suggested a defect of fatty acid oxidation and we have demonstrated a reduction in the activity of 3-hydroxyacyl-CoA dehydrogenases in cultured skin fibroblasts.

Because the long-chain 3-hydroxyacyl-CoA dehydrogenase has not been purified to homogeneity, the substrate specificity of the enzymes that metabolize hydroxyacyl-CoA esters has not been defined. For our studies, to define the nature of the deficiency we therefore raised antibodies to the short-chain enzyme. Saturating amounts of the antibody decreased the activity to the short-chain substrate to 14 and 18% of initial values in controls, and in the two patients to 16 and 10%. The antibody to the short-chain enzyme decreased the activity to the long-chain substrate to 74 and 76% of the initial value in controls, whereas in the patients there was a much greater fall in the already low level (to 33 and 25% of initial levels). These results suggest that the short-chain enzyme has activity toward the long-chain substrate. The residual enzyme activity after immunoprecipitation of the short-chain enzyme in the patients' fibroblasts could be due to a number of factors, including a small amount of residual long-chain enzyme, reduced activity of an altered long-chain enzyme, incomplete immunoprecipitation of the short-chain enzyme, or action of the peroxisomal 3-hydroxyacyl-CoA dehydrogenase. The results of these immunoprecipitation experiments are similar to those reported by Hale *et al.* (10). They incubated the cell supernatant with antibody to the short-chain enzyme for 30 min at room temperature before assay. This inhibited 75% of the residual activity to the long-chain substrate in the patient's fibroblasts.

In the fibroblasts from both patients, there was low activity of the long-chain enoyl-CoA hydratase and the long-chain thiolase, whereas the activity of all the short-chain enzymes and the long-chain acyl-CoA dehydrogenase was normal. We assume that the changes in the long-chain enoyl-CoA hydratase and long-chain thiolase are secondary to the abnormality in the long-chain 3-hydroxyacyl-CoA dehydrogenase. The organization of the β-oxidation enzymes in the mitochondrial matrix is unknown. However, recent evidence suggests that some β-oxidation enzymes are associated with CPT II (21). Thus, a defect of long-chain 3-hydroxyacyl-CoA dehydrogenase may affect this organization and result in lower amounts of the other long-chain enzymes.

3-Hydroxydicarboxylic acids are seen as part of a hypoketotic dicarboxylic aciduria pattern in patients with deficiency of medium- or long-chain acyl-CoA dehydrogenases, but usually in relatively small amounts. When the amounts of 3-hydroxydicarboxylic acids excreted approach those of the unsubstituted dicarboxylic acids, and particularly if C_{12} and C_{14} dicarboxylic acids are prominent, the pattern may be classified as a "hydroxydicarboxylic aciduria." This is a qualitative interpretation based on pattern recognition but may be a useful pointer to long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; the urinary organic acid patterns in both patients described here had been classified as hydroxydicarboxylic acidurias before the enzyme assays had been performed. However, hydroxydicarboxylic aciduria is also seen in a familial disease associated with progressive cirrhosis of the liver and may occur during transient secondary impairment of hepatic fatty acid oxidation with Reye-like presentation (11).

Both patients had a metabolic acidosis with a high blood lactate concentration, and this has also been reported in one of the previous cases (10, 22). A lactic acidosis is not a common feature in patients with defects of β-oxidation. The mechanism of the lactic acidosis is unknown, although several possibilities exist. The intermediates of fatty acid oxidation that accumulate in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency may inhibit pyruvate dehydrogenase or one of the respiratory chain complexes. Long-chain 3-hydroxyacyl-CoA dehydrogenase generates NADH and may be closely associated with complex I of the respiratory chain. Absence of the long-chain 3-hydroxyacyl-

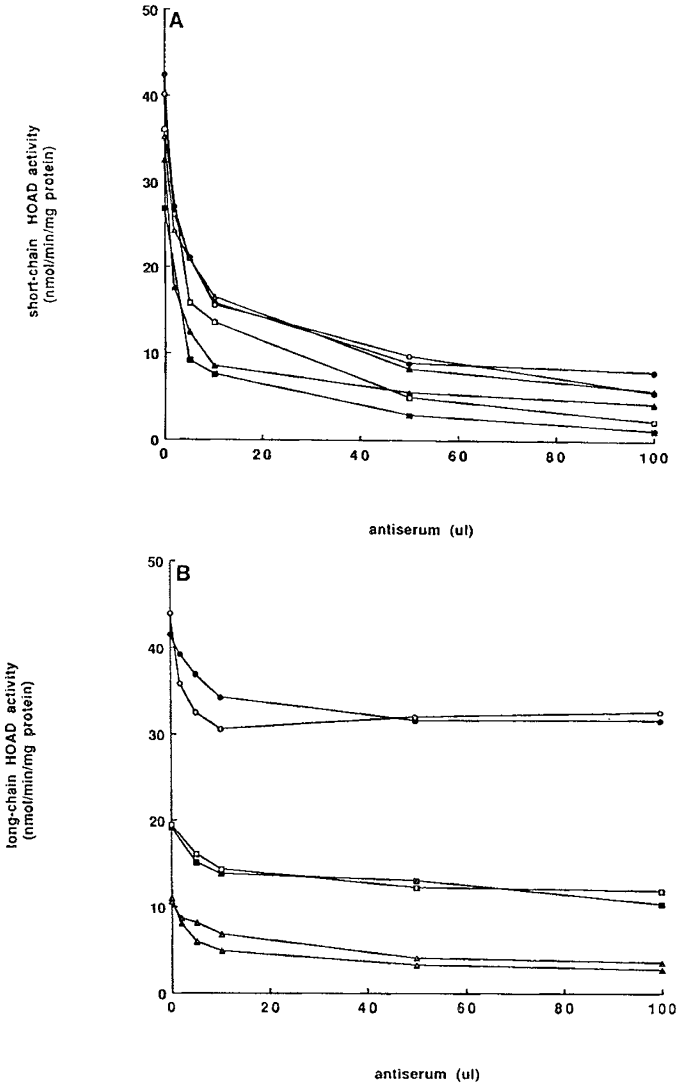

Fig. 2. Immunoprecipitation of short-chain 3-hydroxyacyl-CoA dehydrogenase from fibroblast homogenates. The 3-hydroxyacyl-CoA dehydrogenase activity was measured using acetoacyl-CoA (*A*) and 3-ketohexadecanoyl-CoA (*B*) as substrates. The activities were measured in fibroblast homogenates from patient 1 (▲), patient 2 (△), father of patient 2 (□), mother of patient 2 (■), and two controls (●, ○). The short-chain enzyme was immunoprecipitated using increasing concentrations of anti-short-chain 3-hydroxyacyl-CoA dehydrogenase antibody (316 μg in 100 μL) (see Materials and Methods). Preimmune serum after ammonium sulphate precipitation did not decrease activity with either the short-chain or long-chain substrate (not shown).

CoA dehydrogenase may significantly affect the function of complex I and thus cause the lactic acidosis seen in these patients.

Two previous cases of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency have been reported (8–10, 22). One child was well until 9 mo of age when he developed an illness characterized by vomiting, listlessness and hypotonia after a respiratory infection. He developed hepatomegaly and a liver biopsy showed mild fibrosis and severe fatty infiltration. Fasting for 11 h induced marked hypoglycemia and a lactic acidosis. Studies of glycogen and glucose metabolism were normal. The child had a very prolonged admission (6 mo), predominantly due to poor hepatic function. He was discharged on a regime of regular nasogastric feeds, but was readmitted at 19 mo for reassessment. Free carnitine concentrations were low in muscle, liver, and plasma, but the concentration of long-chain acylcarnitine was high. He died aged 19 mo after catastrophic illness associated with profound hypoglycemia. The patient's twin sister also had episodes of hypoglycemia, lactic acidosis, and liver dysfunction associated with infections. She was treated with D,L-carnitine and regular high carbohydrate meals. Despite this, she developed a myopathy and cardiomyopathy, both of which responded well to a medium-chain triglyceride diet. The biochemical defect in this patient is presumably the same as that in her twin brother. Another child presented at the age of 5 mo when after a few days of vomiting and diarrhea she became lethargic, associated with hypoketotic hypoglycemia and a massive 3-hydroxy dicarboxylic aciduria (8, 9). She recovered rapidly after i.v. glucose administration. The first child of this family, a boy, had died suddenly and unexpectedly on the 3rd d of life. Analysis of a blood sample obtained at *post-mortem* revealed marked hypoglycemia, no ketonemia, and a moderate dicarboxylic aciduria. This latter child presumably had a defect of long-chain 3-hydroxyacyl-CoA dehydrogenase. One of the children in our study also had a sibling who died suddenly in the neonatal period, suggesting that defects of this enzyme, as well as medium-chain acyl-CoA dehydrogenase, could be an important and potentially treatable cause of sudden infant death.

The inheritance of defects of β-oxidation is assumed to be autosomal recessive. This pattern would seem likely in at least one of our patients inasmuch as the parents have intermediate enzyme activity. Interestingly, the mother of this child, although clinically normal now, had a severe fatty liver during pregnancy. She had a low short-chain 3-hydroxyacyl-CoA dehydrogenase, and this suggests that heterozygotes may be symptomatic at times of stress. In addition, the activity of the long-chain enoyl-CoA hydratases in the parents' fibroblasts was also low, suggesting that even with partial defects the organization of the β-oxidation enzymes is disrupted.

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