

Reversible mitochondrial myopathy with cytochrome *c* oxidase deficiency

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Abstract

Two siblings, a boy and a girl born in a non-consanguineous marriage, presented with a similar clinical course. Sucking and breathing difficulties appeared within a few weeks of birth. Clinical examination revealed profound muscular hypotonia, hepatomegaly, increased serum creatine kinase activities, and lactic acidosis. Both infants were treated with gavage feeding, the boy also needing ventilatory support. Clinically they improved gradually. Now, the boy aged 4 years and the girl aged 28 months are free of clinical signs. Muscle biopsy specimens taken at 3 months showed, in both, ragged red fibres, abnormal mitochondria, and reduced cytochrome *c* oxidase (COX) staining. Biochemical analysis showed COX activity to be reduced to about 25% of the normal mean. The second biopsy specimen from the boy at 16 months was normal on morphological examination, but the girl's second specimen at 13 months still showed abnormal features. These cases are examples of the rare benign reversible COX deficiency. Early diagnosis is crucial to provide intensive treatment until spontaneous clinical improvement appears.

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Infantile myopathies with cytochrome *c* oxidase (COX) deficiency are rare conditions with both benign and fatal outcome.¹⁻³ The mode of inheritance and the mechanism of the reversibility are still unknown.

We describe a pair of siblings, who both show COX deficiency with a benign clinical course.

Case reports

CASE 1

The boy was the first child from a non-consanguineous marriage. Both parents were healthy and the family history was unremarkable with no neurological disorders. The fetal movements were weak. The delivery was uncomplicated, and the Apgar score at 1 and 5 minutes was 5 and 8, respectively. The boy showed hyperbilirubinaemia, but was discharged at the normal time of 7 days. At home he developed sucking difficulties and lost weight. When readmitted to hospital at 3 weeks he was lethargic, dehydrated, and severely hypotonic. The liver was 5 cm below the costal margin and he had profound acidosis. After correction of the dehydration and acidosis, he improved temporarily, but at the age of 6 weeks his condition worsened again making artificial ventilation necessary for 14 weeks. Blood

chemistry showed severe metabolic acidosis. Blood lactate concentration was 17.4 mmol/l (reference values <1.8 mmol/l). Serum creatine kinase activity was also considerably increased 1402 U/l (<220 U/l). Blood ammonia was slightly increased at 99 µmol/l (<50 µmol/l). Serum total carnitine was normal at 36.5 µmol/l (>35 µmol/l), but only 3% of it was in unesterified form. He received carnitine supplementation (100 mg/kg/day) from the age of 2 months. Gradually his condition started to improve. Abnormal blood lactate and serum creatine kinase values declined to near normal values during the first year of life, but periodic increases in creatine kinase were detected afterwards (fig 1). Muscular hypotonia showed slow but steady improvement. He learned to walk by 20 months and now at 4 years he can pedal a tricycle for several kilometres and shows no muscle weakness. His intellectual development has been normal. Carnitine supplementation was stopped at the age of 27 months.

CASE 2

The younger sister was admitted to hospital at 12 weeks for sucking difficulties, poor weight gain, and severe hypotonia. She also had increased blood lactate concentration at 16.8 mmol/l (fig 1) and serum creatine kinase activity at 798 U/l. Serum carnitine concentration was 36.0 µmol/l (7% unesterified). She received oral carnitine supplementation (100 mg/kg/day) from the age of 3 months until 18 months. She had gavage feeding for six months after hospitalisation, but no artificial ventilation. Her motor development was slightly delayed. At 18 months she was able to climb up from the sitting position and take a few steps without support, and now at 28 months her muscle strength is normal.

Investigations

MUSCLE BIOPSY

Surgical muscle biopsy specimens were taken with informed consent from the parents from vastus lateralis muscles at the age of 3 months. A second biopsy was carried out in case 1 at the age of 16 months and in case 2 at the age of 13 months.

MORPHOLOGICAL METHODS

Cryostat sections were stained with haematoxylin and eosin, periodic acid Schiff stain (PAS), reduced nicotinamide adenine dinucleotide (NADH) tetrazolium reductase, modified Gomori stain, as well as ATPase after preincubations at pH 10.4, 4.6, and 4.3. COX staining was carried out according to Dubowitz.⁴ Samples

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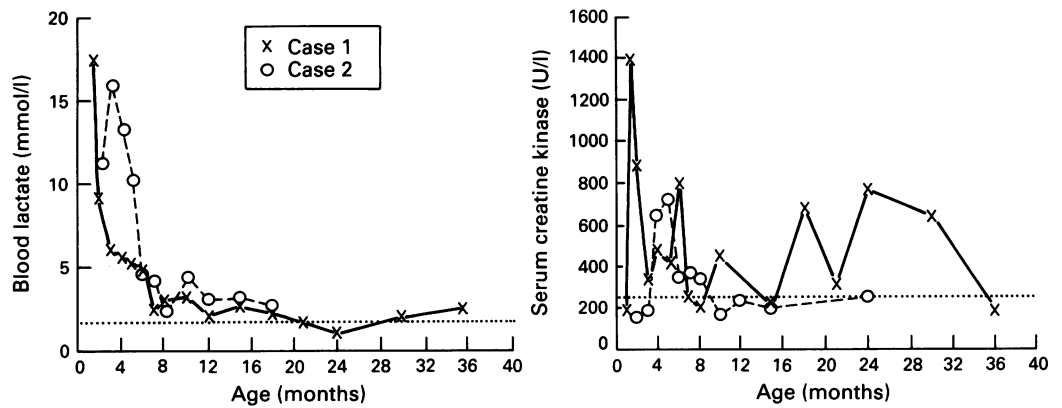


Figure 1 Blood lactate concentrations (left) and serum creatine kinase activities (right) during follow up.

for electron microscopy were processed according to standard technique.

BIOCHEMICAL ANALYSES

Portions of the first biopsy specimens of both cases were immediately frozen in liquid nitrogen and stored at -70°C until analysed. Biopsy specimens were then homogenised in nine volumes of 0.15 M potassium chloride with 50 mM Tris hydrogen chloride (pH 7.4) in all glass motor driven homogenisers. COX and other mitochondrial enzymes were measured in the supernatant fluid after centrifugation at 1000 g for 15 minutes by methods previously described.³

SKIN FIBROBLAST CULTURES

Fibroblasts were grown from a skin biopsy specimen taken from case 1 and cultured in

Eagle's basal medium supplemented with 10% fetal calf serum. Enzyme activities of pyruvate carboxylase and pyruvate dehydrogenase were kindly measured by Dr E Holme (University of Gothenburg, Sweden) and those of long and medium chain fatty acid β -oxidation by Dr C Vianey-Liaud (Hospital Debrousse, Lyon, France).

Results

MORPHOLOGICAL FINDINGS

The first muscle biopsy specimen taken from case 1 at 3 months showed profound changes including necrotic fibres, increased ventilation of fibre size, split fibres, increased amount of internal nuclei, and fatty infiltration (fig 2A). Numerous ragged red fibres were shown with modified Gomori stain suggesting mitochondrial involvement. An electron micrograph from the same specimen showed swollen mitochondria containing tightly packed concentric cristae embedded in abundant glycogen matrix (fig 2B). Some of the mitochondria were remarkably increased in size. Histochemical COX staining of the same biopsy specimen

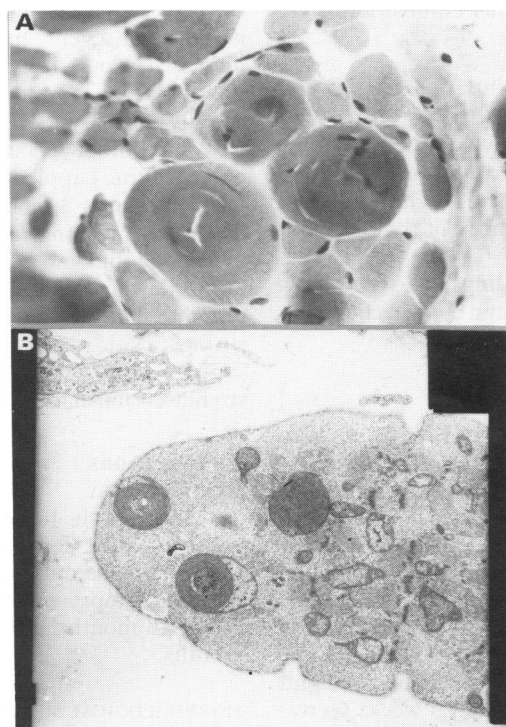


Figure 2 (A) Muscle biopsy specimen from vastus lateralis muscle (case 1) at the age of 3 months showing fibre splitting, a whorled fibre, fibre size variation, and internal nuclei. (Stained with haematoxylin and eosin, magnification $\times 250$.) (B) Electron micrograph from the same specimen showing swollen mitochondrial with tightly packed concentric cristae embedded in abundant glycogen matrix (magnification $\times 18\ 000$).

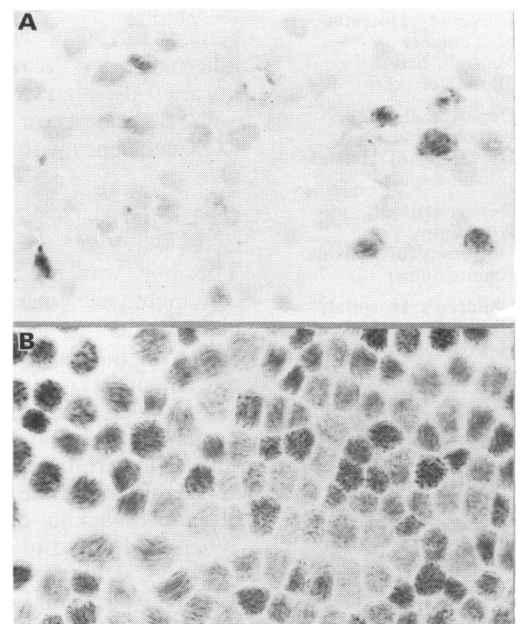


Figure 3 Muscle biopsy specimens from vastus lateralis muscle from case 1. (COX staining, magnification $\times 120$.) (A) Specimen taken at 3 months showing normal staining in a few fibres only. (B) Second specimen taken at 16 months showing normal staining in most fibres.

Table 1 Mitochondrial enzyme analyses in muscle tissue biopsy specimens from the two cases and 38 control patients. (Activities are expressed as μmol of substrate used per min/g of tissue)

	Case 1 at 3 months	Case 2 at 3 months	Controls: Mean (SD)
Cytochrome c oxidase	0.74	0.73	2.86 (0.53)
Succinate cytochrome c reductase	1.07	2.10	0.75 (0.20)
Rotenone sensitive NADH cytochrome c reductase	1.32	0.59	1.17 (0.47)
Citrate synthase	15.73	39.70	11.35 (2.4)
NADH dehydrogenase	59.85	8.71	35.21 (6.3)
Succinate dehydrogenase	1.07	3.40	1.68 (0.45)

Table 2 Clinical and biochemical findings in reversible COX deficiency

	DiMauro et al 1983 ¹	Zeviani et al 1987 ²	Own cases	Siblings
Family history	—	—	Siblings	Siblings
Pregnancy and delivery	Normal	Normal	Normal	Normal
Onset of symptoms (weeks)	<2	<6	<3	<12
Weak cry	+	—	+	+
Sucking difficulties	+	+	+	+
Muscular hypotonia	+	+	+	+
Extraocular muscle weakness	—	+	—	—
Macroglossia	+	—	—	—
Hepatomegaly	+	+	+	—
Gavage feeding	+	+	+	+
Respiratory support	—	+	+	—
Blood lactate at onset (mmol/l)	28	12	18	16
COX activity at 1–4 months	6%	8%	25%	25%
Blood lactate at 11–14 months	3	Normal	3	3
COX activity at 11–18 months	nd	33%	nd	nd
Residual signs	Waddling gait (33 months)	Cannot walk (20 months)	None (4 years)	None (28 months)

nd= not determined.

Reference values for blood lactate are 0.70–1.80 mmol/l. COX activity refers to relative values as compared with controls.

showed abnormal reaction in most fibres. Some fibres showed a faint reaction, whereas the majority remained completely non-reactive (fig 3A). In the second biopsy specimen taken at 16 months the general structure of the muscle was already normal. No ragged red fibres were observed and COX staining revealed a relatively homogenous reaction (fig 3B).

A muscle biopsy specimen from the case 2 at 3 months was identical to the first specimen from case 1, but her second specimen, taken at the age of 13 months, still showed abnormalities compatible with mitochondrial myopathy, including an uneven COX staining pattern.

BIOCHEMICAL FINDINGS

A consistent finding in the two biopsy specimens taken at age 3 months was the reduction of COX activity to about 25% of the controls (table 1). Pyruvate carboxylase and pyruvate dehydrogenase as well as fatty acid β -oxidation activities were normal in the fibroblast cultures obtained from case 1.

Discussion

Reversible mitochondrial myopathy in these two siblings bears close resemblance to two previous cases.^{1,2} Although reversal of COX activity was not documented by enzyme analysis, it is clearly apparent from the COX staining in the successive biopsy specimens.

The four cases share several clinical characteristics in common (table 2). The first symptoms appear soon after birth. Muscle tissue is severely affected, transient hepatomegaly and macroglossia may appear, but the myocardium and central nervous system do not seem to be affected. Hospitalisation is needed, and in some

cases ventilatory support also. The pace of recovery may vary, as seen in our two siblings, but clear improvement is usually detected within the first year of life. The low serum free carnitine concentration prompted us to start carnitine supplementation. Yet, because we had no measurement of its concentration in muscle, it is impossible to estimate if there was a true carnitine deficiency. Clinical improvement seems to be associated with a decline in blood lactate concentrations. The first described patient¹ had still a waddling gait at the age of 33 months, and the second patient² was unable to walk at the age of 20 months, causing some uncertainty about the reversibility of the syndrome. Our first patient has the longest follow up, and at the age of 4 years he has no muscle weakness at all. Our second patient is keeping up with her peers at the age of 2 years, suggesting that functional recovery may be complete in these cases. It should be noted, however, that our first patient showed periodic increases in serum creatine kinase activity, despite his normal muscle strength.

These are the first familial cases reported. The condition is likely to be inherited. Reversibility of COX deficiency is a unique finding among inherited metabolic diseases, and it is not completely understood at the moment. The enzyme is composed of 13 subunits, three of which are encoded by mitochondrial DNA. There is also evidence of tissue specific isoforms.⁵ All four cases manifested purely as a myopathy. As there is no evidence of mitochondrial transmission, a mutation in developmentally regulated nuclear encoded subunits seems a plausible explanation.

Reduced COX activity is associated with various clinical syndromes, some of them resembling Leigh's syndrome and affecting predominantly brain, some manifesting predominantly as a myopathy.⁶ In clinical situations the differential diagnosis is mainly between the fatal and the benign reversible myopathy. It is possible that the use of monoclonal antibodies against COX subunits may bring a solution to the problem,⁷ although more experience is needed to prove their diagnostic value. Until then, careful follow up with adequate life support is needed until the benign form can be differentiated from the fatal one.

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