Biochemical studies in mitochondrial encephalomyopathy

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SUMMARY The alpha-keto acid dehydrogenase complex and its component enzymes, lactate dehydrogenase, pyruvate carboxylase, cytochrome c oxidase, succinate-cytochrome c reductase, NADH-cytochrome c reductase, and the concentration of cytochromes and enzymes of beta-oxidation in muscle from a patient with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes were studied and no specific defect was found. These results raise the possibility that the mitochondrial changes in the patient may be secondary.

A syndrome which has been labelled MELAS¹ (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) is characterised by seizures and stroke-like symptoms as well as lactic acidosis, ragged-red fibres and dementia. Although the clinical features are relatively distinctive, the biochemical abnormalities reported so far have not been uniform. We studied several enzymes and the concentration of cytochromes in isolated muscle mitochondria from a patient with the syndrome, and found no specific defect of the respiratory chain.

Case report

A girl, the second child of unconanguineous healthy parents had normal growth and development until age 10 years but she could never run as fast as her peers. At age 11, she began to complain of nausea and headache during swimming and had recurrent febrile illnesses. She also had visual problems and concentric field constriction was found by an ophthalmologist. CT scan at that time was normal but EEG showed bilateral synchronous spike and wave bursts. At age 12 febrile episodes spontaneously subsided but school per-

formances gradually deteriorated. She became nauseated during exercise without pain or weakness. At age 14 she had two generalised seizures. Five months after the first attack she suddenly developed right hemiparesis, numbness of the tongue and speech disturbances, followed by several attacks of jerky involuntary movements of the right arm and difficulty in calculation. Her family history was unremarkable.

On admission her weight was 38 kg (−1.6 SD) and her height 151 cm (−1.0 SD). She was alert, but showed mild memory disturbances, paraphasia, agraphia, acalculia and constructional apraxia. She had right homonymous hemianopia with concentric constriction of the visual field. Optic fundi and external ocular movement were normal and there was no ptosis. Auditory acuity was normal. There was moderate weakness of the right limbs. Deep tendon reflexes were moderately hyperactive in both legs. Muscle tone was normal. She had mild impairment of the light touch and joint position sense on the right half of the body. No cerebellar ataxia was found.

Normal laboratory tests included: complete blood count, blood coagulation and platelet function tests, serum electrolyte, cholesterol, triglyceride and free fatty acids, serological tests including viral studies, thyroid and adrenal function tests, serum copper, ceruloplasmin, thiamine, ammonia and galactose, basal metabolic rate, CSF protein concentration, echocardiogram. The following tests gave abnormal results (normal values in parentheses): urinalysis showed ketonuria, serum creatine kinase (CK) was 400 IU/l (15–60), lactate dehydrogenase (LDH) was 984 IU/l (120–250), and glutamic-oxaloacetic transaminase (GOT) was 127 IU/l (less than 40); serum lactate varied between 40.2 and 83.7 mg/dl (4–16), and serum pyruvate between 0.82 and 1.89 mg/dl

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Fig (A) Haematoxylin-eosin stain, $\times 250$. Muscle fibres are variable in size and connective tissue is increased. Basophilic fibres are scattered. (B) Electron microscopy, $\times 10,000$. Abnormal mitochondria with paracrystalline inclusion bodies. (C) Electron microscopy, $\times 4,000$. The upper fibre contains many giant mitochondria.

(0.3–0.9); arterial blood showed pH 7.37, pO₂ 105 mm Hg, pCO₂ 32 mm Hg, base excess 5.4 mmol/l; CSF lactate was 76.9 mg/dl (mean:19.0) and pyruvate was 3.0 mg/dl (mean:1.02). EEG showed marked slowing of the background activity, irregular slow waves at the left temporal area and bilateral synchronous spike and wave bursts. Periodic sharp waves were transiently seen at the left temporal area. A high-amplitude somatosensory evoked potential (SEP) was evoked bilaterally by median nerve stimulation with a wave form abnormality similar to that of myoclonic epilepsy. A giant SEP with C response was evoked by the left median nerve stimulation. No visual evoked potential was evoked by stimulation of the right hemifield in checkerboard pattern reversal, but normal P100 was evoked by stimulation of the left hemifield. Auditory brainstem responses were normal. Needle EMG revealed low amplitude polyphasic motor unit potentials in the muscles of the right arm; no abnormalities were noted in the other muscles. Nerve conduction velocities were normal in the right median, sural and tibial nerves.

Brain CT scan on admission showed a low density area with irregular enhancement in the left temporo-parietal lobe, which disappeared 2 weeks later when another low density area was found in the left occipital lobe. Cerebral angiography showed hypervascularity at the left occipital lobe, which was considered to reflect hyperaemia after infarction.

Marked elevation of blood lactate and pyruvate was noted after slight exercise. No hypoglycaemia was found during 36 hours of fasting. Blood glucose increased normally after glucagon injection and venous lactate increased normally after forearm ischaemic exercise. Glucose, alanine and galactose tolerance tests caused elevation of lactate. Hypoglycaemia was not induced by fructose, glycerol or alanine loads.

The patient's clinical state and blood lactate and pyruvate levels were not influenced by high-fat or high-carbohydrate diets or by intravenous fat infusion.

Paraphasia and memory disturbances disappeared soon after admission. The reading difficulty worsened during the first month of admission, then improved and reading was normal by the tenth week. Agraphia also improved but incompletely. Serum enzymes returned to normal values within 2 weeks. She had an episode of status epilepticus after intense exercise, followed by postictal stupor. This episode was accompanied by marked elevation of serum lactate and pyruvate and severe metabolic acidosis.

Results

Morphology With the haematoxylin-eosin stain, muscle fibres ranged 20–60 μ m (mainly 40–50 μ m) in diameter and a slight increase in endomysial connective tissue was seen. With the modified trichrome stain approximately 5 to 10% of fibres were so-called "ragged-red" fibres, which showed increased subsarcolemmal activity in NADH tetrazolium reductase stain. Increased subsarcolemmal activities in scattered fibres were also seen in succinic dehydrogenase and PAS stain. But Sudan III stain did not show apparent lipid deposits. Acid phosphatase was negative. With the myosin ATPase normal checkerboard pattern was preserved. With ubiquinone and cytochrome oxidase stain 5–10% of fibres with dense subsarcolemmal staining were seen scattered respectively and no apparent decrease in overall activity compared with the controls was observed. With mitochondrial ATPase there was no difference between the case and the control. Electron microscopy showed increased number and size of the mitochondria, packed cristae and peculiar inclusion bodies (fig).

Biochemistry The muscle biopsy was stored at –80°C until use. Alpha-keto acid dehydrogenase complex and component enzymes, pyruvate carboxylase and lactate dehydrogenase were assayed by the methods of Koike *et al.*,² Atkin *et al.*³ and Okabe

Table 1 Enzymes concerned with pyruvate metabolism

	Patient	Control
Pyruvate dehydrogenase complex	35.1	1–15
Pyruvate dehydrogenase	3.1	1–2.3
Lipoate acetyl transferase	106	100–400
Lipoamide dehydrogenase	3600	1000–3000
Pyruvate carboxylase	3.9	1–18
Lactate dehydrogenase	69.7 $\times 10^3$	50–250 $\times 10^3$
2-oxoglutarate dehydrogenase complex	44.7	2–20
2-oxoglutarate dehydrogenase	13.8	2–24

Values expressed as nanomoles substrate utilised/h/mg protein.

Table 2 Mitochondrial enzymes

	Crude preparation		Mitochondrial fraction	
	Patient	Control range	Patient	Control range
Cyt. c oxidase	2.65	1.76–3.74 (31)	572	202–534 (12)
Succinate-cyt. c reductase	1.25	0.51–1.89 (21)	514	137–370 (12)
NADH-cyt. c reductase (rotenone sensitive)			228	162–248 (9)
Citrate synthase	15.02	4.12–11.77 (13)	350	412–735 (6)
NADH dehydrogenase	39.37	16.13–45.2 (18)	1,280	1,980–2,888 (4)
Succinate dehydrogenase	1.28	0.51–1.65 (14)		
Carnitine acyltransferase	2.36	0.97–2.73 (21)	111	93–155 (4)

Values expressed as μ mol substrate utilised/min/gm fresh tissue (crude preparation) or mmol/min/mg protein (mitochondrial fraction). Number of controls in parenthesis.

Table 3 Content of cytochromes in muscle mitochondria calculated from reduced-minus-oxidised spectra.³

	Patient	Control range
Cytochrome b	488	427– 774 (15)
cc ₁	487	376–1009 (15)
aa ₃	305	372– 960 (15)

Cytochromes were reduced by addition of dithionite. Number of controls in parenthesis.

Table 4 Beta-oxidation enzymes in muscle biopsies of the patient and controls

Beta-oxidation enzymes	Patient	Controls
I. Butyryl-CoA dehydrogenase	0.88	0.89 ± 0.34 (13)
Octanoyl-CoA dehydrogenase	0.83	0.73 ± 0.20 (11)
Palmitoyl-CoA dehydrogenase	1.01	0.84 ± 0.22 (11)
II. Crotonase	12.77	15.30 ± 4.60 (12)
III. Beta-hydroxy-acyl-CoA dehydrogenase	3.91	3.40 ± 1.35 (12)
IV. Aceto-acetyl-CoA thiolase (KCl)	5.93	5.10 ± 1.97 (13)
3-Keto-acyl-CoA thiolase	2.43	1.95 ± 0.50 (13)

Enzyme activities are expressed as $\mu\text{mol/min/g}$ fresh tissue. Control values are means $\pm$ SD. Number of controls in parenthesis.

al,⁴ respectively. Activities of PDH complex, pyruvate carboxylase, lactate dehydrogenase and 2-oxoglutarate dehydrogenase complex were normal (table 1).

Enzymatic studies of isolated muscle mitochondria⁵ showed normal activities of cytochrome c oxidase, succinate-cytochrome c reductase and NADH-cytochrome c reductase (table 2). The concentration of cytochromes were calculated from reduced-minus-oxidised spectra (table 3): they were normal except for a slight reduction of cytochrome aa₃. The activities of all enzymes of beta-oxidation⁶ were normal (table 4).

Discussion

Among the various syndromes included under the heading of mitochondrial encephalomyopathy, Kearns-Sayre syndrome,^{7,8} myoclonic epilepsy with ragged-red fibres^{9,10} and MELAS¹ are the best defined clinical entities.¹¹ MELAS is characterised by early normal development, short stature, episodic headache and vomiting, cortical blindness, hemianopia, hemiparesis, seizures and dementia. Some patients with MELAS lack muscle symptoms,^{12,13} while most of them have muscle weakness and atrophy before the development of the CNS symptoms. Although our patient lacked muscle symptoms initially, she can be classified as a case of MELAS on the basis of her CNS symptomatology.

Elevated resting levels of lactate and pyruvate in blood and CSF, which are further increased by slight exercise, are commonly observed in most mitochondrial encephalomyopathies and are believed to result from the disturbance of oxidative pathways. Two cases of MELAS reported by Pavlakis *et al*¹ had a partial defect of cytochrome c oxidase in skeletal muscle. Holliday *et al*¹² suggested that a syndrome similar to MELAS might be due to a defect of NADH-CoQ reductase. Stronger evidence that defects of complex I of the respiratory chain may be involved in at least some cases of MELAS comes from biochemical studies of Morgan-Hughes *et al*¹³ and Kobayashi *et al*¹⁴ in three patients. In our patient, mitochondrial enzymes, including those of the respiratory chain and beta-oxidation, were normal. Enzymes of glycogen metabolism and gluconeogenesis were also considered normal because of the normal responses to ischaemic exercise, fasting, loading test with various sugars and glucagon administration. These results raise the possibility that mitochondrial changes in our patient may be secondary. Morphological abnormalities of mitochondria are observed in various conditions,^{15–18} including chronic hypoxia. The serum lactate level and the lactate/pyruvate ratio are dependent on the state of tissue oxidation. The increased lactate level and lactate-pyruvate ratio in our patient could have resulted from the chronic circulatory insufficiency and the subsequent anaerobic metabolism of muscle. The hypothesis that mitochondrial changes in some MELAS patients may be due to chronic hypoxia would be in agreement with the report by Kobayashi *et al*¹⁹ who found severe alteration of the endothelial cells of muscle capillaries.

In cytochrome c oxidase deficiency, a patient with myopathy showed the defect confined to the skeletal muscle and normal activities in the other organs such as heart, liver, kidney and brain.⁵ Myopathic patients with the similar defect who had cardiac or renal disorder showed the decreased activity in the heart or kidney respectively in addition to the defect in the skeletal muscle.²⁰ Our patient showed minimal muscle symptoms and a smaller number of "ragged-red" fibres compared with the previous cases. It is possible that the biochemical defect which was not found in the skeletal muscle might involve the other organs including the brain in particular which our patient's disease predominantly affected.

Two cases with MELAS examined postmortem showed loss of neurons, formation of cysts and proliferation of vessels, findings compatible with progressive poliodystrophy.^{21,22} A case of mitochondrial encephalomyopathy without a stroke-like symptom revealed wide-spread cortical infarcts at necropsy which did not correspond to the territories

of the main vessels,²³ thus suggesting a pathogenic mechanism different from thrombosis or embolism. There are few descriptions of brain CT findings in patients with MELAS.^{12 19 21 22 24} In our case and in the case described by Kobayashi *et al*¹⁹ there was an initial low density area which later disappeared while another low density appeared at a different site. This finding again suggests a process different from common cerebrovascular accidents.

References

- Pavakis SG, Phillips PC, DiMauro S, DeVivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes: a distinctive clinical syndrome associated with ragged-red fibers. *Ann Neurol* 1983;14:364-5.
- Koike M, Koike K. Biochemical properties of mammalian 2-oxo acid dehydrogenase multienzyme complexes and clinical relevancy with chronic lactic acidosis. *Ann NY Acad Sci* 1982;378:225-35.
- Atkin BM, Utter MF, Weinberg MB. Pyruvate carboxylase and phosphoenolpyruvate carboxykinase activity in leukocytes and fibroblasts from a patient with pyruvate carboxylase deficiency. *Pediatr Res* 1979;13:38-43.
- Okabe K, Hayakawa T, Hamada M, Koike M. Purification and comparative properties of human lactate dehydrogenase isozymes from uterus, uterine myoma and cervical cancer. *Biochemistry* 1968;7:79-90.
- Bresolin N, Zeviani M, Bonilla E, *et al*. Fatal infantile cytochrome c oxidase deficiency: decrease of immunologically detectable enzyme in muscle. *Neurology* 1985;35:802-12.
- Trevisan CP, Reichmann H, DeVivo DC, DiMauro S. Beta-oxidation enzymes in normal human muscle and in muscle from a patient with an unusual form of myopathic carnitine deficiency. *Muscle Nerve* 1985;8:672-5.
- Berenberg RA, Pellock JM, DiMauro S, *et al*. Lumping or splitting? "Ophthalmoplegia-plus" or Kearns-Sayre syndrome? *Ann Neurol* 1977;1:37-54.
- Kearns TP, Sayre GP. Retinitis pigmentosa, external ophthalmoplegia and complete heart block. Unusual syndrome with histologic study in one of two cases. *Arch Ophthalmol* 1958;60:280-9.
- Fitzsimons RB, Clifton-Bligh P, Wolfenden WH. Mitochondrial myopathy and lactic acidemia with myoclonic epilepsy, ataxia and hypothalamic infertility. A variant of Ramsay-Hunt syndrome. *J Neurol Neurosurg Psychiatry* 1981;44:79-82.
- Fukuhara N, Tokiguchi S, Shirakawa K, Tsubaki T. Myoclonus epilepsy associated with ragged-red fibers (mitochondrial abnormalities): disease entity or a syndrome? Light- and electron-microscopic studies of two cases and review of literature. *J Neurol Sci* 1980;47:117-33.
- DiMauro S, Bonilla E, Zeviani M, Nakagawa M, DeVivo DC. Mitochondrial myopathies. *Ann Neurol* 1985;17:521-38.
- Holliday PL, Climie ARW, Gilroy J, Mahmud MZ. Mitochondrial myopathy and encephalopathy: three cases—a deficiency of NADH-CoQ dehydrogenase? *Neurology* 1983;33:1619-22.
- Morgan-Hughes JA, Hayes DJ, Clark JB, *et al*. Mitochondrial encephalomyopathies. Biochemical studies in two cases revealing defects in the respiratory chain. *Brain* 1982;105:553-82.
- Kobayashi M, Morishita H, Sugiyama N, *et al*. Two cases of NADH-CoQ reductase deficiency: relationship to MELAS. *Ann Neurol* (in press).
- Chou SM. "Megaconial" mitochondria observed in a case of chronic polymyositis. *Acta Neuropathol (Berl)* 1969;12:68-89.
- Norris FH, Panner BJ. Hypothyroid myopathy. Clinical, electromyographical and ultrastructural observations. *Arch Neurol* 1966;14:574-89.
- Shafiq SA, Milhorat AT, Gorycki MA. Crystals in muscle fibers in patients with diabetic amyotrophy and neuropathy. *Neurology* 1968;18:785-90.
- Teravainen H, Makitie J. Striated muscle ultrastructure in intermittent claudication. *Arch Pathol Lab Med* 1977;101:230-5.
- Kobayashi Y, Miyabayashi S, Takada G, *et al*. Ultrastructural study of the childhood mitochondrial myopathic syndrome associated with lactic acidosis. *Eur J Pediatr* 1982;139:25-30.
- Zeviani M, Moggio M, Bonilla E, *et al*. Cytochrome-c-oxidase deficiency: clinical and biochemical heterogeneity. *Neurology* 1985;35(Suppl 1):95.
- Hart ZH, Chang CH, Perrin EVD, Naerunjun JS, Ayyar R. Familial poliodystrophy, mitochondrial myopathy and lactic acidemia. *Arch Neurol* 1977;34:180-5.
- Shapira Y, Cederbaum SD, Cancilla PA, Nielsen D, Lippe BM. Familial poliodystrophy, mitochondrial myopathy and lactic acidemia. *Neurology* 1975;25:614-21.
- Kuriyama M, Umezaki H, Fukuda Y, *et al*. Mitochondrial encephalomyopathy with lactate-pyruvate elevation and brain infarctions. *Neurology* 1984;34:72-7.
- Skoglund RR. Reversible alexia, mitochondrial myopathy and lactic acidemia. *Neurology* 1979;29:717-20.