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Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy

(metabolic disease/mitochondrial electron transport system/lactic acidemia)

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ABSTRACT **The electron transport system of muscle mitochondria was examined in a familial syndrome of lactacidemia, mitochondrial myopathy, and encephalopathy. The propositus, a 14-year-old female, and her 12-year-old sister had suffered from progressive muscle weakness, abnormal fatigability, and central nervous system dysfunction since early childhood. In the propositus, the state 3 respiratory rate of muscle mitochondria with NADH-linked substrates and with succinate was markedly reduced. The levels of cytochromes $a + a_3$, b, and $c + c_1$ were normal. The activities of complexes I, II, III, and IV of the electron transport chain were normal or increased. By contrast, the activities of complex I-III and of complex II-III, both of which need coenzyme Q_{10} (CoQ_{10}), were abnormally low. On direct measurement, the mitochondrial CoQ_{10} content was 3.7% of the mean value observed in 10 controls. Serum and cultured fibroblasts of the propositus had normal CoQ_{10} contents. In the younger sister, the respiratory activities and CoQ_{10} level of muscle mitochondria were similar to those observed in the propositus. The findings establish CoQ_{10} deficiency as a cause of a familial mitochondrial cytopathy and suggest that the disease results from a tissue-specific defect of CoQ_{10} biosynthesis.**

The transduction of oxidative energy to a proton motive force and the utilization of the protonic energy for ATP synthesis are catalyzed at the inner mitochondrial membrane by five discrete multisubunit complexes (complexes I, II, III, IV, and V), ubiquinone, or coenzyme Q (CoQ), and cytochrome *c*. Complex I [NADH dehydrogenase (NADH:ubiquinone oxidoreductase, EC 1.6.5.3)], complex II [succinate dehydrogenase (succinate:ubiquinone oxidoreductase, EC 1.3.5.1)], complex III [ubiquinol–cytochrome-*c* reductase (ubiquinol:ferricytochrome-*c* oxidoreductase, EC 1.10.2.2)], complex IV [cytochrome-*c* oxidase (ferrocytochrome-*c*:oxygen oxidoreductase, EC 1.9.3.1)], CoQ, and cytochrome *c* comprise the electron transport chain that generates the proton gradient utilized by complex V [ATP synthase (H^+-transporting ATP phosphohydrolase, EC 3.6.1.34)] for ATP synthesis (for reviews, see refs. 1 and 2). CoQ, a lipid quinone, is the electron carrier between flavine-linked dehydrogenases (complexes I and II) and complex III. CoQ may also function as a transmembrane hydrogen carrier, thereby stoichiometrically coupling proton translocation to respiration (3–5).

Since the description of a hypermetabolic mitochondrial myopathy by Luft *et al.* (6), many disorders of mitochondrial energy metabolism have been reported (for reviews, see refs. 7 and 8). For example, clinical and biochemical features of syndromes associated with deficiencies of complex I (9, 10), III (11, 12), IV (13, 14), and V (15, 16) have been described. However, previous studies of mitochondrial energy metabolism have focused on the electron transport complexes and their subunits; unambiguous, marked deficiency of CoQ in humans has not been observed to date. We here show that a selective deficiency of CoQ_{10} is the primary cause of abnormal energy metabolism in a familial syndrome of lactacidemia, mitochondrial myopathy, and encephalopathy.

MATERIALS AND METHODS

Clinical Data. Two sisters (patients 1 and 2), now 14 and 12 years of age, are the only children of unrelated parents. Both were products of a normal gestation and delivery and had normal early development. After the age of 3 years, they showed abnormal fatigability on exertion and developed slowly progressive weakness of trunkal and proximal limb muscles. The cranial muscles were unaffected. After the age of 5, learning disability was noted in both sisters. At age 7, the younger sister developed a generalized seizure disorder. This was eventually controlled with 750 mg of sodium valproate per day. The older sister never had seizures, but the electroencephalogram in both sisters showed similar bioccipital slowing and epileptiform abnormalities. Since age 12, the older sister has had slowly progressive cerebellar symptoms consisting of dysarthria, limb ataxia, and tremor of the head and trunk. Myoglobinuria occurred in the older sister after an upper respiratory infection at age 10. The younger sister had episodes of myoglobinuria after some of her major seizures. Since the age of 5, the sisters' heights have remained between the 2nd and 5th percentile. Neither sister had clinical or laboratory evidence for retinal, cochlear, hepatic, renal, or cardiac dysfunction. There was no history of a similar illness in the family.

Both sisters had lactacidemia at rest (3.6–10 mmol/liter) and a 2- to 20-fold elevation of the serum creatine kinase level. Serum amino acid analyses gave normal results. Electromyographic studies were consistent with a myopathy. Biceps muscle specimens of both sisters in 1983 and a vastus lateralis muscle specimen from the older sister in 1986 and from the younger one in 1988 showed mitochondrial and lipid excess in type I muscle fibers and scattered necrotic fibers. The free and total carnitine levels in serum were normal in both sisters in 1985, but they were 20–30% below normal in 1988; free and total carnitine levels in muscle were normal in the older sister in 1986, but total muscle carnitine was 30% below normal in the younger sister in 1988.

Control subjects were patients with a family history of susceptibility to malignant hyperthermia in whom this diagnosis was excluded by the caffeine and halothane contracture tests or patients with muscle symptoms. All were eventually shown to be free of muscle disease by clinical and histological criteria.

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Abbreviation: CoQ, coenzyme Q.

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Isolation of Muscle Mitochondria. Mitochondria were isolated from the vastus lateralis muscle by a minor modification of the procedure of Makinen and Lee (17). Protease digestion of the minced tissue was omitted. Isolation medium A consisted of 100 mM KCl, 50 mM Tris·HCl (pH 7.4), 1 mM ATP, 5 mM $MgCl_2$, 1 mM EGTA, and 0.5% (wt/vol) bovine serum albumin. Isolation medium B consisted of 100 mM KCl, 50 mM Tris·HCl (pH 7.4), 0.2 mM ATP, 1 mM $MgCl_2$, 0.2 mM EGTA, and 1% bovine serum albumin. The initial centrifugation of the muscle homogenate was at 650 × *g* for 10 min. The pellet was resuspended in isolation medium A, rehomogenized, and centrifuged at 650 × *g* for 10 min. The combined supernatants were centrifuged at 650 × *g* for 10 min, and the pellet was discarded. The supernatant was centrifuged at 14,000 × *g* for 10 min. The pellet was resuspended in isolation medium B and centrifuged at 8000 × *g* for 10 min. The fluffy layer of the pellet was removed by rinsing its surface with 0.25 M sucrose. The pellet was washed twice with 0.25 M sucrose and then suspended in 0.25 M sucrose at about 10 mg of protein per ml. Protein was measured by the method of Lowry *et al.* (18).

Polarographic Studies. Mitochondrial respiration was analyzed with a Clark electrode at 30°C. The respiratory medium contained 0.225 M sucrose, 10 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 10 mM potassium phosphate buffer, and 10 mM Tris·HCl buffer (pH 7.4). Substrates included 5 mM pyruvate/1 mM malate, 5 mM glutamate/1 mM malate, and 5 mM succinate/1 μg of rotenone per ml. ADP was added in amounts of 100–300 nmol. The respiratory control and ADP/O ratios were calculated by the method of Chance and Williams (19).

Cytochrome Analysis. The cytochrome content of mitochondria was determined from the reduced-minus-oxidized difference spectra recorded at room temperature, as described by Bookelman *et al.* (20).

Enzyme Assays. Sucrose suspensions of mitochondria stored at −80°C were thawed and diluted with a solution containing 150 mM KCl, 1 mM $MgCl_2$, 50 mM Tris·HCl (pH 7.4), and 0.5% bovine serum albumin to a mitochondrial protein concentration of 0.5 mg/ml. The suspensions were then sonicated on ice three times at 50 W for 5-sec intervals interrupted by 15-sec cooling periods. NADH dehydrogenase (complex I) activity was measured by following the oxidation of NADH at 340 nm in the presence of CoQ_1 (21). The rotenone-sensitive initial activity was assumed to be the activity of complex I. Succinate dehydrogenase (complex II) activity was determined by the reduction of 2,6-dichloroindophenol in the presence of CoQ_1 (22). Ubiquinol-cytochrome-*c* reductase (complex III) was assayed as described by Hatefi (23), but with reduced CoQ_3 as substrate. The antimycin-sensitive initial activity was assumed to be the activity of complex III. Cytochrome-*c* oxidase (complex IV) activity was measured by the method of Wharton and Tzagoloff (24). The final concentration of reduced cytochrome *c* was 58 nmol/ml. Rotenone-sensitive NADH:cytochrome-*c* oxidoreductase (NADH dehyrogenase, EC 1.6.99.3) (complex I-III) activity was determined by the method of Hatefi and Rieske (25), and succinate:cytochrome-*c* oxidoreductase (EC 1.3.99.1) (complex II-III) activity was assayed as described by Tisdale (26).

Measurement of CoQ_{10}. CoQ_{10} was extracted at 4°C from muscle mitochondria, fibroblasts, and serum with ethanol/*n*-hexane, 2:5 (vol/vol) and determined by reverse-phase HPLC and UV detection at 275 nm, as described by Takeda *et al.* (27). CoQ_9 was used as an internal standard. The stationary phase was octadecylsilane in a 250- × 4-mm Bio-Sil ODS-5S column (Bio-Rad). The mobile phase was prepared by dissolving 7 g of $NaClO_4 \cdot H_2O$ in 1000 ml of ethanol/methanol/70% $HClO_4$, 700:300:1 (vol/vol) (27). The flow rate was 1 ml/min. The HPLC measurements were performed at room temperature.

Fibroblast Cultures. Skin fibroblasts were cultured in monolayers in Eagle's essential medium containing 10% (vol/vol) fetal bovine serum and used between the fifth and seventh passages (28).

Reagents. Coenzymes Q_1, Q_3, Q_9, and Q_{10} were gifts from Eisai (Tokyo). All other reagents were from standard commercial suppliers.

RESULTS

Polarographic Studies of Muscle Mitochondria. In both patients, the oxygen consumption of muscle mitochondria in state 3 with NADH-linked substrates or succinate plus rotenone was only 16–30% of the corresponding mean control values (Table 1). The addition of 1 mM dinitrophenol did not cause higher oxygen consumption than that in state 3 (data not shown).

In patient 1, the mitochondria lost respiratory control after the first addition of ADP, and the respiratory control and ADP/O ratios could not be calculated. In patient 2, the mitochondria lost respiratory control after the second addition of ADP; the single respiratory control and ADP/O ratios obtained with each substrate were lower than normal, except with succinate for which the ADP/O ratio fell in the normal range (Table 1).

Mitochondrial Cytochrome Content and Electron Transport Complex Activities. These were determined in patient 1. Cytochrome *b* and $c + c_1$ levels were normal, but the cy-

Table 1. Polarographic assays of mitochondrial respiratory activities

	State 3 respiration*				Respiratory control ratio				ADP/O			
	Controls†		Patient‡		Controls†		Patient‡		Controls†		Patient‡	
Substrate	Mean ± SEM	Range	1	2	Mean ± SEM	Range	1	2	Mean ± SEM	Range	1	2
Pyruvate (5 mM) + malate (1 mM)	125 ± 9.62	80–195	24	30	5.3 ± 0.51	2.9–8.2	ND	1.5	3.3 ± 0.11	2.5–3.8	ND	2.2
Glutamate (5 mM) + malate (1 mM)	145 ± 8.55	88–207	24	38	6.0 ± 0.45	3.6–9.8	ND	1.4	3.1 ± 0.11	2.6–3.7	ND	2.2
Succinate (5 mM) + rotenone (1 μg/ml)	175 ± 10.16	110–242	40	52	3.8 ± 0.16	2.7–4.9	ND	1.4	1.8 ± 0.11	1.5–2.1	ND	1.7

ND, not detectable.

*Values indicate nanoatoms of oxygen per min per mg of mitochondrial protein.

†Fourteen control mitochondrial preparations were studied. With each preparation and substrate, state 3 respiration was induced three times by three consecutive additions of ADP, and the state 3 respiratory rates, ADP/O ratios, and respiratory control ratios (RCRs) were averaged. RCRs were calculated by dividing a given state 3 respiratory rate by the state 4 respiratory rate that appeared following the expenditure of ADP (19).

‡In patient 1, respiratory control was lost after the first addition of ADP, and the RCR and ADP/O ratio could not be calculated. In patient 2, respiratory control was lost after the second addition of ADP; the RCR and ADP/O ratio for each substrate indicate the results of a single determination.

Table 2. Cytochrome content of muscle mitochondria

Cytochrome	Content, pmol/mg of mitochondrial protein		
	Patient 1	Controls (n = 9)	
		Mean ± SEM	Range
$a + a_3$	542	410 ± 34	272–530
b	335	383 ± 37	251–614
$c + c_1$	399	582 ± 50	342–750

tochrome $a + a_3$ level was slightly higher than normal (Table 2). The activities of complexes I, III, and IV were normal and that of complex II was higher than normal (Table 3). By contrast, the activities of complex I-III and of complex II-III were reduced to 14–22% of the control mean (Table 3).

CoQ_{10} Levels in Muscle Mitochondria, Fibroblasts, and Serum. The CoQ_{10} content of muscle mitochondria was only 3.7% of the normal mean in patient 1 and 5.4% of the normal mean in patient 2 (Table 4 and Fig. 1). No abnormal peaks suggesting the accumulation intermediates of CoQ_{10} biosynthesis were detected in the patients' mitochondrial extracts by HPLC (Fig. 1). The fibroblast CoQ_{10} level, determined in patient 1, was also normal. The serum CoQ_{10} levels were normal in both patients (Table 4).

DISCUSSION

The impaired state 3 respiration of muscle mitochondria with complex I-dependent and complex II-dependent substrates could have been due to a defect involving both complex I and II, a defect involving only complex III, IV, or V, or to a deficiency of cytochrome c or CoQ_{10}. The failure of the uncoupler 2,4-dinitrophenol to increase the respiratory rate beyond the low level observed in state 3 implied a metabolic defect proximal to complex V (15, 16). The activities of complexes I, II, III, and IV and the mitochondrial cytochrome levels were not diminished. On the other hand, the activities of complex I-III and II-III, which require the presence of CoQ_{10} in the inner mitochondrial membrane, were markedly reduced. These findings strongly suggested that the CoQ_{10} content of the mitochondria was reduced, and this was confirmed by direct measurement.

The results establish that in the present disorder a marked CoQ_{10} deficiency is an isolated and, therefore, a primary defect in the mitochondrial electron transport system. The mode of transmission of the herein described disorder is uncertain, but the family history is consistent with autosomal recessive inheritance.

A decreased CoQ_{10} content of muscle mitochondria has been previously noted in some patients with the Kearns–Sayre syndrome (28). However, in these patients the mitochondrial CoQ_{10} levels were 20–30% of the control mean, and other components of the mitochondrial electron transport complex were also reduced (29). The combined abnormalities in these patients could stem from a yet unidentified perturbation of the organization or expression of mitochondrial respiratory chain components. A patient with oculoskeletal myopathy, exercise intolerance, and lactacidemia but without multisystem features also had mild CoQ_{10} deficiency in muscle mitochondria (30). However, the mitochondrial CoQ_{10} level [1.6 nmol (1380 ng) per mg of protein] was 28% of that observed in a single control, and this level is only slightly lower than the lowest CoQ_{10} level observed in our 10

Table 3. Enzyme activities of electron transport complexes in muscle mitochondria

Enzyme	Activity, nmol/min per mg of mitochondrial protein		
	Patient 1	Controls (n = 8)	
		Mean ± SEM	Range
Complex I*	151	139 ± 15.6	91–223
Complex II	444	309 ± 19.1	250–385
Complex III	3016	2872 ± 315	1163–3913
Complex IV	1946	2237 ± 179	1445–3061
Complex I-III*	80	367 ± 37.8	253–621
Complex II-III	80	564 ± 50	345–792

*Rotenone-sensitive activity.

Table 4. CoQ_{10} levels in muscle mitochondria, serum, and fibroblasts

Source	CoQ_{10} concentration*			
	Patient		Controls†	
	1	2	Mean ± SEM	Range
Muscle mitochondria	67	98	1811 ± 99	1440–2260
Fibroblasts	55	ND	48 ± 1.3	43–51
Serum	648	461	637 ± 84	264–941

ND, not determined.
*Concentrations are expressed as ng/mg of protein for mitochondria and fibroblasts and as ng/ml for serum.
†n = 10 for mitochondria; n = 5 for fibroblasts; and n = 8 for serum.

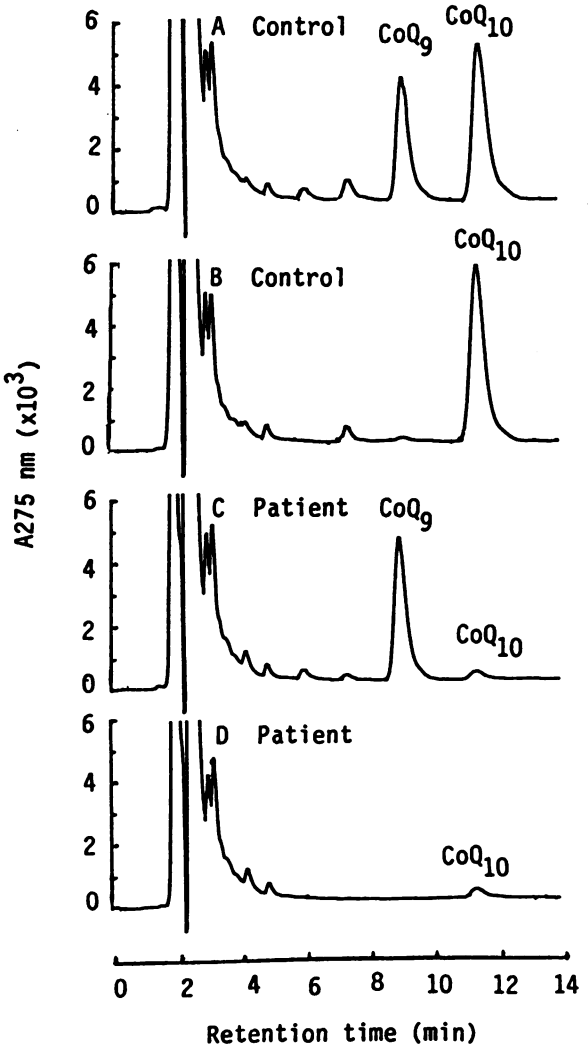

FIG. 1. HPLC chromatograms of n-hexane extracts of mitochondria from control muscle (A and B) and from muscle of patient 1 (C and D). Extracts corresponding to 200 μg of mitochondrial protein were applied. Two hundred nanograms of CoQ_9 was added to the mitochondrial suspension as an internal standard in A and C. Only trace amounts of CoQ_{10} can be detected in C and D.

control mitochondrial preparations (Table 4). Unambiguous and severe mitochondrial CoQ_{10} deficiency associated with a familial mitochondrial encephalomyopathy has not been documented to date.

The lipid excess in skeletal muscle fibers in CoQ deficiency is attributed to impaired fatty acid oxidation. This can be readily explained by the fact that during β-oxidation of fatty acids there is obligatory transfer of electrons to CoQ from electron-transferring flavoprotein and from complex I. Impaired fatty acid oxidation may also explain the mild carnitine depletion in serum and muscle (reviewed in ref. 31).

Recurrent myoglobinuria has not been reported in patients with deficiency of an electron transport complex (7, 8). The episodes of myoglobinuria in our patients, which appeared when muscle energy metabolism was stressed by fever or convulsions, attest to the central and essential role of CoQ_{10} in cellular energy metabolism.

Serum CoQ_{10} may be derived from CoQ_{10} in the diet or from an endogenous source (32). The normal CoQ_{10} level in serum and the very low CoQ_{10} level in muscle mitochondria in our patients indicate that the CoQ_{10} level in muscle mitochondria must be maintained by *de novo* synthesis. Thus, the normal CoQ_{10} content of fibroblasts and serum and the lack of involvement of other organs, such as heart, kidney, and liver, suggest a deficiency of a tissue-specific isoenzyme in the CoQ_{10} biosynthetic pathway in skeletal muscle and brain.

In eukaryotes the first committed step in CoQ biosynthesis, the alkylation of *p*-hydroxybenzoate by polyprenyl pyrophosphate, occurs at the inner mitochondrial membrane. The subsequent steps involve modification of the *p*-hydroxybenzoate moiety by hydroxylation, methylation, and decarboxylation (33). A number of CoQ-deficient mutants of bacteria and yeast have been described. In these mutants, accumulating intermediates of CoQ biosynthesis have been identified by TLC (33–36). However, we could not detect an accumulating intermediate in our patients' mitochondrial extracts by HPLC. Thus, although a block in CoQ_{10} biosynthesis seems likely, the present data do not eliminate the possibility of increased destruction of CoQ_{10}. Further studies will be required to clarify the mechanism of the CoQ_{10} deficiency in our patients.

Recognition of the CoQ_{10} deficiency syndrome is important because oral replacement therapy may prove to be of clinical benefit. After receiving 50 mg of CoQ_{10} three times daily for the past 3 months, the two sisters appear to fatigue less rapidly on physical exertion and are generally more alert.

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