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A case of mitochondrial encephalomyopathy associated with a muscle coenzyme Q₁₀ deficiency

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Abstract

We report severe coenzyme Q₁₀ deficiency of muscle in a 4-year-old boy presenting with progressive muscle weakness, seizures, cerebellar syndrome, and a raised cerebro-spinal fluid lactate concentration. State-3 respiratory rates of muscle mitochondria with glutamate, pyruvate, palmitoylcarnitine, and succinate as respiratory substrates were markedly reduced, whereas ascorbate/N,N,N',N'-tetramethyl-*p*-phenylenediamine were oxidized normally. The activities of complexes I, II, III and IV of the electron transport chain were normal, but the activities of complexes I+III and II+III, both systems requiring coenzyme Q₁₀ as an electron carrier, were dramatically decreased. These results suggested a defect in the mitochondrial coenzyme Q₁₀ content. This was confirmed by the direct assessment of coenzyme Q₁₀ level by high-performance liquid chromatography in patient's muscle homogenate and isolated mitochondria, revealing levels of 16% and 6% of the control values, respectively. We did not find any impairment of the respiratory chain either in a lymphoblastoid cell line or in skin cultured fibroblasts from the patient, suggesting that the coenzyme Q₁₀ depletion was tissue-specific. This is a new case of a muscle deficiency of mitochondrial coenzyme Q in a patient suffering from an encephalomyopathy. © 1998 Elsevier Science B.V.

Keywords: Respiratory chain; Coenzyme Q₁₀; Tissue-specificity; Encephalomyopathy

1. Introduction

A decreased activity of the oxidative phosphorylation system leading to a reduced cellular energy production and functional cell impairment is often due to a defect in one or more respiratory complex(es) of the mitochondrial respiratory chain (DiMauro, 1993). This multienzyme system, located in the inner mitochondrial membrane, is made up of five polypeptide complexes (I–V) and two mobile electron carriers: coenzyme Q (a lipid-soluble, quinone derivative with ten isoprene units in man) (Olson and Rudney, 1983) and cytochrome *c* (a small, extrinsic protein). Cytochrome *c* donates electrons from complex III

to complex IV (COX). Coenzyme Q₁₀ behaves as a homogeneously pooled redox carrier between flavin dehydrogenases and the cytochrome system (Kröger and Klingenberg, 1973), transferring reducing equivalents from complexes I and II to complex III. It may also translocate protons from the mitochondrial matrix to the intermembrane space, contributing to the energy conservation occurring at coupling site 2 of the respiratory chain (Trumpower, 1990). Reduced coenzyme Q₁₀ (ubiquinol-10) also acts as an antioxidant (Takayanagi et al., 1980; Ernster and Forsmark-Andrée, 1993; Ernster and Dallner, 1995), protecting mitochondrial inner membrane lipids and proteins, and mitochondrial DNA against oxidative damage. Several cases of multisystemic, partial coenzyme Q₁₀ defects have been reported in the literature (Ogasahara et

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al., 1985; Fischer et al., 1986; Zierz et al., 1989; Matsuoka et al., 1991). However, they were moderate and associated with defects of respiratory chain complexes (Yorifuji et al., 1985). By contrast, there has been only few reports of a near complete depletion of coenzyme Q₁₀ in the muscle tissue of patients presenting with mitochondrial encephalomyopathies (Ogasahara et al., 1989; Servidei et al., 1996; Sobreira et al., 1997). Here, we describe another patient presenting with generalized epilepsy, cerebellar ataxia, retinopathy, proximal muscle deficiency and a raised cerebro–spinal fluid (CSF) lactate concentration associated with a severe depletion of coenzyme Q₁₀ in muscle mitochondria.

2. Materials and methods

2.1. Case report

The patient was the second child of healthy, nonconsanguineous parents. He walked at 26 months but had proximal motor weakness. He presented myopathic symptoms with an elevated muscle creatine phosphokinase (CPK) at 3500 IU (normal value: 150 IU). At 2 years of age, he exhibited cerebellar symptoms with ataxia and tremor. At the same time, he had seizures with generalized spikes on the electroencephalogram. They were controlled by clonazepam. At 3 years of age, he had a proximal weakness with bilateral ptosis, cerebellar syndrome and a dysarthria with delayed language development. He looked hypotrophic (body weight: 14 kg; length: 84 cm). The electromyogram showed features of myopathy and both motor and sensory nerve conduction velocities were normal. He presented also a mild pigmentary degeneration of the retina evidenced by an abnormal electroretinogram. He had neither cardiac nor renal dysfunction. Magnetic resonance imaging showed a severe cerebellar atrophy without leucodystrophy. Biochemical assays revealed elevated lactatemia before and after meals with an elevated lactate/pyruvate ratio, but no paradoxal ketosis. The CSF lactate concentration was also elevated at 4 mmol/l. The organic acids and amino acids chromatographic profiles of serum, urine and CSF were normal, as were free fatty acids, triglycerides and cholesterol concentrations in the blood. The muscle biopsy showed irregular type I fibers with accumulation of succinate dehydrogenase (SDH) positive mitochondria (atypical ragged-red fibers) and of lipid droplets. After the biochemical investigations, a replacement therapy with coenzyme Q₁₀ was given orally (Ubiten: 60 to 250 mg per day). There was no neurological improvement, but the patient appeared to be less weak after 6 months of therapy. He could stand up alone and speak better. The biochemical abnormalities (hyperlactatemia and raised CSF lactate concentration) were similar except for the CPK value which normalized. Muscle and

skin biopsies, as blood samples, were obtained with the informed consent of the parents.

2.2. Skin fibroblasts and B-lymphoblastoid cell lines preparation

Skin fibroblasts and Epstein–Barr virus-transformed lymphocytes were grown in Royal Park Memorial Institute (RPMI) 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 100 µg/ml streptomycin and 100 IU/ml penicillin at 37°C under standard conditions. Cells (one dish of 75 cm² for fibroblasts or 50 millions of lymphoblasts) were pelleted and resuspended in phosphate buffered saline. The cells were submitted to three cycles of freezing–thawing before doing the spectrophotometric assays.

2.3. Muscle mitochondria preparation

Muscle mitochondria were isolated (Morgan-Hughes et al., 1977) by differential centrifugations following nagarse digestion and Ultra-Turrax homogenization of a muscle fragment from patient's quadriceps. Purified mitochondria were resuspended in the isolation medium. Protein concentration was determined by the bicinchoninic acid procedure (Smith et al., 1985).

2.4. Oxygen consumption assays

Oxygen consumption by freshly isolated skeletal muscle mitochondria (~100 µg) was measured with a Clark-type oxygen electrode in a total volume of 0.5 ml respiratory buffer (75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM mono[tris(hydroxymethyl)-aminoethane] (Trizma[®]) phosphate, 50 µM K₂EDTA, 10 mM Tris–HCl, pH 7.4) at 25°C using a Gilson oxygraph (Model 5/6).

Various respiratory substrates generating reducing equivalents to different sites of the respiratory chain system were investigated. The NAD⁺-linked substrates pyruvate (5 mM), glutamate (10 mM) and palmitoylcarnitine (80 µM), each added with malate (2.5 mM), the complex II substrate succinate (10 mM+rotenone 3 µM) and the complex IV substrates ascorbate (2 mM) plus N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD, 50 µM) were used.

The state-3/state-4 transition respirations were induced with additions of ADP (150 µM).

2.5. Enzymatic assays of respiratory chain enzymes and of the mitochondrial marker citrate synthase (CS)

Isolated muscle mitochondria, and fibroblast and lymphoblastoid cell line homogenates were used for enzymatic assays. The activities of complexes I and II were measured according to Birch-Machin et al. (1994). Complex III activity was measured according to Rustin et

al. (1994). Complexes I+III (NADHCR) activity was measured according to Ichiki et al. (1988); complexes II+III (SCR) and complex IV activities, according to Cooperstein and Lazarow (1951). Citrate synthase (CS) activity was assayed according to Srere (1969).

2.6. Coenzyme Q₁₀ determination

Coenzyme Q₁₀ concentrations were determined in skeletal muscle homogenates and mitochondria by reversed-phase high-performance liquid chromatography on a C₁₈ column (150 mm×4 mm; 5 μm Nucleosil), after extraction at 4°C with ethanol–*n*-hexane (2:5, v/v) and evaporation to complete desiccation under a stream of nitrogen. The mobile phase was prepared by dissolving 7 g of NaClO₄, H₂O in 1000 ml of ethanol–methanol–HClO₄ 70% (700:300:1). The flow-rate was 1 ml/min at room temperature. Coenzyme Q₁₀ was detected by UV light absorption (275 nm) and quantified every third sample, using decylubiquinone as a standard. Coenzyme Q₁₀ was concomitantly determined in skeletal muscle homogenates and mitochondria from 20 and five healthy patients, respectively.

3. Results

3.1. Polarographic studies of freshly isolated skeletal muscle mitochondria

The oxidative phosphorylation activity of freshly isolated mitochondria from normal skeletal muscle samples and propositus' tissues was studied by determining their oxygen consumption with and without ADP. Results are shown in Table 1. Normal skeletal muscle mitochondria exhibited high oxygen consumption rates in the presence of ADP with the various respiratory substrates used. Moreover, the normal respiratory control ratios found with each substrate suggest that we isolated tightly coupled, functionally intact mitochondria. By contrast, the oxygen consumption rates of the propositus' muscle mitochondria using NAD⁺-linked substrates, as well as succinate, were

dramatically reduced. However, the oxygen consumption rate with complex IV substrates ascorbate/TMPD ranged within normal values (Table 1).

3.2. Respiratory chain enzymes and mitochondrial marker

Respiratory complexes (I, II, III, I+III, II+III and IV) and the mitochondrial marker CS were assayed in isolated mitochondria from skeletal muscle of the patient and normal subjects. Respiratory complexes I, II, III and IV, and the mitochondrial marker CS from the patient's mitochondria showed normal activities (Table 2). Particular attention was paid to the kinetic parameters of complex III activity. The kinetic studies of the oxidoreductase activity in human skeletal muscle homogenates did not show any difference in the K_m for decylubiquinol between the propositus ($K_m=10.8\pm 2.2$ mM) and controls ($K_m=11.1\pm 2.3$ mM). However, complexes I+III and complexes II+III, both of which require coenzyme Q₁₀, presented highly reduced activities, representing 13% and 4% of the normal values, respectively (Table 2). The addition of 50 mM decylubiquinone in the SCR (complexes II+III) assay of the propositus' mitochondria restored the activity to 90% of the normal value (Table 2).

The activities of the respiratory complexes and of CS were also investigated in cultured skin fibroblasts and a lymphoblastoid cell line established from the patient's blood (Table 3). Complex IV and notably complexes II+III (the latter system being dependent on coenzyme Q₁₀ for normal function) were in the normal range in both tissues. Moreover, CS activity, an index of the mitochondrial population, was also normal in both tissues, suggesting a normal mitochondrial content.

3.3. Coenzyme Q₁₀ concentration in skeletal muscle homogenates and mitochondria

The coenzyme Q₁₀ concentration was determined in the homogenates and mitochondria from propositus' and control skeletal muscles (Table 4). The patient's muscle presented a dramatic decrease of the coenzyme Q₁₀ content

Table 1
Respiratory activity of skeletal muscle mitochondria from controls and the propositus

	NAD ⁺ -linked substrates						FAD-linked substrate		Complex IV substrates	
	Glutamate+malate		Pyruvate+malate		Palmitoylcarnitine+malate		Succinate (+rotenone)		Ascorbate+TMPD	
	State-3	RCR	State-3	RCR	State-3	RCR	State-3	RCR	State-3	RCR
Controls (n=13)	232±99	7.4±2.6	188±52	5.7±2.9	194±60	5.2±1.9	232±90	2.7±1.5	325±129	1
Propositus	<15	1	<15	1	<15	1	<15	1	380	1

Freshly isolated mitochondria (around 100 μg) were incubated in 0.5 ml respiratory buffer (75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM Trizma phosphate, 50 μM K₂EDTA, 10 mM Tris–HCl, pH 7.4) at 25°C. Substrate concentrations: 10 mM for glutamate and succinate; 5 mM for pyruvate; 2.5 mM for malate; 80 μM for palmitoylcarnitine; 2 mM for ascorbate and 50 μM for TMPD. Oxygen consumption was monitored with a Clark-type oxygen electrode as described in Section 2. Results (mean±S.D.) are expressed in ngatoms O/min/mg protein and refer to respiration in the presence of ADP (150 μM). The RCR data (mean±S.D.) correspond to the ratio of state-3 respiration (in presence of ADP) on state-4 respiration (after ADP expenditure). *n*, number of controls.

Table 2

Enzymatic activities of respiratory chain complexes and of the mitochondrial marker CS in skeletal muscle mitochondria from controls and the propositus

	Controls	Propositus
Complex I ^a	480±80	603
Complex II ^a	1322±389	1058
Complex III ^a	1495±259	1233
Complex IV ^b	4217±1541	2337
Complexes I+III ^a	890±188	117 (13%)
Complexes II+III ^c	1146±464	48 (4%)
Complexes II+III+decylubiquinone (50 µM) ^a	1146±464	1031
CS ^b	3313±637	3908

^a n=6.^b n=29.^c n=28.

Results (mean±S.D.) are expressed in nmol/min/mg protein. The percentage of remaining activity is given in parentheses. n, number of controls.

Table 3

SCR and COX activities in skin cultured fibroblasts and lymphoblastoid cell lines from controls and the propositus

	Fibroblasts		Lymphoblasts	
	Controls (n=40)	Propositus	Controls (n=8)	Propositus
COX (Complex IV)	65±6	75	81±14	75
SCR (Complexes II+III)	28±7	36	37±7	31
Citrate synthase (CS)	86±31	114	120±2	102
COX/CS	0.8±0.27	0.66	0.61±0.02	0.71
SCR/CS	0.25±0.12	0.19	0.25±0.06	0.22

Results are expressed in nmol/min/mg protein, mean±S.D. for the control values. n, number of controls.

in both homogenate and mitochondria, representing only 16% and 6% of the normal levels, respectively (Table 4).

3.4. Ultrastructure

Mitochondria isolated from the patient's muscle were either swollen or empty, while others had sparse, thin, or vesiculated cristae. The intermembrane space was increased and the electron density of the matrix was reduced. Only very few normal mitochondria may be seen, looking generally smaller than those isolated from a normal control muscle fragment.

4. Discussion

There is an increasing number of publications reporting either an increase or a decrease in coenzyme Q₁₀ levels in various pathological conditions in man and animals. These

changes are often partial and multisystemic. For example, coenzyme Q₁₀ was found increased in neuro-degenerative diseases, such as Alzheimer's disease (Söderberg et al., 1992), or slightly decreased in others, including cardiomyopathy (Mortensen, 1993), hepato-cellular carcinomas (Eggen et al., 1989) or Kearns–Sayre syndrome (Ogasahara et al., 1985; Fischer et al., 1986; Zierz et al., 1989; Matsuoka et al., 1991).

Here, we report another case of coenzyme Q₁₀ deficiency in the skeletal muscle tissue of a patient presenting with an encephalomyopathy. Muscle mitochondria were isolated and assayed polarographically and spectrophotometrically. A markedly reduced state-3 respiration was observed with all respiratory substrates tested, except for ascorbate/TMPD. These data suggest an impairment of the oxidative phosphorylation upstream to complex IV, either at the level of complex III which reduces cytochrome c, or at the level of coenzyme Q₁₀, which accepts reducing equivalents from complex I and complex II. Although the

Table 4

Coenzyme Q₁₀ levels in skeletal muscle mitochondria and homogenates from controls and the propositus

	Controls		Propositus	
	Homogenates (n=20)	Mitochondria (n=5)	Homogenate	Mitochondria
Coenzyme Q ₁₀ concentration	19±4.8	1856±112	3 (16%)	114 (6%)

Results (mean±S.D.) are expressed in µg coenzyme Q₁₀/g of tissue for homogenates and in µg coenzyme Q₁₀/mg protein for mitochondria. The coenzyme Q₁₀ levels are expressed for the propositus in % of the control values. n, number of controls.

polarographic data do not exclude the possibility of a defect in complex I and/or complex II and/or III activity, the impairment of any individual respiratory complexes was ruled out by spectrophotometric assessment. By contrast, complexes I+III (NADHCR) and complexes II+III (SCR) activities were strongly reduced in the patient's muscle mitochondria. Interestingly, the patient's muscle SCR activity was near fully restored by adding decylubiquinone in the reaction mixture, suggesting a defect in the mitochondrial coenzyme Q₁₀ content. Indeed, the coenzyme Q₁₀ level was found to be very low in the patient's muscle tissue (homogenate and mitochondria). By contrast, SCR and COX activities ranged within normal values in the skin fibroblasts and lymphoblasts homogenates. These results strongly support a decreased mitochondrial coenzyme Q₁₀ level in the propositus' skeletal muscle. The muscle biopsy showed a lipid excess likely due to an impaired fatty acid oxidation since electrons from complex I and from electron-transferring flavoprotein must be accepted by coenzyme Q₁₀ during this process (Engel, 1986). Electron microscopy showed many abnormal, swollen and emptied mitochondria. The morphological abnormalities, including the dearth of cristae are reminiscent of those obtained after perfusion of rat hearts with free radicals-generating media (Burton et al., 1984) and most likely reflect an impaired energy production due to the lack of oxidation of the substrates linked to complexes I and II of the respiratory chain. Taken together, these findings suggest that the present patient is affected by a lack of muscle mitochondrial coenzyme Q₁₀, similar to that described by Ogasahara et al. (1989); Servidei et al. (1996); Sobreira et al. (1997) in patients presenting with mitochondrial encephalomyopathy. Our case concerns the second child of healthy, nonconsanguineous parents. The pedigrees are consistent with an autosomal recessive inheritance. A specific enzymatic defect in the biosynthesis of the coenzyme Q₁₀ has not yet been established. In the present case, with the used methods, we have not detected any accumulation of intermediates neither from tyrosine to 4-hydroxybenzoate nor from fatty acids to decaprenyl-pyrophosphate in the blood, CSF and urine. Moreover, the elution profile of the propositus' homogenate and mitochondrial extracts did not reveal any additional peaks evocative of an accumulation of intermediates in the course of coenzyme Q₁₀ biosynthesis (data not shown). However, these results do not demonstrate a normal coenzyme Q₁₀ biosynthesis in the patient's skeletal muscle, and do not eliminate the possibility of an increased destruction (Ogasahara et al., 1989). A rapid degradation of coenzyme Q₁₀ in our patient's muscle due to a lack of coenzyme Q₁₀-binding protein in the complex III is unlikely since the K_m of complex III for decylubiquinol is similar to that of the control values. Furthermore, Western blotting analysis of the coenzyme Q₁₀-binding protein in complex III showed the presence of this 9.5 kD protein in normal amounts (data not shown).

Since the coenzyme Q₁₀ defect was only found in the muscle tissue of our patient, as in the patients described by Ogasahara et al. (1989), the coenzyme Q₁₀ level in muscle must be maintained by a de novo synthesis (Ogasahara et al., 1989). One may also speculate that the CNS and muscle symptoms are due to a similar mitochondrial coenzyme Q₁₀ depletion. After 6 months therapy by coenzyme Q₁₀, a muscle, but not cerebral (persistence of electroencephalogram abnormalities), improvement has been observed in the present case as in the two sisters described by Ogasahara et al. (1989). This only partial benefit could be explained by the presence of a severe cerebellar atrophy before the therapy started. These observations emphasize the essential function of coenzyme Q₁₀ in the mitochondrial energy metabolism, and suggest that both brain and muscle tissues share a common step in the synthesis of coenzyme Q₁₀. Obviously, further studies will be required to fully understand the tissue-selectivity of coenzyme Q₁₀ deficiencies.

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