



Acquired coenzyme Q10 deficiency in children with recurrent food intolerance and allergies

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ARTICLE INFO

Article history:

Received 12 April 2010

Received in revised form 6 August 2010

Accepted 24 August 2010

Available online 15 September 2010

Keywords:

Allergy

Coenzyme Q10

Food intolerance

Mitochondria

Muscle pathology

Respiratory chain

Electron transport chain

Ubiquinone

ABSTRACT

The current study evaluated 23 children (ages 2–16 years) with recurrent food intolerance and allergies for CoQ10 deficiency and mitochondrial abnormalities. Muscle biopsies were tested for CoQ10 levels, pathology, and mitochondrial respiratory chain (MRC) activities. Group 2 (age >10 years; n = 9) subjects had significantly decreased muscle CoQ10 than Group 1 (age <10 y; n = 14) subjects ($p = 0.001$) and 16 controls ($p < 0.05$). MRC activities were significantly lower in Group 2 than in Group 1 ($p < 0.05$). Muscle CoQ10 levels in study subjects were significantly correlated with duration of illness (adjusted $r^2 = 0.69$; $p = 0.012$; $n = 23$). Children with recurrent food intolerance and allergies may acquire CoQ10 deficiency with disease progression.

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Abbreviations: ATP, adenosine triphosphate; CF, cystic fibrosis; CoQ10, coenzyme Q10; CS, citrate synthase; CSF, cerebrospinal fluid; EGID, eosinophilic gastrointestinal disease; EM, electron microscopy; GERD, gastroesophageal reflux disease; HPLC, high performance liquid chromatography; LM, light microscopy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; MRC, mitochondrial respiratory chain; OXPHOS, oxidative phosphorylation; PKU, phenylketonuria; RWE, ragweed pollen extract; SSMA, subsarcolemmal mitochondrial aggregates; UQCRC2, ubiquinol-cytochrome c reductase core II protein; I, respiratory complex I, i.e. NADH-ferricyanide reductase; II, respiratory complex II, i.e. succinate dehydrogenase; III, respiratory complex III, i.e. decylubiquinol-cytochrome c reductase; IV, respiratory complex IV, i.e. cytochrome c oxidase; respiratory complex I + III, NADH-cytochrome c reductase (rotenone-sensitive); II + III, respiratory complex II + III, i.e. succinate-cytochrome c reductase (antimycin-sensitive).

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1. Introduction

Although the importance of coenzyme Q10 (CoQ10) in mitochondrial function is widely recognized, the significance of CoQ10 deficiency has only recently been considered to have clinical importance. Causes of CoQ10 deficiency have been described as primary or secondary. Primary CoQ10 deficiency has been associated with mutations of several genes involved in CoQ10 biosynthesis (Quinzii et al., 2007; Rötig et al., 2007), and seems to be relatively rare (Quinzii et al., 2007). However, there is growing evidence that secondary or acquired CoQ10 deficiency may be much more common than primary deficiency. Low levels of CoQ10 in plasma and serum have been reported in a number of disorders, including phenylketonuria (PKU) (Artuch et al., 1999), asthma (Gazdik et al., 2002), migraine headaches (Hershey et al., 2007), Friedreich's ataxia (Cooper et al., 2008), cystic fibrosis (Laguna et al., 2008), and congestive heart failure (Molyneux et al., 2008). However, none of these reports was able to conclusively demonstrate the presence of CoQ10 deficiency by evaluation of CoQ10 content in tissue.

In this report we describe CoQ10, pathologic, and mitochondrial respiratory chain (MRC) abnormalities in a selected group of children with suspected eosinophilic gastrointestinal disease (EGID). We hypothesize that children with food intolerance requiring an elemental (amino acid-based) diet may be at risk of CoQ10 deficiency and mitochondrial dysfunction.

2. Material and methods

2.1. Preliminary considerations

This study was a prospective cohort study of children with severe food intolerance, who were suspected of having a mitochondrial disorder. Subjects in this select subgroup came from a larger population of children who were seen in a multidisciplinary eosinophilic GI disease clinic for second opinion regarding diagnosis or treatment. These children had histories of feeding intolerance, food allergies, and eosinophilia of the gastrointestinal mucosa requiring elemental (amino acid-based) formula or total parenteral nutrition. Although their nutritional status was supported by the formula or TPN, they continued to experience symptoms from intestinal dysmotility, abdominal pain, or other neuromuscular complaints that raised concern for an underlying mitochondrial disorder. None had a history of previous CoQ10 supplementation. All had undergone open muscle biopsies to evaluate mitochondrial respiratory chain (MRC) activities, pathology, and muscle CoQ10 content. Plasma CoQ10 levels were obtained at the time of muscle biopsy whenever possible. This study was approved by the Institutional Review Board of the Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, Ohio.

2.2. Pathology methods and procedures

Muscle specimens were collected from the *quadriceps femoris* of patients (<17 years) between November, 2005 and July, 2009. Specimens were delivered to the CCHMC Division of Pathology and Laboratory Medicine immediately after resection. Specimens were evaluated for light microscopic (LM) and electron microscopic (EM) evidence of mitochondrial disease by a pediatric pathologist (LM), who has extensive experience in neuromuscular diseases. The pathologist was blinded from MRC and CoQ10 analysis results. The percentage of myofibers with subsarcolemmal mitochondrial aggregates (SSMA) was counted in a representative high-power field of 100–300 myofibers using succinate dehydrogenase (SDH) stained slides (Miles et al., 2005). A subgroup of SSMA, i.e. large SSMA, was also determined. The large SSMA group was defined as the thickness of SSMA >3 μ m by using SDH stained slides (Miles et al., 2006). The numerical proportion of type 1 myofibers was estimated by using ATPase stained slides. Type 1 predominance was defined by the presence of 60% or more of type 1 myofibers.

All muscle specimens were evaluated by EM. Pathological mitochondria were defined by the presence of unequivocally pathologic mitochondria, i.e. based upon the abnormal arrangement or deficiency of cristae, abnormal matrix density, or matrix inclusions. Matrix density abnormalities were only assessed in specimens with optimal fixation. For a detailed description of procedures see the earlier description (Miles et al., 2006).

2.3. MRC activity assessment

A portion of each muscle specimen was flash frozen within 10 min after collection, and then stored at -70°C until shipment to the Center for Inherited Disorders of Energy Metabolism at Rainbow Babies and Children's Hospital, Cleveland, Ohio, for MRC complex activity analysis. Abnormal MRC complex activity is defined as complex activity <20% of the mean laboratory control. This cutoff

was suggested as a major diagnostic criterion for respiratory chain disorders in children (Bernier et al., 2002).

2.4. CoQ10 analysis

Excess residual muscle (approximately 20–40 mg) was also flash frozen within 10 min of removal, and stored at -70°C until CoQ10 analysis. CoQ10 content was measured in muscle and plasma using validated methods by high performance liquid chromatography (HPLC) with electrochemical detection in the CCHMC Clinical Laboratory (Tang et al., 2001, 2004). Total protein content of each specimen was determined as an index for CoQ10 content as described previously (Tang et al., 2004). Laboratory personnel were blinded from subject identifiers.

2.5. Controls

Clinical, MRC, and CoQ10 data of age-matched controls were extracted from the results of an earlier study (Miles et al., 2008). Controls were selected according to the following criteria: 1) age 1–16 years; 2) citrate synthase (CS) >60% (% mean control value) activity; 3) all MRC complex enzyme activities >50% (% mean control value); and 4) no pathologically or clinically diagnosed muscle disease.

2.6. Data stratification

Study data were stratified according to subject age, i.e. Group 1 (<10 y) and Group 2 (>10 y). Dichotomization of data was based upon recent reports which suggest the natural history of EGIDs, e.g. eosinophilic esophagitis, and gastrointestinal reflux disease (GERD), may progress from feeding difficulties in young children to food impaction and esophageal stricture in older children and adolescents (Fig. 1) (Ferguson and Foxx-Orenstein, 2007; Putnam and Rothenberg, 2009; Sant'Anna et al., 2004; Spergel et al., 2009). Based upon these reports, data were stratified according to age <10 years (Group 1) and >10 years (Group 2) in order to evaluate changes in CoQ10 and mitochondria which might occur in association with early vs. late disease features.

2.7. Data analysis

The Wilcoxon rank-sum test was used for dichotomous group comparisons of continuous variables, and exact methods were used because of the relatively small sample sizes, though the results did not change much from using the chi-square approximation. Spearman rank-order correlation coefficients (r_s) were determined between muscle and plasma CoQ10 levels, and between muscle and plasma

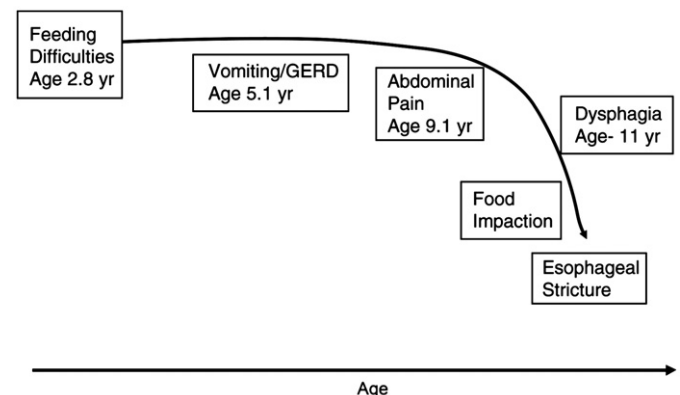


Fig. 1. Potential natural history of EGID/GERD (mean age of presentation). EGID, eosinophilic gastrointestinal disease; and GERD, gastroesophageal reflux disease. (Spergel et al., 2009; with permission).

CoQ10 vs. individual MRC activities. Factors associated with CoQ10 deficiency were evaluated using regression modeling of muscle CoQ10 vs. age, BMI, MRC complex activities (I, II, III, IV), and time interval from the onset of feeding difficulty symptoms to the date of muscle biopsy. Data are expressed as mean (SD) unless specified otherwise. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Clinical and laboratory findings

A total of 25 patients were screened and evaluated for CoQ10, pathology, and MRC complex defects during the study period. Two subjects were excluded from the study, including one with insuffi-

cient muscle specimen for CoQ10 analysis and another with a history of normal dietary intake. Group 1 (<10 years) and Group 2 (>10 years) characteristics, nutritional sources, and clinical features are summarized in Table 1. Study groups are similar for most clinical findings, except for expected growth-related differences including weight, height, and BMI (Table 1). Growth-related percentiles for weight, height, and BMI are similar for Groups 1 and 2 (Table 1). All subjects had a history of allergy disorders (Table 1). The nutritional status and dietary support of subjects in both groups are similar, except for the mean age at onset of feeding difficulty and the mean time interval between the onset of feeding difficulties to the muscle biopsy date, which are significantly increased in Group 2 subjects (Table 1). No significant differences between study Groups 1 and 2 are evident in laboratory testing and pathology findings (Table 2).

3.2. Muscle pathology

LM examination of muscle specimens revealed no definitive evidence of mitochondrial myopathy, such as ragged-red, ragged-blue, or cytochrome c oxidase (COX) negative myofibers. Type 1 myofiber predominance and SSMA frequency are not significantly different in both study groups (Table 2). Type 1 myofiber predominance is present in six (43%) Group 1 subjects and one (11%) Group 2 subject (Table 2). SSMA >2%, a minor criterion for MRC disorders in children (Bernier et al., 2002), are present in 11 (79%) Group 1 subjects and six (67%) Group 2 subjects (Table 2). The presence of large SSMA, which is an indicator of significant mitochondrial proliferation and suggestive of an underlying mitochondrial abnormality (Miles et al., 2006), was found only in three Group 1 subjects (Table 2). Evaluation of mitochondrial ultrastructure determined that two subjects, one in each study group, had pathological mitochondria (Fig. 2, A–F). Because many of these abnormalities were observed in the same field with normal mitochondria, the possibility of artifact due to laboratory processing error or other discrepancy seems very unlikely.

3.3. MRC enzymology

A summary of MRC complex testing results for study groups and age-matched controls is provided in Table 3, and are compared in

Table 1

Clinical features of 23 subjects with food intolerance and allergies according to age, i.e. Group 1 (<10 y; n = 14) and Group 2 (>10 y; n = 9). Mean (SD) except where indicated otherwise.

	Group 1	Group 2	p
<i>Demographic and growth features</i>			
Age (years)	4.4 (2.2)	13.2 (2.4)	<0.0001
Female, n (%)	5 (36)	5 (56)	0.42
Weight (kg)	17.4 (6.5)	44.5 (13.0)	<0.0001
Weight (percentile)	43.7 (29.7)	33.5 (32.8)	0.34
Weight <5th percentile, n (%)	1 (7)	2 (22)	0.54
Height (cm)	100.0 (15.7)	146.6 (12.4)	<0.0001
Height (percentile)	36.1 (33.3)	26.7 (27.0)	0.53
Height <5th percentile, n (%)	4 (29)	2 (22)	1.0
BMI (kg/m ²)	16.9 (1.8)	20.3 (3.8)	0.01
BMI (percentile)	65.5 (26.5)	60.2 (28.6)	0.66
BMI <5th percentile, n (%)	0	0	1.0
<i>GI abnormalities</i>			
Age at feeding difficulty onset (years)	0.3 (0.5)	4.0 (3.3)	0.0002
Time from onset of feeding difficulties to muscle biopsy (months)	50.1 (28.5)	112.4 (22.6)	<0.0001
Oral food intolerance/aversion, n (%)	14 (100)	9 (100)	1.0
Abdominal pain, n (%)	13 (93)	7 (78)	0.54
Constipation, n (%)	12 (86)	3 (33)	0.02
Vomiting/GERD, n (%)	7 (50)	7 (78)	0.23
Nausea, n (%)	4 (29)	6 (67)	0.10
Dysphagia, n (%)	7 (50)	0	0.02
Liver/pancreatic disease, n (%)	2 (14)	3 (33)	0.34
<i>Allergic abnormalities</i>			
Food allergies, n (%)	13 (93)	9 (100)	1.0
Eosinophilic esophagitis, n (%)	11 (79)	4 (44)	0.18
Asthma/chronic rhinitis, n (%)	7 (50)	4 (44)	1.0
Drug/food additive allergies, n (%)	5 (36)	5 (56)	0.42
<i>Neurological abnormalities</i>			
Weakness/easy fatigability, n (%)	4 (29)	5 (56)	0.38
Non-migraine headaches, n (%)	3 (21)	6 (67)	0.08
Developmental delay, n (%)	4 (29)	1 (11)	0.61
Migraines, n (%)	2 (14)	1 (11)	1.0
Seizures, n (%)	2 (14)	1 (11)	1.0
<i>GI medications</i>			
Proton pump inhibitor, n (%)	9 (64)	8 (89)	0.34
Topical (oral) corticosteroids, n (%)	10 (71)	5 (56)	0.66
Systemic corticosteroids, n (%)	7 (50)	4 (44)	1.0
<i>Nutritional requirements</i>			
Elemental formula (per tube) with food supplementation, n (%)	10 (71)	6 (67)	1.0
Elemental formula (per tube) without food supplementation, n (%)	2 (14)	1 (11)	1.0
Parenteral nutrition with oral supplementation, n (%)	2 (14)	1 (11)	1.0
Parenteral nutrition without oral supplementation, n (%)	0	1 (11)	0.39

GERD, gastroesophageal reflux disease.

Table 2

Comparison of laboratory evaluations of 23 subjects with food intolerance and allergies according to age, i.e. Group 1 (<10 y) and Group 2 (>10 y). Mean (SD) except where indicated otherwise.

	n	Group 1	n	Group 2	p
<i>Clinical laboratory testing</i>					
Lactate (mmol/L)	13	2.7 (2.8)	8	1.7 (0.7)	0.40
Lactate/pyruvate ratio	12	16.5 (5.9)	7	17.7 (4.3)	0.76
AST (U/L)	11	53.4 (25.6)	8	63.3 (41.6)	0.70
ALT (U/L)	13	42.0 (28.9)	8	88.9 (106.6)	0.28
Conjugated bilirubin (mg/dL)	13	0	8	0	1.0
Unconjugated bilirubin (mg/dL)	13	0.47 (0.29)	8	0.58 (0.31)	0.37
GGT (U)	13	20.8 (16.3)	8	54.3 (101.7)	0.68
Absolute eosinophils, blood (k/ μ L)	14	0.18 (0.15)	8	0.28 (0.23)	0.37
Peripheral eosinophilia >0.1 k/ μ L, n (%)	13	8 (62)	9	5 (56)	1.0
Basal plasma CoQ10, (μ g/mL)	8	0.64 (0.17)	7	0.49 (0.24)	0.14
Basal plasma CoQ10 <0.50 μ g/mL, n (%)	8	1 (13)	7	4 (57)	0.12
Plasma total cholesterol (mg/dL)	7	120.9 (13.3)	7	133.0 (30.0)	0.14
Basal plasma CoQ10:total cholesterol index (μ g/mg)	7	0.52 (0.18)	7	0.40 (0.14)	0.23
<i>Muscle pathology</i>					
Type 1 myofibers (%)	14	50.3 (14.3)	9	45.3 (10.8)	0.31
Type 1 myofiber predominance, n (%)	14	6 (43)	9	1 (11)	0.18
SSMA (%)	14	11.4 (12.7)	9	5.8 (5.9)	0.30
SSMA >2%, n (%)	14	11 (79)	9	6 (67)	1.0
Large SSMA, n (%)	14	3 (21)	9	0	0.25
Abnormal ultrastructure, n (%)	14	1 (7)	9	1 (11)	1.0

SSMA, subsarcolemmal mitochondrial aggregates.

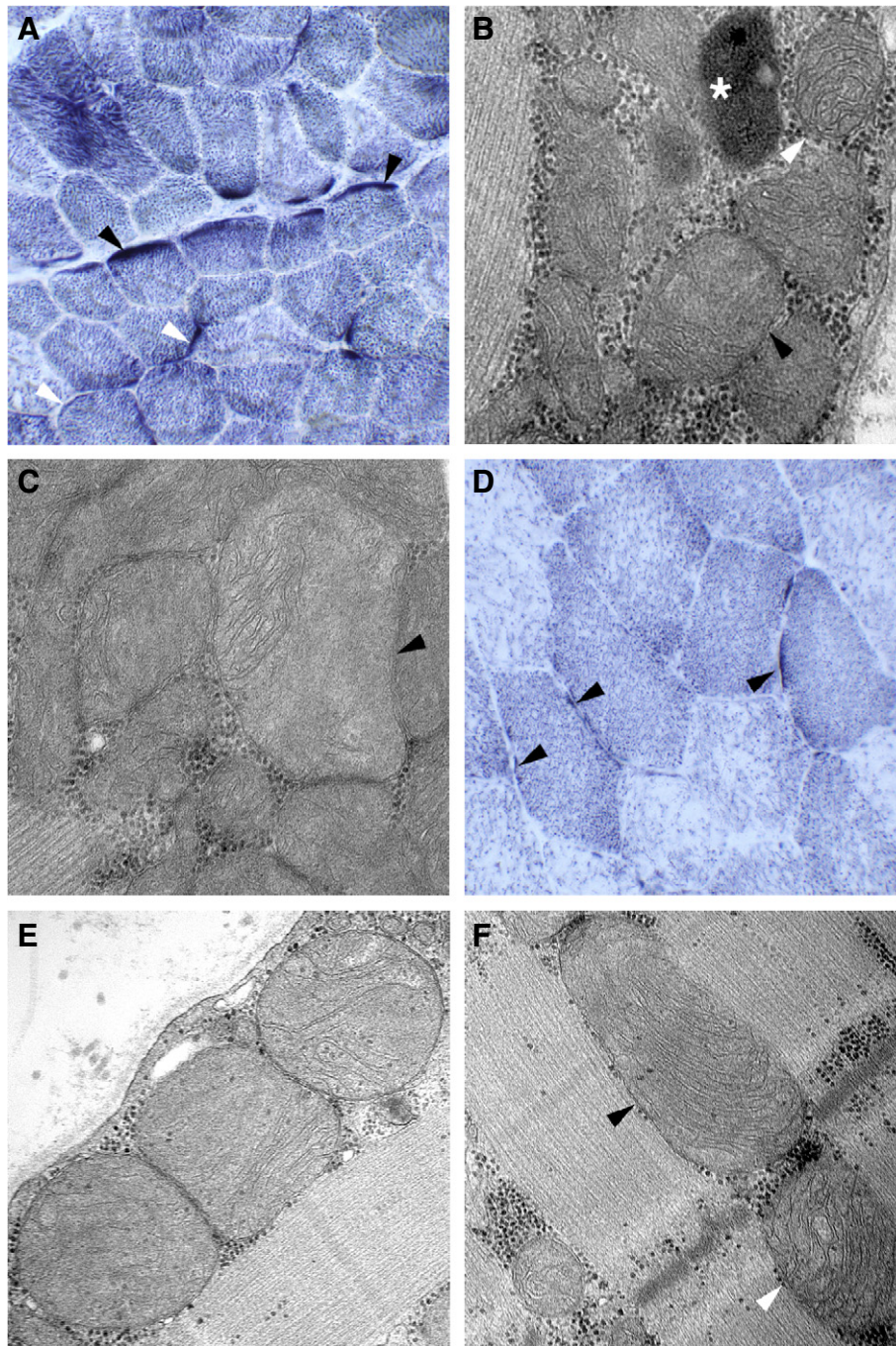


Fig. 2. Abnormal mitochondrial morphology from 2 subjects with pathological ultrastructure. Subject 1 is a 3 year-old male with (A) 5% large (black arrowheads) and 25% small (white arrowheads) subsarcolemmal mitochondrial aggregates (SHD, $\times 400$); (B) mitochondria with fragmented cristae (black arrowhead) and secondary lysosomes (asterisk) with normal mitochondria (white arrowhead) ($\times 40,000$); and (C) large mitochondria with fragmented and smudged cristae and matrix clearing (black arrowhead) ($\times 40,000$). Subject 2 is a 12 year-old female with (D) 5% SSMA (black arrowheads) (SHD, $\times 400$); (E) mitochondria with simplified and fragmented cristae with matrix clearing ($\times 30,000$); and (F) mitochondria with concentric or "fingerprint" arrangement of cristae (black arrowhead) with normal mitochondria (white arrowhead) ($\times 30,000$).

Fig. 3. No subject has CS <40% or MRC complexes I or II <20% of the mean laboratory control. CS activity, a biomarker for mitochondrial mass, is not significantly different in study and control groups (Table 3). Mean MRC complex activities for complexes I, II, III, IV, I + III, and II + III are decreased in both Groups 1 and 2 compared with controls (Table 3). A total of ten subjects, five in Group 1 (36%) and five in Group 2 (56%), have significant deficiency (<20% of the mean laboratory control) of one or more MRC complex activities. The most common MRC complex abnormalities, which are the CoQ10-linked complexes I + III and II + III, account for 80% of the deficiencies in each study group.

3.4. Muscle and plasma CoQ10 levels

Muscle CoQ10 levels in Group 1 were not significantly different from controls. However, Group 2 levels were significantly decreased compared with Group 1 and compared with controls >10 years (Table 3). Weak, but significant, correlation was found between muscle CoQ10 and CS of study subjects, but not with other MRC complex activities (Table 4).

Regression modeling of muscle CoQ10 vs. age, BMI, MRC complex activities (I, II, III, IV), and duration of illness, indicated that only the duration of illness is significant (adjusted $r^2 = 0.69$; $p = 0.012$; $n = 23$).

Table 3

Comparison of muscle CoQ10 and mitochondrial respiratory chain (MRC) complex testing results of 23 study subjects vs. 16 age-matched controls. Mean (SD).

	Study groups		Control groups	
	Group 1 <10 years (n = 14)	Group 2 >10 years (n = 9)	<10 years (n = 10)	>10 years (n = 6)
Muscle CoQ10 (nmol/g protein)	210.0 ^a (44.9)	118.0 ^b (47.7)	235.7 (44.2)	191.5 (37.8)
Complex I (% mean control)	74.6 ^{c,d} (33.5)	91.2 ^b (79.4)	114.5 (48.3)	114.1 (25.0)
Complex II (% mean control)	112.9 ^{c,d} (72.6)	70.8 ^b (44.1)	183.5 (71.9)	204.9 (115.6)
Complex III (% mean control)	50.6 ^{c,d} (25.0)	45.0 ^b (23.8)	113.1 (52.8)	106.6 (25.9)
Complex IV (% mean control)	47.0 ^{c,d} (22.5)	42.5 ^b (19.8)	99.2 (30.7)	139.0 (75.2)
Complex I + III (% mean control)	33.3 ^{c,d} (20.3)	33.0 ^b (22.1)	102.9 (49.9)	139.2 (62.5)
Complex II + III (% mean control)	43.5 ^{c,d} (22.2)	44.0 ^b (28.3)	109.8 (55.5)	117.9 (87.7)
Citrate synthase (% mean control)	101.1 (29.9)	75.1 (26.8)	103.1 (21.4)	88.4 (24.1)

^a Group 1 (<10 years) vs. Group 2 (>10 years); $p = 0.001$.

^b Group 2 (>10 years) vs. Controls >10 years; $p < 0.05$.

^c Group 1 (<10 years) vs. Controls <10 years; $p < 0.05$.

^d Group 1 (<10 years) vs. Controls >10 years; $p < 0.05$.

(Fig. 4). It should be noted that nine subjects (39%), all of whom have muscle CoQ10 levels <140 nmol/g protein, had histories of long-standing disease >87 months; and eight of the nine are in Group 2 (Fig. 5). Three of the nine subjects with long-standing disease >87 months, who are all in Group 2, had muscle CoQ10 levels below the lower limit of the previously reported reference range (Miles et al., 2008), i.e. 107 nmol/g protein (Fig. 5). The single Group 1 individual, previously mentioned, was the oldest member of Group 1, i.e. 9.7 years, and also had long-standing disease >87 months (Fig. 5). For reference, muscle CoQ10 levels <140 nmol/g protein were well below the published cutoff value for muscle CoQ10, i.e. 186 nmol/g protein, which was established to predict increased risk of MRC defects (Miles et al., 2008).

Basal plasma CoQ10 levels were reported for 15 subjects at the time of muscle biopsy (Table 2). Correction of basal plasma CoQ10 levels for total cholesterol had no effect on group differences (Table 2). Five of 15 subjects (33%), including one in Group 1 and four in Group 2, had CoQ10 levels below the lower limit of the laboratory reference range (0.50 µg/mL). Significant correlation existed between muscle and plasma CoQ10 levels ($r_s = 0.64$; $p = 0.010$; $n = 15$). Weak, but significant, correlations also existed

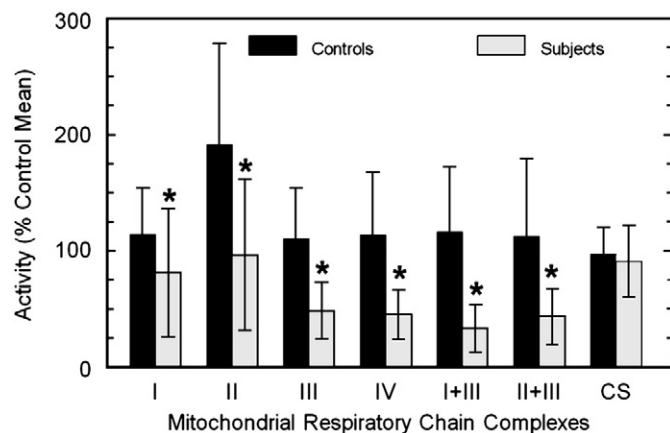


Fig. 3. Comparison of mean (SD) mitochondrial respiratory chain (MRC) activities (% control mean) of 23 subjects and 16 controls. * $p < 0.0001$.

Table 4

Spearman's rank-order coefficients (r_s) of muscle (nmol/g protein) ($n = 23$) and plasma (µg/mL) ($n = 15$) coenzyme Q10 (CoQ10) levels with mitochondrial respiratory chain (MRC) complex activities (percent of mean control value).

	Muscle CoQ10		Plasma CoQ10	
	r_s	p	r_s	p
Complex I ^a	0.199	0.362	0.329	0.231
Complex II ^b	0.371	0.081	0.577	0.024
Complex III ^c	0.196	0.371	0.070	0.805
Complex IV ^d	0.092	0.677	0.143	0.611
Complex I + III ^e	0.120	0.587	0.609	0.016
Complex II + III ^f	0.039	0.860	0.347	0.205
Citrate synthase	0.568	0.005	0.459	0.085

^a NADH-ferricyanide reductase.

^b Succinate dehydrogenase.

^c Decylubiquinol-cytochrome c reductase.

^d Cytochrome c oxidase.

^e NADH-cytochrome c reductase (rotenone-sensitive).

^f Succinate-cytochrome c reductase (antimycin-sensitive).

between plasma CoQ10 and MRC complex I + III and between plasma CoQ10 and complex II activities (Table 4).

4. Discussion

4.1. Clinical considerations

We have detected CoQ10, mitochondrial ultrastructure, and MRC abnormalities in a selected group of children whose EGID and constitutional symptoms were not abolished by the use of an elemental formula (as would be expected with pure food allergic disease) (Putnam and Rothenberg, 2009). In addition to typical problems such as gastrointestinal dysmotility, pain, and allergies, several subjects exhibited non-GI findings, such as neuromuscular, headache, and seizure abnormalities (Table 1). Although commonly seen in children with mitochondrial disorders (Bernier et al., 2002; Haas et al., 2007; Uusimaa et al., 2000), the non-GI features are uncommon in children with EGID (Furuta et al., 2008; Putnam and Rothenberg, 2009; Spergel et al., 2009).

4.2. Significance of CoQ10 deficiency

The process of oxidative phosphorylation (OXPHOS) and ATP production is primarily dependent upon function of the MRC complexes, which are located within the inner membrane of

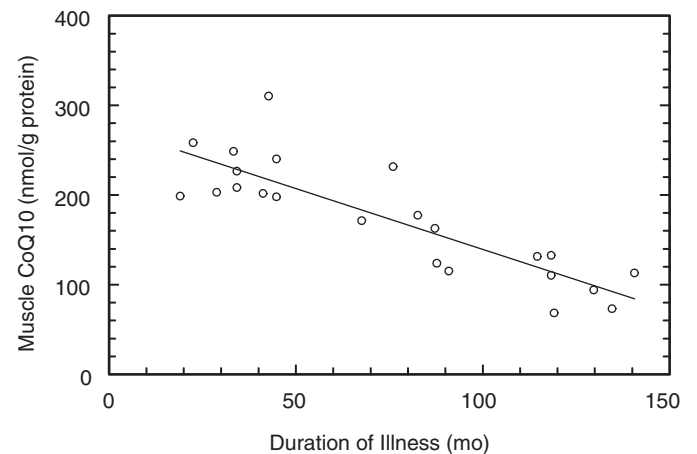


Fig. 4. Correlation of muscle CoQ10 levels with duration of illness, i.e. interval between onset of symptoms and muscle biopsy, in 23 children with recurrent food intolerance and allergies (adjusted $r^2 = 0.69$; $p = 0.012$).

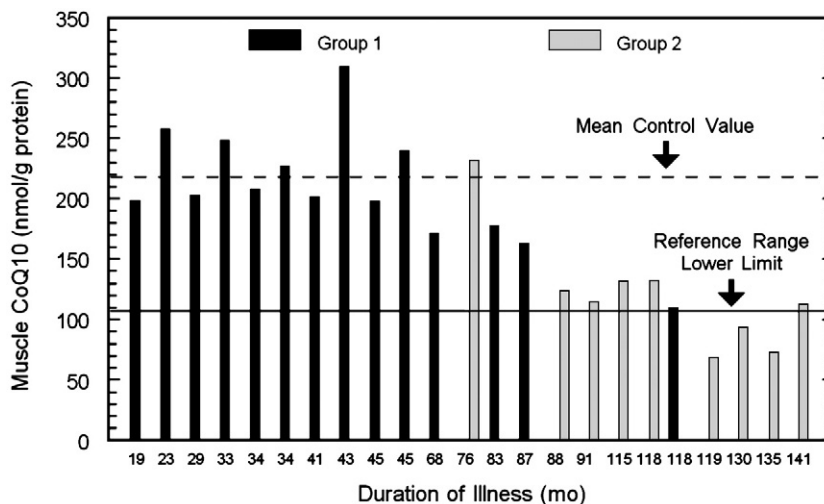


Fig. 5. Individual muscle CoQ10 levels of study subjects in relation to increasing duration of illness. Solid grid line: lower limit of established reference range (Miles et al., 2008). Dashed grid line: mean muscle CoQ10 of study control group.

mitochondria. CoQ10 has been established as an essential MRC component and required for efficient MRC function (Quinzii et al., 2008). MRC dysfunction, which is frequently associated with CoQ10 deficiency (Rötig et al., 2007), may lead to degradation of mitochondria by mitophagy (Rodríguez-Hernández et al., 2009). CoQ10 deficiency may also contribute to increased generation of reactive oxygen species, induction of mitochondrial permeability transition, and collapse of mitochondrial membrane potential (Rodríguez-Hernández et al., 2009). Further evidence, which suggested that CoQ10 deficiency may be a precursor to mitophagy and mitochondrial dysfunction, was reported very recently (Cordero et al., 2010). The appearance of secondary lysosomes in a muscle of a 3 year-old subject in proximity to abnormal mitochondria is suggestive of increased mitochondrial turnover (Fig. 2B), an unusual finding in a young child with no evidence of muscle disease.

In humans profound depletion of muscle CoQ10 content has been associated with mutations of genes required for CoQ10 biosynthesis, i.e. primary CoQ10 deficiency (Quinzii et al., 2008; Rötig et al., 2007). Thus far, secondary CoQ10 deficiency has been associated with mutations of three genes not involved in CoQ10 biosynthesis, including *ATPX* (involved in nuclear DNA single strand break repair) (Quinzii et al., 2005), *ETFDH* (associated with multiple acyl-CoA dehydrogenase deficiency and electron transport defects) (Gempel et al., 2007), and *BRAF* (mechanism unknown) (Aeby et al., 2007). There is increasing evidence that secondary CoQ10 deficiency may be much more common than primary deficiency (Moslemi and Darin, 2007; Uusimaa et al., 2000; Zeviani and Di Donato, 2004). A very recent report noted muscle CoQ10 deficiency was present in 37% of patients with mitochondrial phenotypes, and 32% of CoQ10-deficient individuals harbored pathogenic mutations (Sacconi et al., 2010). The current results suggest that children with food intolerance and allergies, who require an elemental (amino acid-based) diet, may develop secondary CoQ10 deficiency in relation to the progression of their disease (Figs. 4 and 5).

Biochemical abnormalities associated with MRC complexes I + III and II + III are most frequently related to CoQ10 deficiency (Haas et al., 2008; Miles et al., 2008; Rötig et al., 2007). The current results concur with previous reports in that 80% of subjects with MRC deficiency, i.e. <20% of the mean control, have complex I + III and/or complex II + III deficiency. Quantitation of CoQ10 content in muscle has been recommended to confirm the presence of CoQ10 deficiency in patients with abnormal MRC complexes I + III and II + III (Haas et al., 2008; Rötig et al., 2007). Muscle CoQ10 deficiency in the current study appears to be significant in Group 2 subjects (>10 years) (Table 3; Fig. 5).

4.3. Evidence of mitochondrial pathology

Although a subject of controversy in the past, an expert panel recently suggested that evaluation of mitochondrial morphology and ultrastructure should be considered for children with suspected mitochondrial disease (Haas et al., 2008). It should be noted that while classic findings of mitochondrial myopathy, such as ragged-red and COX-deficient myofibers, are common in adults, these findings are relatively rare in children (Bernier et al., 2002; Haas et al., 2008). Thus, the current study findings, showing pathologic mitochondria in muscle of two children without clinical evidence of muscle disease (Fig. 2), are quite unexpected and deserve further discussion.

The younger of the two subjects is a 3 year-old male with a history of severe food intolerance, multiple food and medication allergies, and a seizure disorder. His clinical features include severe food intolerance with abdominal pain, seizures, speech delay, leucopenia, and recurrent infections for approximately 2.2 years. He required both elemental and parenteral nutrition because of his severe food intolerance. Skin prick testing showed dramatic positives to milk and egg white. Because of certain clinical features he was evaluated for mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Plasma thymidine and deoxyuridine levels were normal, which essentially excluded the possibility of MNGIE. His MRC testing indicates a low-normal complex III activity, but no other abnormalities. His muscle CoQ10 level, 258 nmol/g protein, is slightly higher than the mean value for age-matched controls (Fig. 5). A plasma CoQ10 level was not obtained. LM shows 25% myofibers with SSMA and 5% with large SSMA (Fig. 2A). Increased SSMA >2% has been suggested as a minor criterion for MRC disorders in children (Bernier et al., 2002). The appearance of large SSMA in children has been associated with mitochondrial myopathy in a report by several of the current investigators (Miles et al., 2006). On EM his muscle shows pathological mitochondria with fragmented cristae and dense secondary lysosomes in the same field with normal mitochondria (Fig. 2B), as well as mitochondria with smudged cristae and matrix clearing (Fig. 2C). In summary, pathology examination of this boy provides evidence highly suggestive of a mitochondrial disorder.

The older subject with pathological mitochondria is a 12 year-old female with a history of asthma, multiple food allergies, seizures, and long-standing food intolerance for ~11 years. Although she received elemental formula (amino acid-based) by feeding tube for much of her life, her growth and development are reported as normal. Her MRC activities are also normal. However, her muscle CoQ10 level, i.e. 73 nmol/g protein, is one of the lowest reported in this study (Fig. 5).

Her plasma CoQ10 level, 0.39 µg/mL, is also one of the lowest reported, and is well below the lower limit of laboratory reference range (0.50 µg/mL). On LM she has 5% myofibers containing SSMA and no large SSMA (Fig. 2D). In addition, many pathological mitochondria with ultrastructure abnormalities are observed, including mitochondria with simplified and fragmented cristae and matrix clearing (Fig. 2E) and with concentric or “fingerprint” arrangement of cristae (Fig. 2F). It is also interesting to note that ultrastructural changes similar to those described in this subject have also been reported in CoQ10 deficient fibroblasts (Rodríguez-Hernández et al., 2009). Although her MRC testing is normal, this subject's pathology findings and marked CoQ10 deficiency provide substantial evidence of an underlying mitochondrial defect.

4.4. Evidence of MRC defects

Biochemical testing of MRC complex activities has been considered a mainstay for the diagnosis of MRC disorders for many years, but is associated with a number of limitations. These limitations include non-standardized laboratory methods, inter-laboratory variability, unvalidated cutoff values, poorly-defined control ranges, and deleterious freeze-thaw effects (Gellerich et al., 2004; Hui et al., 2006; McFarland et al., 2007; Taylor et al., 2004). In addition, MRC testing has been associated with false-positive (Hui et al., 2006; Jongpiputvanich et al., 2005; Lee et al., 2007; Vallance, 2004) and false-negative results (Oglesbee et al., 2006). Because the effects of freezing on tissue mitochondria are unpredictable, the reliability of MRC complexes I + III and II + III results has also been questioned (Thorburn et al., 2004). Although muscle specimens for the current study required freezing prior to MRC testing and CoQ10 analysis, it should be emphasized that muscle biopsy specimens were meticulously resected surgically without tissue clamps, rapidly frozen, and maintained at -70°C or below throughout this study. Also freeze-thaw effects on muscle CoQ10 have been reported as minimal (Tang et al., 2004). Therefore, we contend that the relatively high frequency of abnormal MRC complexes I + III and II + III, especially in conjunction with normal CS activities, should be considered as additional evidence of an underlying MRC defect in this study population.

4.5. Novel findings

To the best of our knowledge the current study is the first to associate muscle CoQ10 levels with the duration of a progressive clinical disorder. An early report noted that muscle CoQ10 tended to decrease with disease progression in nine patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), but found no correlations between CoQ10 in muscle, serum, and cerebrospinal fluid (CSF) (Matsuoka et al., 1991). More recently cystic fibrosis (CF) patients with pancreatic insufficiency were found to have CoQ10 deficiency based upon serum levels, but no tissue analysis for CoQ10 was performed (Laguna et al., 2008). The current results, which show significant CoQ10 deficiency in muscle, MRC and ultrastructure abnormalities, and a strong correlation between muscle CoQ10 levels and duration of disease symptoms, are compelling evidence of an underlying mitochondrial abnormality.

Reports showing correlations between plasma/serum CoQ10 and tissue CoQ10 are rare. One report attempted to correlate CoQ10 in plasma, muscle, and blood mononuclear cells (Duncan et al., 2005). They found close association between CoQ10 in blood mononuclear cells and muscle in 13 disease controls, but found no relationship between plasma and muscle CoQ10 or between plasma and blood mononuclear cell CoQ10 (Duncan et al., 2005). The current results, indicating correlations between basal plasma CoQ10 levels and activities of MRC complexes II and I + III (Table 4), have not been described previously as far as these authors can determine. The

correlation between muscle and plasma CoQ10, which was noted earlier, deserves further investigation.

4.6. Nutritional factors associated with CoQ10 deficiency

Although the majority of CoQ10 in the human body is produced endogenously, it has been estimated that approximately 25% of CoQ10 is derived from dietary sources (Weber et al., 1997). Most dietary CoQ10, approximately 2–3 mg/day, is provided in meat, oily fish, nuts, and certain oils (Kamei et al., 1986; Weber et al., 1997). Fruit, vegetables, eggs, dairy products, and cereals provide <1 mg/day (Kamei et al., 1986; Weber et al., 1997). Although the clinical effects of disease in relation to restricted intake of dietary CoQ10 are not well-studied, a decrease from basal serum CoQ10 levels of approximately 40% was observed in 95 adults following one week of parenteral nutrition (Okamoto et al., 1986). In that report low serum CoQ10 levels, ~ 0.40 µg/mL, were constant during a 4-week study period in their patients (Okamoto et al., 1986).

Nutrition is a key consideration in the current study population, because virtually 100% of patients with food intolerance and allergies will improve with food avoidance and an amino acid-based diet (Liacouras, 2008). In the most severe cases patients will require periods of parenteral nutrition, as was noted in four of the current subjects (Table 1). Unfortunately, most of these patients will have recurrence of their food intolerance with the re-introduction of a normal diet (Liacouras, 2008). All subjects in this study had histories of recurrent food intolerance requiring restriction from certain foods, but few had clinical evidence of a mitochondrial disorder.

We suggest that two factors, in particular, may contribute to CoQ10 depletion with prolonged food intolerance in this study population (Figs. 4 and 5). First, we offer the possibility that nutritional support failed to provide adequate CoQ10 supplementation, because of prolonged requirement for an elemental diet. As far as we can determine, none of the enteral or parenteral formulas provided the study subjects contained more than trace amounts of CoQ10. A somewhat analogous situation was reported in CF patients (Laguna et al., 2008). Significantly lower serum CoQ10 levels in 346 CF patients with pancreatic insufficiency were significantly lower than 35 CF patients with pancreatic sufficiency. However, no correlations were found between CoQ10 serum levels and age, sex, weight, or height. Because CoQ10 serum levels correlated positively with beta-carotene, retinol, and alpha-tocopherol levels, the authors concluded that low CoQ10 levels were most likely a result of malabsorption (Laguna et al., 2008).

Another example of nutrition-related CoQ10 deficiency was reported in children and adolescents with PKU. Artuch et al. observed that 37% of 41 patients with PKU had serum CoQ10 levels below a reference population (Artuch et al., 1999). It has been suggested that children with PKU may be prone to CoQ10 deficiency because of dietary restrictions, which often limit the intake of the primary sources of dietary CoQ10, e.g. meats, poultry, soy bean products, and nuts, (Hargreaves, 2007). Unfortunately, investigators in neither of the two previous examples (Artuch et al., 1999; Laguna et al., 2008) evaluated tissue for CoQ10 levels or for mitochondrial abnormalities. One author suggested that assessment of intracellular CoQ10 is needed to establish a relationship between CoQ10 deficiency and the pathogenesis of any disease (Hargreaves, 2007). In our opinion the current study has accomplished that objective.

We propose that patients with disorders associated with either insufficient supplementation or uptake of dietary CoQ10, e.g. CF and PKU, may be at risk of developing CoQ10 deficiency.

4.7. Possible factors associated with mitochondrial abnormalities in EGIDS

Animal studies indicating mitochondrial dysfunction in allergic inflammation may provide explanation for some of the current study

findings. Mitochondrial ultrastructural changes similar to the two cases mentioned previously, including loss of cristae, and MRC dysfunction, have been reported in a murine model of experimental allergic asthma (Mabaliarajan et al., 2008). Another group identified nine oxidatively damaged MRC complexes and associated proteins after cellular insult by ragweed pollen extract (RWE) (Aguilera-Aguirre et al., 2009). These investigators observed that mitochondrial dysfunction following RWE challenge in airway epithelium of sensitized BALB/c mice, was associated with deficiency of ubiquinol-cytochrome c reductase core II protein (UQCRC2) (Aguilera-Aguirre et al., 2009). UQCRC2 is a structural protein associated with MRC complex III. Preexisting mitochondrial dysfunction and deficiency of UQCRC2 were associated with increased Ag-induced accumulation of eosinophils, mucin levels in the airways, and ultrastructural changes, including matrix clearing and loss of cristae (Aguilera-Aguirre et al., 2009). It seems plausible that mitochondrial dysfunction in muscle and tissues of the gastrointestinal tract of patients with food intolerance and allergies may also enhance allergic inflammation.

We propose that CoQ10 deficiency may be an important factor, which contributes to the development of mitochondrial pathology, MRC dysfunction, and allergic inflammation in children with recurrent food intolerance and allergies. Although all subjects were closely followed by experienced dietitians, it is possible that other nutritional deficiencies, e.g. vitamin B deficiency (Depeint et al., 2006), may have contributed to the mitochondrial abnormalities observed in this study. The current findings may also be important, because identification of pathogenic mechanisms leading to EGIDs has been recommended as a priority topic for researchers in this field (Furuta et al., 2008). In addition, CoQ10 deficiency and mitochondrial dysfunction may help clinicians to distinguish between patients who will progress to more severe complications from others who will simply have chronic symptoms.

4.8. Study limitations

Several limitations of the current study are evident. The study design does not allow determination of cause and effect relationships in the study population. It cannot be concluded that CoQ10 deficiency actually exacerbated or caused food intolerance. The limited sample size is also a consideration. There is the possibility that deficiency of other nutrients may have adversely affected mitochondrial function in these patients. Other factors may also be involved, e.g. unknown effects associated with medications, diet, and heredity. Further studies are needed to confirm the current findings.

5. Conclusions

The current results provide compelling evidence that CoQ10 deficiency and mitochondrial abnormalities in muscle were determined in children and adolescents with recurrent food intolerance and allergies. In addition, CoQ10 deficiency is more severe in children with long-standing clinical disease. Dietary, disease-related, and hereditary factors may contribute to the development of acquired CoQ10 deficiency and mitochondrial abnormalities in these patients. Clinicians, who care for patients with dietary restrictions which may limit CoQ10 intake, may want to consider CoQ10 supplementation for those patients. Longer term follow-up of these patients is needed to evaluate the possible link between CoQ10 and mitochondrial abnormalities and development of more severe co-morbidities. Investigators interested in the pathogenesis and natural course of EGIDs should consider factors associated with CoQ10 deficiency and mitochondrial dysfunction in future studies.

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