Nutritional cofactor treatment in mitochondrial disorders

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ABSTRACT

Mitochondrial disorders are degenerative diseases characterized by a decrease in the ability of mitochondria to supply cellular energy requirements. Substantial progress has been made in defining the specific biochemical defects and underlying molecular mechanisms, but limited information is available about the development and evaluation of effective treatment approaches. The goal of nutritional cofactor therapy is to increase mitochondrial adenosine 5'-triphosphate production and slow or arrest the progression of clinical symptoms. Accumulation of toxic metabolites and reduction of electron transfer activity have prompted the use of antioxidants, electron transfer mediators (which bypass the defective site), and enzyme cofactors. Metabolic therapies that have been reported to produce a positive effect include Coenzyme Q_{10} (ubiquinone); other antioxidants such as ascorbic acid, vitamin E, and lipoic acid; riboflavin; thiamin; niacin; vitamin K (phylloquinone and menadione); creatine; and carnitine. A literature review of the use of these supplements in mitochondrial disorders is presented. *J Am Diet Assoc. 2003;103: 1029-1038.*

Mitochondrial disorders result from a progressive decrease in the ability of mitochondria to meet cellular demands for adenosine 5'-triphosphate (ATP). Oxidative phosphorylation (OXPHOS) is carried out by 5 mitochondrial enzyme complexes, which collectively produce the majority of the cellular energy requirement. These enzyme complexes are situated in the inner mitochondrial membrane and are designated as complex I (NADH:ubiquinone oxidoreductase), complex II (succinate:ubiquinone oxidoreductase), complex III (ubiquinol:ferrocytochrome *c* oxidoreductase), complex IV (cytochrome *c* oxidase), and complex V (ATP synthase). The relationship among respiratory complexes, electron flow, proton translocation, and how the cofactors may influence ATP production is illustrated in the Figure.

Defects in this process encompass a large array of mitochondrial disorders whose onset can occur at any time. Mitochondrial diseases include encephalopathies, myopathies, neuropathies, and cardiomyopathies and are characterized by clinical, biochemical, and genetic heterogeneity (1). A subset of these mitochondrial disorders are a result of inherited or de novo mutations in the mitochondrial or nuclear DNA, which lead to

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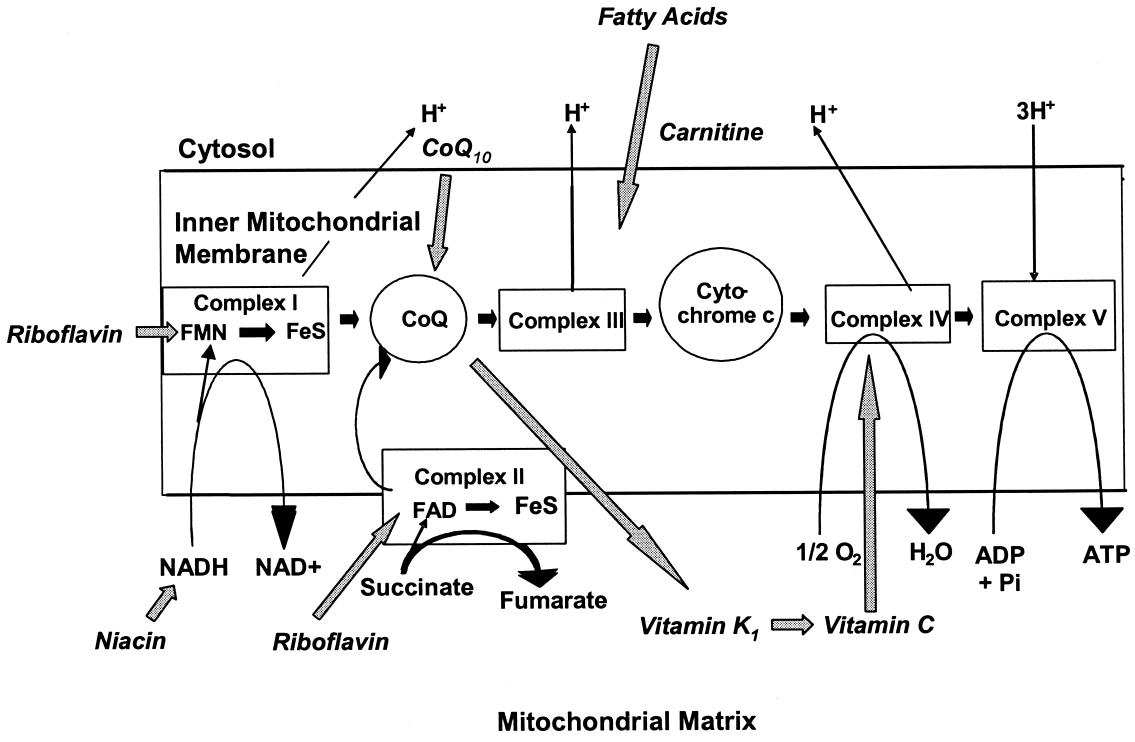

FIG. Mitochondrial Respiratory Chain. Protons (H^+) are pumped from the mitochondrial matrix to the intermembrane space through complexes I, III, and IV. Complex V utilizes the proton gradient as a source of energy to produce ATP. Coenzyme Q_{10} transfers electrons from complexes I and II to complex III. Riboflavin is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The amide form of niacin, (nicotinamide) is a precursor for nicotinamide adenine dinucleotide (NAD). Vitamin K_3 in combination with vitamin C serve as electron acceptors to bypass a deficiency in complex III. Carnitine functions to transfer long chain fatty acids across the mitochondrial membrane.

respiratory chain dysfunction. Disorders affecting OXPHOS may be as common as 1 in 5,000 to 1 in 10,000 births (2). Substantial progress has been made in the past 15 years in defining the specific biochemical defects and genetic causes of these defects, but information about the development and evaluation of effective treatment approaches is limited.

The goal of nutritional cofactor therapy is to increase mitochondrial ATP production and to slow or arrest the progression of clinical symptoms. The accumulation of toxic metabolites and the reduction in oxidative capacity have led to the use of antioxidants, electron transfer mediators (which bypass the defective site), and enzyme cofactors. Coenzyme Q_{10}, other antioxidants such as ascorbic acid, vitamin E, and lipoic acid; riboflavin; thiamin; niacin; vitamin K (phylloquinone and menadione); creatine; and carnitine have been used in the treatment of OXPHOS disorders to augment energy production. In the following paragraphs, we discuss these cofactors and their clinical use in the treatment of OXPHOS disorders.

EVALUATION OF COFACTOR THERAPY STUDIES

Therapeutic trials in mitochondrial disorders are difficult to conduct because the diseases are rare and demonstrate vast clinical and genetic heterogeneity. These disorders affect many organ systems, making it difficult to develop treatment trials examining the efficacy of a particular supplement on all possible affected organ systems. Mitochondrial disorders may be classified on the basis of a genetic defect, but we are unable to predict the natural history of the disease and methods for defining severity of illness are limited. Many reports of treatment have been case studies with limited patient numbers and varied periods of follow up. Treatment trials that are not conducted over a sufficient length of time could possibly reject a therapy of potential benefit. In addition, the biochemical parameters commonly investigated (lactate, pyruvate) may not be adequate indicators of therapeutic efficacy. The unpredictable and variable natural history of these disorders and the lack of reliable clinical outcome measures also make it difficult to evaluate these reports. Large, double-blind, placebo-controlled trials of potentially beneficial therapies for OXPHOS diseases have been difficult to do because of the rarity of the disorders, which prevents recruitment of sufficient numbers of patients for statistical significance. For the reasons discussed, substantial proof that any supplement or combinations of therapies will be effective in the treatment of mitochondrial disorders is unlikely. A review of the studies examining supplement use in the treatment of these disorders is presented in Tables 1 and 2.

Table 1
Coenzyme Q_{10} treatment in mitochondrial disorders

Dosage	Disorder[a]	Study details	Effects[b]	Reference
120-300 mg/d	MELAS	Case Report 1 patient 2 months	↓ CSF lactate and pyruvate Improved muscle weakness	7
150 mg/d	COX deficiency	Case Report 1 patient 24 months	↓ Serum lactate and pyruvate ↑ Post-exercise recovery (NMR)	9
300 mg/d	MELAS	Case Report 1 patient 8 months	↓ Serum lactate and pyruvate Clinical improvement	10
60-120 mg/d	Kearns-Sayre syndrome	Case Report 1 patient 3 months	↓ Serum lactate and pyruvate Improvement in atrioventricular block and ocular movements	11
90 mg/d	MELAS	Case Report 1 patient 14 months	↓ Serum lactate Improved muscle weakness	12
210 mg/d	MELAS	Case Report 2 patients 8 months	Improved peripheral nerve function Improved muscle weakness	13
200 mg/d	MELAS	Case Report 2 patients 2 weeks	Improved oxygen utilization during exercise (tissue oximetry)	14
150 mg/d	MELAS	Case Report 2 patients 10 months	↑ Post-exercise recovery (NMR)	16
150 mg/d	Mitochondrial myopathy	Open Study 9 patients 6 months	↓ Serum lactate and pyruvate (4 patients)	23
30-210 mg/d	MELAS	Open Study 11 patients 3-5 months	Improvement in neuromuscular symptoms	19
150 mg/d	Mitochondrial myopathy	Open Study 11 patients 6 months	↑ Post-exercise recovery (Effect mainly due to a single therapy responder)	20
150 mg/d	Mitochondrial cytopathy	Open Study 6 patients 6 months	Improved ATP synthesis in brain and skeletal muscle (MRS)	21
120 mg/d	Kearns-Sayre syndrome	Open Study 7 patients 12 months	↓ Serum lactate and pyruvate	8
160 mg/d	MELAS (4) MERFF (3) CPEO (1) (# of patients)	Double-Blind Crossover 8 patients 3 months	Improved global muscle strength	18
100 mg/d	Muscular Dystrophy[1] Neurogenic Atrophy[2]	Double-Blind Trials [1]12 patients [2]15 patients 3 months	Improvement in cardiac function and physical performance	17
2 mg/kg/d	Mitochondrial myopathy	Double-Blind 44 patients 6 months	Reduction in post-exercise lactate levels (16 patients)	22
150 mg/d	MELAS (28 MIDD) (28 IGT) (15 NGT)	Open Study 44 patients 3 years	↑ Insulin secretory response and improved lactate response post-exercise (MIDD group)	24
200-3,000 mg/d	Muscle CoQ_{10} deficiency	Case Report 6 patients 1 month	Improvement in strength, seizure control, muscle weakness and ataxia	25

[a]MELAS=mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; MERFF=mitochondrial encephalopathy, ragged-red fibers; CPEO=chronic progressive external ophthalmoplegia; MIDD=maternally inherited diabetes and deafness; IGT=impaired glucose tolerance; NGT=normal glucose tolerance.
[b]NMR=nuclear magnetic resonance; MRS=magnetic resonance spectroscopy.

Table 2
Vitamin and cofactor treatment in mitochondrial disorders

Cofactor	Dosage	Disorder[a]	Study details	Effects[e]	Reference
Riboflavin	100 mg/d	Complex I deficiency	Case Report 1 patient 6 months	Improvement in exercise capacity	47
	50 mg/d	Complex I deficiency	Case Report 1 patient 3 years	Improved exercise tolerance and muscle tone	49
	120 mg/d	Complex I deficiency	Case Report 1 patient 3 to 17 months	↓ Serum lactate and pyruvate Improvement in motor development	53
	9-60 mg/d	Complex I deficiency	Case Report 5 patients 3 to 6 months	Improvement in myopathic form (2/5) ↑ Complex I activity	48
Riboflavin plus Carnitine	9 mg/d 2 g/d	Complex I deficiency	Case Report 1 patient 7 months	Improved muscle weakness ↑ Complex I activity	52
Riboflavin plus Carnitine	100-200 mg/d 2 g/d	Complex I deficiency	Case Reports 4 patients 1 to 2 years	Improvement in exercise capacity ↑ Complex I activity (1/4)	51
Riboflavin plus Nicotinamide	100 mg/d 3 g/d	MELAS	Case Report 1 patient 18 months	Improvement in encephalopathic symptoms and nerve conduction	50
Vitamin K_3	10 mg/kg/d	Complex I deficiency	Case Report 1 patient 3 months	↓ Serum lactate and pyruvate Clinical improvement	62
Vitamin K_3 plus Ascorbate	80 mg/d 4 g/d	Complex III deficiency	Case Report 1 patient 1 year	Improved exercise capacity Clinical improvement	36
Vitamin K_3 plus Ascorbate	40 mg/d 4 g/d	Complex III deficiency	Case Report 1 patient 5 months	Improvement in ataxia	61
Lipoic Acid	600 mg/d	CPEO	Case Report 1 patient 7 months	Improvement in muscle and brain bioenergetics (NMR)	46
Creatine	10 g/d (2 weeks)	Mitochondrial myopathy (6 MELAS)	Randomized Controlled Trial 7 patients 6 weeks	Improved high intensity activities No effect on lower intensity exercise	69
Carnitine	50-200 mg/d	Mitochondrial myopathy (Carnitine deficiency)	Open Study 21 patients 1 to 24 months	Improvement in muscle strength Cardiac improvement (8/8 patients)	75
Combined Therapy	[b]	Diverse Mitochondrial Disorders	Case Reports 16 patients 1 to 13 years	Subset of patients appeared to have improved morbidity	78
	[c]	Diverse Mitochondrial Disorders	Open Study 16 patients 8 months[d]	No significant reproducible objective improvement	79

[a]MELAS=mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; CPEO=chronic progressive external ophthalmoplegia.
[b]CoQ_{10} (30 to 120 mg/day), vitamin K_3 (20 to 60 mg/day) ascorbate (2 g/day) and methylprednisolone (2 to 16 mg every other day).
[c]CoQ_{10} (300 mg/day), vitamin K_3 (60 mg/day) ascorbate (2 g/day), thiamin (100 mg/day), riboflavin (25 mg/day), niacin (200 mg/day).
[d]2 months treatment and 2 months off treatment—2 cycles of each.
[e]NMR=nuclear magnetic resonance.

Coenzyme Q_{10} (Ubiquinone)

Coenzyme Q_{10} (CoQ_{10}) is a fat-soluble quinone that transfers electrons from complexes I and II to complex III, a process that is coupled to ATP synthesis (3). In its reduced form (ubiquinol), CoQ_{10} also inhibits lipid peroxidation and can protect mitochondrial inner-membrane proteins and DNA from oxidative damage (4). CoQ_{10} also helps stabilize the OXPHOS complexes within the inner mitochondrial membrane by maintaining optimal membrane fluidity (5). CoQ_{10} is the most widely used supplement in the treatment of mitochondrial disorders.

Endogenous synthesis and dietary sources, primarily animal products, contribute to normal CoQ_{10} levels in plasma. The mean daily intake of CoQ_{10} from the diet was estimated to be 3 to 5 mg per day in the Danish population (6).The optimal dietary requirement for CoQ_{10} is unknown. The contribution of dietary sources to CoQ_{10} levels in plasma, compared to the amount contributed through de novo synthesis, is also unknown.

CoQ_{10} has been reported to have a beneficial effect on clinical outcomes and biochemical parameters in a variety of OXPHOS disorders. The positive effects have included a reduction of cerebrospinal fluid (CSF) and serum lactate and pyruvate (7-12), improvement in cardiac conduction defects and ocular movements (11), reduced muscle weakness (7,12,13) and improved exercise tolerance (8,10), improved oxygen utilization during exercise (14), decreased peripheral nerve damage (13), improvement in neurological function (8), increased respiratory chain activity (8,15), and acceleration of post-exercise recovery detected by nuclear magnetic resonance (^{31}P-NMR) spectroscopy (9,16). Most reports about treatment have been case studies or anecdotal reports with limited numbers of patients, variable treatment periods, and CoQ_{10} dosages ranging from 30 to 300 mg/day.

Several short-term studies have shown variable results with CoQ_{10} treatment. Two trials in patients with muscular dystrophies and neurogenic atrophies showed an improvement in cardiac function and physical performance (17). In another short-term study (3 months CoQ_{10}, 1 month placebo) in patients with mitochondrial encephalomyopathies, a trend of effectiveness of CoQ_{10} was noted by improved muscle endurance, decreased fatigability from daily activities, and decreased serum lactate and pyruvate levels, but statistical significance was only noted in global muscle strength (18). In both of these studies, the authors questioned whether the dosages of 100 and 160 mg/day, respectively, were too low and whether the short-term administration was adequate. In a study with CoQ_{10} supplementation in patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), there was an improvement in neuromuscular symptoms, but no difference in fasting glucose or glycemic control (19).

Longer-term studies of 6 months' duration have evaluated the effectiveness of CoQ_{10}. A report of CoQ_{10} treatment in mitochondrial myopathies, using ^{31}P-NMR to objectively measure muscular function, indicated an improvement in the mean ratio of phosphocreatine (PCr) to inorganic phosphate (P_i), but the beneficial effect was mainly due to a single therapy responder (20). The phosphorylation potential (PCr/P_i ratio) at rest is lower, and there is a delay in post-exercise PCr/P_i recovery in mitochondrial patients. Phosphorous magnetic resonance spectroscopy (^{31}PMRS) was used to study the effect of CoQ_{10} treatment on brain and skeletal muscle mitochondrial function, and baseline brain and skeletal muscle metabolism was compared with 36 age-matched healthy control subjects. Mitochondrial function in both brain and muscle was reduced by 25% and 29%, respectively, compared with control subjects. Treatment with CoQ_{10} statistically improved phosphorylation potential and calculated ATP synthesis in both brain and skeletal muscle in all patients studied (21).

In a multi-center trial of CoQ_{10} in mitochondrial cytopathies, a 25% decrease in post-exercise lactate levels was observed in more than one third of the patients (22). A bicycle ergometry study in patients with mitochondrial encephalomyopathies treated with CoQ_{10} showed no change in metabolic parameters after 3 months of treatment, but after 6 months of treatment a decrease in lactate:pyruvate ratios at rest and in association with exercise was noted in approximately half of the patients (23). It is unclear why some patients respond and others with the same clinical phenotype and biochemical defect do not show any beneficial effects. Response to treatment was not related to CoQ_{10} level in serum or in platelet mitochondria, or to the type of molecular defect (22). Characterization of responders would contribute greatly to our understanding of the means by which CoQ_{10} exerts its beneficial effect.

The longest-term study examined supplementation of CoQ_{10} in MELAS patients with the 3243 mutation. Insulin secretory response, progression of hearing loss, and lactate response after exercise were evaluated. The patients presented with diabetes and hearing loss, impaired glucose tolerance and normal glucose tolerance. The group of the MELAS patients with diabetes experienced an increased insulin secretory response and improved lactate response after exercise with the CoQ_{10} treatment. In addition, there was no progression of further hearing loss. The CoQ_{10} treatment did not affect the insulin secretory response of the patients with impaired or normal glucose tolerance (24).

Whereas most cases of decreased CoQ_{10} levels are secondary to other causes, primary muscle CoQ_{10} deficiency has been documented in patients with a mitochondrial encephalomyopathy characterized by ragged-red fibers and lipid storage in muscle, recurrent myoglobinuria, seizures, ataxia, and mental retardation (25-27). In patients with muscle CoQ_{10} deficiency, CoQ_{10} administration resulted in dramatic improvements in strength, seizure control, muscle weakness, and ataxia (25).

Commercially prepared CoQ_{10} supplements are available as powder-filled hard-shell capsules, oil-based suspensions in a soft-gel capsule, and emulsions in a soft-gel capsule. There are limited reports on the bioavailability or absorption of these preparations. Most studies have demonstrated that compounds formulated in soft gelatin capsules representing liquid suspensions tend to be absorbed more effectively than a dry-powder blend encapsulated in a hard gelatin capsule (28-30). Animal and human studies have demonstrated that approximately 2% to 10% of the dose administered is taken up into the blood (31,32). Dosages of 90 to 150 mg/day of CoQ_{10} have been shown to increase plasma concentrations by 180% (33).

The efficacy of CoQ_{10} supplementation in mitochondrial disorders is unclear. Many patients report improvement in clinical symptoms, and side effects from large pharmaceutical dosages (ie, 100 to 3,000 mg/d) are extremely rare. Dosage in the treatment of mitochondrial disorders varies, but some clinicians currently recommend 4 to 15 mg/kg/day to determine efficacy in an individual patient (34).

ANTIOXIDANTS

Vitamin C

Vitamin C has been used in OXPHOS disorders for its antioxidant properties (35). Ascorbic acid acts as a reducing agent; prevention of oxygen radical damage is the rationale for vitamin C administration. Vitamin C has been administered in combination with vitamin K_3 (menadione) to donate electrons directly to cytochrome *c* in a patient with complex III deficiency (36). Marked improvement in recovery from exercise as measured by NMR spectroscopy has prompted clinicians to use this regimen in other patients with OXPHOS disorders.

Antioxidants such as vitamin C may slow the process of oxidative damage, but the benefits over time are difficult to measure. Dosages of vitamin C typically range from 250 to 4,000 mg/day (34). Adverse effects, such as diarrhea, although rare, have been attributed to large doses (3 to 10 g/day), due to the osmotic effect of unabsorbed vitamin C in the intestine (37).

Vitamin E

Vitamin E includes 8 compounds, with α-tocopherol having the greatest biological activity. The main function of vitamin E is to scavenge free radicals and inhibit lipid peroxidation, which helps maintain membrane integrity (38). The α-tocopherol: cholesterol ratio has been shown to be reduced in patients and asymptomatic carriers of the 11778 Leber's hereditary optic neuropathy (LHON) mutation (39). Klivenyi et al concluded that the impaired function of complex I increases free radical formation and that the reduced ratio of α-tocopherol:cholesterol reflects the α-tocopherol consumption in the affected tissues. Radical scavenging antioxidants function independently, but may also act synergistically with other antioxidants. The tocopheroxyl radical formed is regenerated to active vitamin E by reaction with ubiquinol or ubisemiquinone (40). Several studies indicate that α-tocopherol and CoQ_{10} are more efficient when acting together. Indeed, both ubiquinol and ascorbate may play a role in the regeneration of active vitamin E (41).

Vitamin E seems to be one of the least toxic vitamins. Although there are no proven benefits with vitamin E treatment in OXPHOS disorders, dosages commonly range from 400 to 1,200 IU per day (34).

Lipoic Acid

Lipoic acid, found naturally in mitochondria, is a coenzyme for the β-ketoacid dehydrogenases. The rationale for its use in mitochondrial disorders is its ability to reduce oxidative stress (42). Animal studies with lipoic acid supplementation have demonstrated decreased oxidative damage, reduced oxidant formation, and improved mitochondrial function (43). In human studies, lipoic acid has been shown to decrease plasma indexes of oxidative stress and reduce lipid peroxides in both normal control subjects and diabetic patients (44,45). In a case study of one patient with chronic progressive external ophthalmoplegia (CPEO), a mitochondrial condition in which mtDNA genes are deleted or duplicated, administration of lipoic acid resulted in a 72% increase in phosphorylation potential and a 55% increase in brain PCr (46). The use of lipoic acid in clinical trials has not been associated with any reported side effects.

RIBOFLAVIN

Riboflavin is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which function as cofactors in complexes I and II. Riboflavin supplementation has been reported to be effective in a number of patients with complex I deficiency (47-53). One theory is that riboflavin may act by inhibiting the proteolytic breakdown of complex I, with a subsequent increase in enzymatic activity (34,54). Riboflavin-deficient rats show abnormalities in both biochemical and morphological aspects of mitochondria, adding support to this hypothesis (55).

Treatment solely with riboflavin has been used in patients with complex I deficiency. Improvement in exercise capacity after riboflavin administration has been noted in a patient with a myopathy due to a complex I deficiency (47). The effect of riboflavin therapy in 5 patients (2 with a pure myopathy and 3 with encephalomyopathic features) was evaluated. Clinical improvement was noted in both patients with the myopathic form of complex I, whereas only 1 of the 5 patients with the encephalomyopathic form improved (48). Complex I activity in 3 out of the 5 patients improved, but the clinical improvement did not correlate well with the increase in enzyme activity (48). The results of the study are further complicated by various dosages of riboflavin and varied length of treatment. An infant with a partial complex I defect was treated with increasing doses of riboflavin (maximum of 13 mg/kg/day or 120 mg/day), with improvement in motor development and normalization of lactate levels (53). In fibroblasts from a patient with a nuclear-encoded complex I deficiency, 5 μmol/L of riboflavin added to the culture medium significantly increased ATP synthesis (56).

Riboflavin has been used for the treatment of respiratory chain disorders in combination with other cofactors. In a young boy with complex I deficiency, myopathy improved dramatically during treatment with riboflavin and carnitine, and after 7 months of treatment complex I activity in muscle normalized (52). Although fatty acid oxidation was normal, muscle carnitine levels before treatment were low and were also normalized after treatment. Further support for the therapeutic benefit of riboflavin alone or in combination with carnitine has been demonstrated in 3 adult family members with complex I deficiency (51). Muscular endurance strength increased (measured by bicycle ergometry), and a muscle biopsy obtained in 1 subject after 2 years of 100 mg/day riboflavin supplementation showed complex I activity increased from 16% to 47% of the mean control levels (51). In a 3-year-old girl treated with riboflavin and carnitine, clinical symptoms improved and the withdrawal of carnitine did not alter the clinical response (49). Exercise tolerance deteriorated and muscle tone decreased when riboflavin was discontinued, thereby suggesting the clinical improvement was solely due to riboflavin (49).

A case study lending support to improvement in encephalomyopathic forms of OXPHOS disorders evaluated treatment of nicotinamide and riboflavin in a patient with MELAS syndrome (50). To confirm clinical benefit, treatment was withdrawn, and changes in ^{31}P-MRS and sural nerve conduction studies coincided with the development of encephalopathy. When the vitamins were restarted, clinical symptoms resolved and sural nerve potential amplitude doubled (50). This study did not identify either vitamin as solely responsible.

Dosages of riboflavin for treatment of OXPHOS disorders have ranged from 9 to 300 mg/day. Because there have not been adverse reactions associated with riboflavin administration, no tolerable upper intake levels have been established (37). The results of treatment vary but demonstrate that in some patients with complex I deficiency, riboflavin alone or in combination with other supplements may provide some benefit.

THIAMIN

Thiamin functions as a coenzyme in the oxidative decarboxylation of both pyruvate and α-ketoglutarate. The use of thiamin in the treatment of some forms of pyruvate dehydrogenase (PDH) deficiency has been well established (57), but thiamin effectiveness in the treatment of OXPHOS disorders is unclear. The therapy is postulated to improve aerobic glycolysis by enhancing pyruvate decarboxylation. Plasma lactate and pyruvate levels improved in a patient with Kearns-Sayre syndrome when thiamin was administered in dosages of 300 mg/day (58). It has also been reported that some patients with lactic acidemia improve clinically after high doses (>100 mg/day) of thiamin. Whether this amelioration is disease-specific and whether it may have implications for other cases of lactic acidosis not caused by a PDH defect is unknown.

Familial thiamin deficiency has been reported in 2 siblings with the 3243 MELAS mtDNA mutation who presented with skeletal muscle myopathy. Thiamin therapy (75 mg/day) improved the myopathy, normalized creatine kinase, and decreased blood lactate and pyruvate levels. Pyruvate dehydrogenase complex activity was less than normal in both patients before thiamin treatment, but it is unclear whether the decreased PDH activity was primary or secondary, caused by the thiamin deficiency. This is the first description of a 3243 mtDNA mutation associated with thiamin deficiency (59). Sato et al questioned whether thiamin metabolism was altered and suggested that thiamin status be investigated in patients with the 3243 mtDNA mutation (59).

In OXPHOS defects, thiamin has generally been used in combination with other cofactors, with variable results. Dosages have ranged from 25 to 300 mg/day, and there are no reported side effects with administration.

NIACIN

Nicotinamide, the amide form of niacin or nicotinic acid, is a precursor for both nicotinamide adenine dinucleotide (NAD/NADH) and nicotinamide adenine dinucleotide phosphate (NADP). The major role of NADH is to transfer electrons from metabolite intermediates to the respiratory chain. Complex I accepts electrons from NADH and passes them to ubiquinone. The rationale for nicotinamide use in OXPHOS disorders is to increase the cellular NADH and NAD concentration and thereby enhance the substrate availability to complex I. In a patient with a 3243 MELAS mutation with decreased complex I activity, nicotinamide treatment (1 g qid) resulted in marked decreases (50%) in both serum lactate and pyruvate and blood NAD levels increased 24-fold after 6 weeks of treatment (60). Majamaa et al speculate that the complex I defect led to an altered interaction between complex I and NADH, and although the affinity of complex I for NADH was similar to that in control subjects, the nicotinamide supplementation enhanced complex I activity by providing an excess of NADH (60). In another study, in a patient with the 3243 MELAS mtDNA mutation treated with both riboflavin and nicotinamide, clinical improvement was noted, but the vitamins were not tested individually (50).

The role of nicotinamide in the treatment of OXPHOS disorders is not clear. Adverse side affects of supplemental niacin use, such as flushing and nausea, are usually associated with dosages of more than 1,500 mg/day, but nicotinamide does not exhibit toxic effects (37).

VITAMIN K (PHYLLOQUINONE AND MENADIONE)

Vitamin K has been used in the treatment of patients with OXPHOS defects because it is assumed to mediate electron transport from NADH to electron acceptors such as coenzyme Q or cytochrome *c*. Vitamin K_3 (menadione) has been administered in combination with vitamin C (ascorbate) to donate electrons directly to cytochrome *c*. Menadione plus ascorbate improved cellular phosphate metabolism, as measured by ^{31}P-NMR, in a patient with complex III deficiency (36). The pretreatment rate of recovery from exercise was 2.5% of normal, and after administration of menadione and ascorbate, recovery rate increased 21-fold (36). Clinical and metabolic improvement continued at 1-year follow-up, and the patient's condition deteriorated after withdrawal of treatment. Recovery of function occurred after the vitamins were restarted (61). In another case study, a 16-year-old girl with complex III deficiency was treated with menadione and ascorbate; although there was a mild improvement of her ataxia, there was no change in lactic acidosis and only slight improvement of muscle bioenergetics. Brain ^{31}P-MRS indexes returned to normal after 5 months of treatment (62). High-dose menadione supplementation in a complex I–deficient patient yielded improvement in clinical and biochemical parameters, supporting its use in this multisystem disorder with lactic acidosis (63).

Which form of vitamin K is preferred for treatment of OXPHOS diseases is not clear. K_1 (phylloquinone) has not been used in any of the reported studies. Phylloquinone has been shown to have better tissue retention and reach higher levels in mitochondria. Menadione is water-soluble and must be alkylated to menaquinone-4 to be biologically active, in contrast to phylloquinone, which is lipid-soluble and biologically active (64). Menadione may cause hemolytic anemia and hyperbilirubinemia in newborns, whereas no side effects have been reported with phylloquinone (65). Whether K_1 (phylloquionine) would have any benefit in the treatment of OXPHOS disorders is unknown.

CREATINE

Creatine is synthesized endogenously in the liver from the amino acids arginine, glycine, and methionine, and is supplied exogenously via the diet. A normal mixed diet provides approximately 1 to 2 g/day, with the highest source being meat products (66). The rationale for creatine use in mitochondrial disorders is to increase phosphocreatine (PCr) stores and thus prevent ATP depletion. Patients with mitochondrial myopathies have been shown to have reductions in PCr in muscle and reduced PCr:ATP ratios in brain (36,67,68). Studies in healthy control subjects and athletes have demonstrated that creatine supplementation is most effective when muscle creatine content is low (69). In a short-term study in patients with mitochondrial disorders, creatine supplementation improved high-intensity anaerobic and aerobic activities, but had no effect on lower-intensity aerobic activities (70). The long-term beneficial effect of creatine use in mitochondrial disorders is unknown. There have been no reported side effects from dosages as high as 330 g/day (69).

CARNITINE

Carnitine transfers long-chain fatty acids across the mitochondrial membrane. It also facilitates branched-chain α-ketoacid oxidation, shuttles acyl CoA products of peroxisomal β-oxidation to the mitochondrial matrix of liver, increases CoA levels in the mitochondria, and esterifies potentially toxic acyl CoA me-

tabolites that impair the citric acid cycle (71). Carnitine may also play a role in membrane stabilization by altering the physiologic properties of mitochondrial membranes (72). Plasma and tissue levels of carnitine are maintained by exogenous dietary sources and de novo synthesis. Approximately 75% of carnitine comes from the diet, with red meat and dairy foods being the primary sources (71).

Endogenous carnitine is synthesized in the liver and kidney from the amino acids lysine and methionine. Skeletal muscle, which contains approximately 90% of the body's carnitine stores, and heart muscle, which contains the highest concentration of carnitine per gram of tissue, cannot synthesize carnitine and must rely on uptake of carnitine from the blood (73). Decreased skeletal muscle and plasma carnitine levels have been reported in many cases of mitochondrial myopathy (11,49,74), although this deficiency is assumed to be secondary to the mitochondrial defect. The carnitine deficiency may occur only in muscle, while the level of carnitine is normal in plasma.

In cytochrome *c* oxidase deficiency, maximal rates of carnitine uptake were decreased by 50% to 80% when studied in cultured skin fibroblasts (75). In one study, abnormal carnitine distribution in muscle was found in 29% (22 of 77) of patients with mitochondrial myopathy (76). The same pattern of abnormal carnitine distribution (increased acyl carnitine:decreased free carntine ratio) in muscle was reported in 11 of 13 patients with idiopathic inflammatory myopathy (77). Six of these 11 patients demonstrated histochemical or biochemical signs of mitochondrial dysfunction. One theory is that impaired mitochondrial function might cause acyl CoA accumulation and increase carnitine esterification, resulting in a low free carnitine level (77). Elevated levels of acyl CoA intermediates also may impair the function of the adenine nucleotide translocase, which exchanges ADP for ATP across the inner mitochondrial membrane. Studies of mitochondrial myopathies have revealed similar carnitine distribution in plasma. Carnitine insufficiency (elevated ratio of esterified to free carnitine) was found in the plasma of 43.8% (21 of 48) patients with mitochondrial myopathies, and both free and total carnitine deficiency was detected in 8.3% of the patients. Carnitine supplementation was started, with subjective improvement in muscle strength and tone noted in 20 of the 21 patients (76). Echocardiographic and clinical evaluation improved in all of the patients who had presented with cardiomyopathy (76).

Further research is warranted to establish the relationship between carnitine metabolism and mitochondrial function. Oral dosages of carnitine in the treatment of these diverse mitochondrial disorders vary greatly, ranging from 100 mg/day to 200 mg/k/day. Side effects from large oral doses (>1 g/day) are minor, but include diarrhea and a fishy body odor in some cases. Carnitine supplementation is generally recommended in conditions in which carnitine deficiency is suspected or identified; however, the potential beneficial effect remains uncertain (78).

COMBINED-THERAPY TREATMENTS

In a study of patients with diverse mitochondrial disorders, a combination of vitamin K_3, ascorbic acid, CoQ_{10}, and methylprednisolone was evaluated by ^{31}P-NMR and clinical and biochemical assessment (79). Length of follow-up varied from 6 months to 13 years. A subset of the patients seemed to live longer with fewer medical complications and functional disability than typically seen in clinical practice. Although the results are encouraging, the effectiveness of treatment is inconclusive.

In another study in which patients were treated with CoQ_{10}, vitamin K_3, vitamin C, thiamin, riboflavin, and niacin, there was no significant, reproducible, objective clinical improvement (80). Serum lactate, exercise testing on a cycle ergometer, ^{31}NMR studies at rest and exercise, and clinical follow-up were used as independent measures of oxidative metabolism. It is difficult to interpret the negative results of the study, but results from previous studies suggest that the 2-month treatment period may have been too short for beneficial effects to become evident. The results are further compromised by small sample size; clinical observation and aerobic exercise performance were assessed in only 10 patients. More studies are required to evaluate the effectiveness of combined therapy over a longer time period in the treatment of mitochondrial disorders.

SUMMARY

The heterogeneity of the phenotype and genotype, as well as the unpredictable natural history of OXPHOS diseases, has complicated the evaluation of treatment regimens. Making definitive conclusions about metabolic therapy is challenging because most of the information available is based on anecdotal reports and case studies. In addition, the lack of reliable, objective measurements impedes interpretation of results. The use of multiple objective measures (nuclear magnetic resonance spectroscopy of brain and muscle, bicycle ergometry, and neurophysiologic studies) will add to the assessment of treatment efficacy. Objective determination of clinical status should be included in assessment to determine whether biochemical improvements are mirrored by improvements in physiological symptoms. The difficulty arises as remissions and exacerbations are characteristic of OXPHOS disorders and may mask the efficacy of a particular treatment; because of this, studies evaluating clinical effectiveness of treatments for these disorders need to be long-term.

Although individual vitamins and combinations of cofactors have been used widely in the treatment of patients with mitochondrial disorders, evidence of a significant effect on the course of the disease has not been demonstrated. It seems unlikely that a standard treatment would have a similar effect on all mitochondrial disorders, and thus studies of a standard treatment on a heterogeneous group of patients may not be applicable to an individual patient. In addition, developing a treatment trial examining the efficacy of cofactors by evaluating the response of all possible organ systems would require an extremely large number of patients. Given the inherent problems mentioned earlier, and the difficulty in identifying large numbers of subjects with these disorders for clinical trials, individual trials in which the patient serves as his or her own control may be a reasonable approach. Because some beneficial effects have been demonstrated by cofactor therapy and there is no proven effective treatment for mitochondrial disorders, common practice is to supplement with cofactor therapy. Provided that safe dosages are used, there is the potential for benefit and minimal risk of harm in trying high-dose nutrient therapy with patients with mitochondrial disorders. The ease of administration, nominal cost, and low level of risk are all advantages, despite the lack of studies demonstrating clinical improvement.

APPLICATIONS

■ This article can serve as a guide to dietitians needing to incorporate vitamin and cofactor supplements in the treatment of mitochondrial disorders. Due to the diverse nature of these conditions, the evaluation of any nutritional therapy for these disorders remains a challenge.

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