

# Nutritional support in mitochondrial diseases: the state of the art

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**Abstract.** – Mitochondrial diseases are a group of rare multisystem disorders characterized by genetic heterogeneity and pleomorphic clinical manifestations. The clinical burden may be heavy for patients and their caregivers. There are no therapies of proven efficacy until now and a multidisciplinary supportive care is therefore necessary. Since the common pathogenic mechanism is the insufficient energy production by defective mitochondria, nutrition may play a crucial role. However, no guidelines are still available.

The article reports the current evidence, highlighting nutrition both as support and as therapy. The estimate of nutritional status, energy needs and nutritional behaviors are firstly discussed. Then, we go in-depth on the scientific rationale and the clinical evidence of the use of anti-oxidants and enzyme-cofactors in the clinical practice. In particular, we analyze the role of Coenzyme Q10, Creatine monohydrate,  $\alpha$ -lipoic acid, riboflavin, arginine and citrulline, folic acid, carnitine, vitamin C, K, and E.

Every attempt at nutritional intervention should be made knowing patient's disease and focusing on his/her energy and nutrients' requirements. For this reason, clinicians expert in mitochondrial medicine and clinical nutritionists should work together to ameliorate care in these fragile patients.

## Key Words

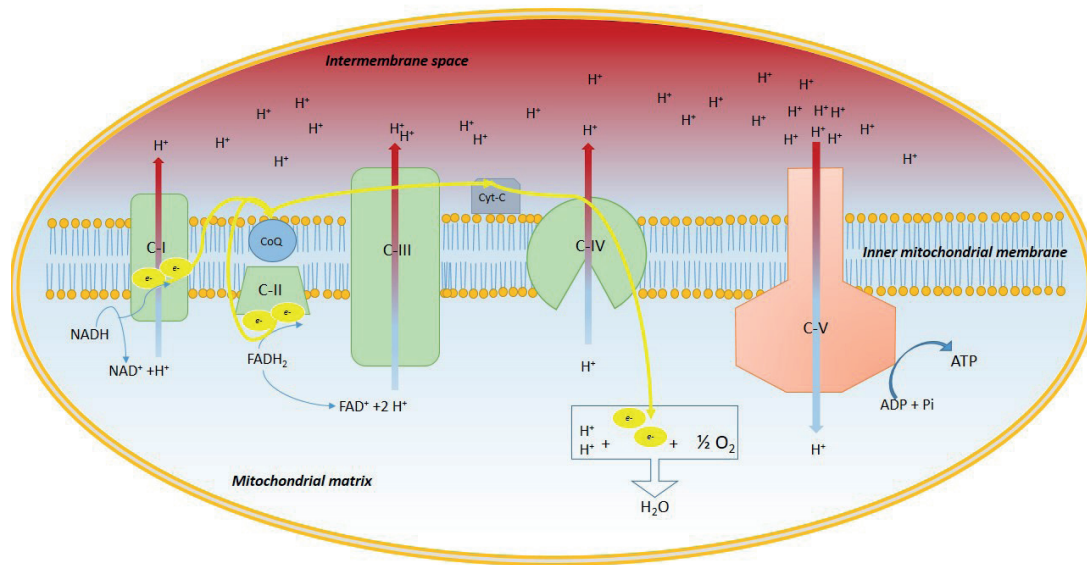
Mitochondrial diseases, Electron transport chain, Ketogenic diet, Coenzyme Q10, Creatine monohydrate,  $\alpha$ -lipoic acid, Riboflavin, Arginine, Citrulline, Folic acid, Carnitine, Vitamin C, Vitamin K, Vitamin E, Personalised medicine.

## Introduction

Mitochondrial diseases (MD) are a group of genetically heterogeneous disorders due to dysfunctional mitochondria and characterized by a broad spectrum of clinical symptoms.

Mitochondria give approximately 90% of the energy provided to our body through an aerobic mechanism called oxidative phosphorylation (OXPHOS): this chain of biochemical reactions is housed in the inner mitochondrial membrane. Energy sources (glucose, fatty acids, amino acids) are there imported from the cytoplasm and enter into metabolic pathways (citric acid cycle,  $\beta$ -oxidation, amino acid oxidation). These cycles produce electron donors such as Nicotinamide Adenine Dinucleotide H (NADH) and Flavin Adenine Dinucleotide H<sub>2</sub> (FADH<sub>2</sub>), transferring electrons into the protein electron transport chain (mitochondrial respiratory chain: complexes I-IV) to molecular oxygen, with the formation of water. The passage of electrons, O<sub>2</sub> and H<sup>+</sup> ions across the inner mitochondrial membrane create an electrochemical proton gradient between the matrix and the intermembrane space, generating adenosine triphosphate (ATP). This last reaction is carried out by the complex V (ATP synthase), condensing adenosine diphosphate (ADP) and Pi<sup>1</sup> (Figure 1).

At the basis of the MD, there are defects of oxidative phosphorylation. These metabolic mistakes are responsible not only for the reduction of ATP synthesis but also for an increased reliance



**Figure 1.** Oxidative phosphorylation in the inner mitochondrial membrane. NADH: Nicotinamide Adenine Dinucleotide H; FADH<sub>2</sub>: Flavin Adenine Dinucleotide H<sub>2</sub>; ATP: adenosine triphosphate; ADP: adenosine diphosphate; CoQ: Coenzyme Q; Cyt-C: Cytochrome C.

on non-aerobic metabolic pathways – such as glycolysis and glycogenesis – to provide ATP, which in turns may result in elevated serum lactate. On the other hand, uncoupled oxygen may generate reactive oxygen species (ROS), contributing to oxidative cell damage.

Mitochondria are the only organelles that host their own DNA. Thus, the pathogenic mechanisms that produce mitochondrial dysfunction rely on mutations that can affect either the mitochondrial DNA (mtDNA) or the nuclear DNA (nDNA). nDNA mutations are inherited in a Mendelian way with autosomal recessive, dominant or more rarely X-linked transmission. Conversely, mtDNA mutations derive almost exclusively from the mother given the lack of mtDNA contribution of the sperm to the zygote cell (matrilineal, non-mendelian transmission). As far as the mtDNA changes, the entity of disease depends on the rate of mutant and wild-type mtDNA genomes housed in the maternal egg cell and with the mitotic segregation randomly distributed in each cell and in the different tissues (*mitochondrial heteroplasmy*)<sup>2</sup>. For the same reason, there is an extreme clinical variability of the disease expression among different individuals carrying the same mutation even in the same family.

MD are overall the most common inherited metabolic disorders and are among the most common inherited neurological disorders<sup>3</sup>. Their incidence

is around 11.5/100,000 worldwide<sup>4</sup>. Instead, the prevalence of childhood-onset (<16 years of age) disorders varies from 5 to 15 cases per 100,000 individuals in different countries, while in the adult population from the North East of England is about 9.6 cases per 100,000 individuals for MD due to mutations in mtDNA and 2.9 cases per 100,000 for mutations in nDNA. Furthermore, it was calculated that 10.8 per 100,000 individuals were potentially at risk of developing mtDNA-linked disorders having an affected first-degree relative<sup>3</sup>.

MD may be syndromic or non-syndromic but, given the ubiquitous distribution of mitochondria (except in red blood cells), are mostly multisystem. However, because muscle and nervous cells have elevated energy needs, they are the main targets of these diseases. Clinical presentation may vary including muscle weakness, lactic acidosis, intolerance to exercise, loss of motor control, dementia, ataxia, parkinsonism, psychomotor regression, developmental delay, pain, seizures, migraine, stroke-like episodes, diabetes mellitus, cardiac diseases, respiratory complications, visual or hearing problems, short stature, gastrointestinal disorders and swallowing difficulties, poor growth, cachexia, liver and kidney disease, psychiatric disorders<sup>3</sup>. Symptoms and signs may represent isolated manifestations, but more often may combine in specific well-characterized phenotypes or widely overlap. The most common phenotype in children is Leigh syndrome and in adult CPEO, both grouping nu-

**Table I.** Principal MD, inheritance way, onset and most common features.

Acronym	Syndrome name	Inheritance	Onset	Common Features
KSS	Kearns-Sayre syndrome	Sporadic	<20 years	PEO, pigmentary retinopathy, heart conduction block, ataxia
MILS	Maternal Inherited leigh syndrome or subacute necrotizing encephalomyopathy	Maternal, mendelian	<2 years	Brain abnormalities: ataxia, seizures, impaired vision and hearing; muscle weakness: dysphagia, impaired speech and eye movements
MDS	Mitochondrial DNA depletion syndrome	Mendelian	<2 years	Muscle weakness, liver failure
MELAS	Mitochondrial encephalomyopathy, Lactic acidosis and Stroke-like episodes	Maternal	2-40 years	Brain abnormalities: stroke-like episodes, seizures, vomiting, migraine-type headaches; Muscle weakness, PEO Hearing loss, diabetes, short stature
MERRF	Myoclonus epilepsy with Ragged red fibers	Maternal	Late adolescence to adulthood	Myoclonus, seizures, ataxia and muscle weakness; pigmentary retinopathy, coordination loss, dementia.
MNGIE	Mitochondrial Neuro Gastro-intestinal encephalomyopathy	Mendelian	<20 years	PEO, peripheral neuropathy, limb muscle weakness, GI disorders (diarrhea, abdominal pain)
NARP	Neuropathy, Ataxia and Retinitis pigmentosa	Maternal	>2 years to adulthood	Neuropathy, ataxia and retinitis
	Pearson syndrome	Sporadic	<2 years	Pancreas abnormalities and anemia
PEO	Progressive external	Ophtalmoplegia Maternal, Mendelian, Sporadic	11-18 years	PEO as main feature. Exercise intolerance
LHON	Leber hereditary optic neuropathy	Maternal	<30 years	Visual loss, pre-excitation cardiac conduction syndromes, spasticity, dystonia and encephalopathy.

Abbreviations: PEO: progressive external ophtalmoplegia; GI: gastro-intestinal

merous different disorders. The better-characterized clinical syndromes are KSS, MELAS, MERRF, and LHON (Table I).

The diagnostic workup of MD often includes a muscle biopsy. The morphological hallmark is the presence of the “ragged red fibers” (RRFs), myofibers that accumulate mitochondria in the subsarcolemmal regions that appeared red with the modified Gömöri trichrome staining. The same fibers strongly react for succinate dehydrogenase and are mostly negative for cytochrome *c* oxidase. We have to keep in mind however that RRFs may be absent in specific genetically-proven MD (i.e., NARP).

Since MD are clinically heterogeneous, treatment should be multidisciplinary, and long-lasting and each symptom needs to be

managed in a proper way. In this sense, a specifically-designed supportive care should be offered to each patient.

Whatever is the clinical presentation, an adequate nutrition should be at the basis of all medical interventions. The main nutritional outcome in MD is to deliver energy to organ and tissues: this could be reached optimizing cells energy production and reducing energy losses. Another endpoint is to reduce free-oxygen radicals’ synthesis and myofibers damage.

Lacking guidelines on this topic, there are two possible nutritional approaches to MD: a general approach, transversal to any syndrome as nutritional support, and a specific one targeted to specific conditions as potential nutritional therapy. We briefly discuss both of them.

### **General Nutritional Approach (Nutritional Support)**

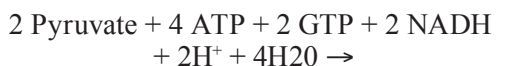
Nutritional support should be at the basis of any approach to each of these syndromes. Indeed, patients affected by MD are at risk of malnutrition. Many of them have not the necessary autonomy to prepare and consume healthy meals, as they need. Moreover, gastroesophageal and pharyngeal motility disorders (dysphagia, swallowing problems and fatigue) and gastrointestinal problems often reduce nutrients' intake and absorption. These aspects have been yet correlated to a low Body Mass Index (BMI) in such population<sup>5</sup>.

A recent observational cross-sectional study, conducted in The Netherlands<sup>6</sup>, collected 3-days nutrition diaries from sixty patients holding a mitochondrial disease, comparing these data with those of healthy Dutch individuals. As compared with healthy subjects, intake of proteins and calcium (as dairy products) was significantly lower, while carbohydrates intake (mainly bread and potatoes) were significantly higher. This is a noteworthy issue, given the increased risk of metabolic disorders such as diabetes in this population. Moreover, a low protein and Vitamin D dietary regime could enhance muscular weakness and fatigue<sup>7</sup>, and Vitamin D deficiency is a well-known risk factor for osteoporosis.

It is proven that, if well sustained in their nutritional needs with an age-appropriate diet and a regular physiotherapy, patients could improve clinical and biochemical parameters<sup>8</sup>.

### **Avoid fasting**

First of all, it is necessary to avoid fasting. Eating small meals frequently should be recommended to patients or their caregivers. Fasting is detrimental to these patients and may worsen fatigue, because of muscular protein breakdown to provide gluconeogenic substrates to the liver. This is a very expensive metabolic pathway, given the employment of ATP and NADH to generate glucose from amino acids, according to the following reaction:



ATP synthesis is so overwhelmed with increased glycolysis and lactic acid production in a vicious cycle. Moreover, during a prolonged period of fasting, gluconeogenesis shifts acetyl-CoA towards the synthesis of ketones bodies, lowering plasma pH, increasing the risk of metabolic acidosis<sup>1</sup>.

### **Meet Energy Needs**

From a nutritional point of view, MD could also benefit from standard measures yet applicable to other neurological diseases, both of peripheral and central nervous system. Recently, the European Society for Clinical Nutrition and Metabolism (ESPEN) published guidelines for clinical nutrition in neurology<sup>9</sup>. The paper focuses on several aspects of neurologic diseases to which we redirect the reader. A general recommendation is to estimate the nutritional status and the energy requirements of the patients. Several tools are available to analyze the nutritional risk, such as body mass index (BMI), BMI loss, lab exams as albumin and serum lipids, bioelectrical impedance (BIA) and dual-energy X-ray absorptiometry (DEXA) to estimate body composition.

Energy needs in neurological patients should be estimated as about 30 kcal/kg body weight depending on physical activity and adapted to weight and body composition evaluation. No recommendation, however, is given about protein content. Even if quantity and quality of protein are not recommended on these guidelines, 1.0-1.5 g/kg of ideal weight should be effective and safe, except for patients with renal failure. Also, amino acids are potentially useful to deal with sarcopenia. Among these, leucine and its derivatives should be preferred given their stimulation of mTOR pathway with a boost in protein synthesis, cell growth, and metabolism<sup>10</sup>.

### **Deal with Dysphagia**

A peculiar topic of the ESPEN guidelines is the management of oropharyngeal dysphagia (OD), often present in MD. Patients should be screened for possible dysphagia with general (structured questionnaires, water and volume viscosity swallow tests) or specialist approaches (video-fluoroscopy, flexible endoscopic evaluation of swallowing). In case of swallowing fatigue or long-lasting meals, patients (or their caregivers) should fractionate meals and enrich their caloric content. Patients may report weight loss: in these case, oral nutritional supplements are recommended. In case of moderate dysphagia, the texture of solid and liquids should be adapted to facilitate swallowing and avoid aspiration; postural maneuvers (such as *chin-tuck* posture) could protect the airway during swallowing. Finally, enteral nutrition (EN) is necessary for patients in whom oral feeding does not meet nutritional needs and in whom malnutrition/dehydration could be responsible for reduced sur-



vival. EN may be delivered by nasogastric tube or Percutaneous Endoscopic Gastrostomy (PEG) in case of medium or long-term supply<sup>9</sup>.

### ***Specific Nutritional Approach (Nutritional Therapy)***

Even if, to date, no specific therapy for MD exists, a specific diet and nutritional supplements may improve, or stabilize, disease signs and symptoms.

There are not structured evidence and large-population studies about this topic; nevertheless, emerging evidence in few patients and anecdotal reports, may help in the ordinary management and pave the path for further robust studies.

The rationale of a nutritional therapy is based on the evidence that diet composition influences mitochondrial function: the relative ratio of dietary macronutrients can select or bypass the complexes of the respiratory chain in which electron enter. For instance, in a *Drosophila* model, it has been demonstrated a lower Complex-I (C-I) activity in flies fed with a low protein: carbohydrates (P:C) ratio (1:12), compared to those fed with a higher P:C ratio (1:3) diet<sup>11</sup>. This evidence may be translated in cases of MD associated to defective C-I. In this sense, diet may have the ability to modulate metabolic defects associated with mitochondrial mutations<sup>12</sup>.

Given the complexity and heterogeneity of MD, to modulate the clinical expression, clinicians should first identify the specific defect, then prompt a patient-tailored nutritional treatment.

Various diets and oral supplements have been proposed. One of the more debated with conflicting evidence is the ketogenic diet.

### ***Ketogenic Diet***

The ketogenic diet (KD) is a high-fat, low carbohydrate diet, commonly used for short periods in metabolic syndrome and obesity<sup>13</sup>. It also has a role in the treatment of refractory epilepsy, in which it has demonstrated to reduce seizures<sup>14</sup>, and has been proposed as a nutritional therapeutic approach in MD with epilepsy. The scientific rationale lies in the evidence that KD stimulates lipid beta-oxidation in mitochondria with ketone bodies production in the liver. This mechanism could be especially exploited in subjects showing C-I deficiencies, given the fact that lipid beta-oxidation enhances electron flow through Complex II (C-II), by-passing partly C-I<sup>15</sup>.

The effects of a high-fat diet (HFD) on the mouse were verified by Wall et al<sup>16</sup> in a specific model called polymerase gamma mutator

(POLG), housing point mutations and deletions in the mitochondrial genome. The Authors showed that an HFD was able to restore thermogenesis in the brown adipose tissue of POLG mice, causing an activation of mitochondrial biogenesis, as confirmed by an increased genome expression of C-I, C-II and Complex III (C-III) of the respiratory chain. Moreover, morphological changes of POLG mouse mitochondria toward a WT-like model were observed.

A human pilot study by Ahola et al<sup>17</sup> experimented a type of KD, called modified Atkins Diet (mAD) (3-9% of carbohydrates, high fat, high protein content) as dietary treatment in five subjects affected by PEO/MM. These patients were compared to age and gender-matched healthy controls. After 2 weeks of a normalized isocaloric standard diet, the subjects were given with mAD. Within the first two weeks of treatment, PEO/MM patients experienced burning sensations and muscle pain. Serum creatin kinase (CK), alanine aminotransferase (ALT) and myoglobin values were increased, suggesting a muscle damage. Healthy subjects did not report any symptoms. The trial was prematurely stopped for ethic reasons. At muscle biopsy, some PEO/MM muscle fibers showed acute degenerative changes affecting RRFs selectively. After 2.5 years, however, three out of four PEO patients showed improved muscle strength. Authors discussed the evidence: mtDNA mutated cells may be especially dependent on pyruvate and glycolysis products for their energy metabolism, whilst they are not able to utilize lipids, amino acids, and ketones as an energy source. The boost in  $\beta$ -oxidation, protein oxidation and ketogenesis, driven by a KD, could lead to a "selective rhabdomyolysis" of RRFs. On the other side, healthy muscle cells may undergo fusion to replenish the sick, damaged cells.

At the actual evidence, KD in MD could be only suggested for children with syndromes in which a refractory epilepsy is the principal clinical feature. In these conditions, ketogenic diet seems to have both a neuroprotective and anti-inflammatory effect (reduction in excitotoxicity and oxidative stress) and also a direct anticonvulsant action. This effect could be reached via a modulation of sodium and potassium voltage-gated channels and a down-regulation of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptor<sup>18</sup>. The efficacy of KD in MD with epilepsy was demonstrated in a few studies. Lee et al<sup>19</sup> have shown a significant reduction of seizures in a cohort of 48 patients with confirmed mitochondrial respiratory

chain defects and medically intractable epilepsy. Zhu et al<sup>20</sup> reported an improved neurological and psychomotor development in children with intractable epilepsy treated with KD. Martikainen et al<sup>21</sup> suggested KD as an adjunct to antiepileptic drugs in POLG-relating epilepsy.

A very low carbohydrate diet should not be considered as the net solution by itself. Firstly, it is not advisable for all patients, in particular for patients affected by liver, heart and kidney diseases and those at risk of osteoporosis of biliary stones. Moreover, metabolic acidosis elicited by a high fat, high protein diet may worsen fatigue and create an unfavorable metabolic setting for the daily living (impaired concentration, headache, constipation), especially if the patients are not under clinical advice.

In conclusion, in the absence of controlled studies and with the risk of inducing or worsening a metabolic crisis, the use of ketogenic diet should be restricted only to children with refractory epilepsy under a strict surveillance of the metabolic equilibrium.

### **Nutritional Supplements**

The role of nutritional supplements (NS) is not yet truly understood. However, many of these are largely used in the clinical setting, with the hope to ameliorate care. It remains valid the concept that the real benefit of each supplement could vary among each syndrome.

### **Coenzyme Q<sub>10</sub> and Idebenone**

Coenzyme Q<sub>10</sub> (CoQ) is an electron acceptor of the mitochondrial respiratory chain, shuttling electrons from C-I-II and electron transferring flavoprotein dehydrogenase (ETF<sub>1</sub>) to C-III (1). It is also an antioxidant. The rationale of an exogenous supplementation of CoQ is to bypass defects in the respiratory chain, reducing electron leak and restoring ATP synthesis. *In vitro* studies suggest an increase of mitochondrial ATP production in cultured cells from patients affected by MD due to CoQ supplementation.

CoQ deficiency underlies several types of mitochondrial diseases depending on the modified gene<sup>22</sup>. It may be present in mitochondrial disorders as primary or secondary deficits<sup>23</sup>. Even when the deficit of CoQ is proven, CoQ supplementation may not be effective, being the pathogenic mechanism not fully understood in all syndromes<sup>24</sup> (Table II).

Nevertheless, irrespectively from the phenotype, CoQ is widely used in MD, including ME-

LAS in which a small randomized trial reported improvement of muscle weakness, fatigability, and serum lactate<sup>25</sup>. However, CoQ it has a limited effect on the central nervous system because it does not cross the blood-brain barrier. Conversely, its analog Idebenone can cross the blood-brain barrier with a potential role in stroke-like episodes in MELAS<sup>26</sup>.

Idebenone is instead approved for the treatment of Leber's hereditary optic neuropathy (LHON), although some issues still remain to define as timing, dose, and frequency of administration and which are the patients to treat<sup>27</sup>.

The dosage suggested for oral CoQ supplementation as ubiquinone is up to 3000 mg, but the average dose is around 300 mg/daily. Ubiquinone is insoluble in water, and it has a poor intestinal absorption. A recent, reduced formulation, ubiquinol, seems to be better absorbed<sup>28</sup>.

### **Creatine Monohydrate**

Creatine monohydrate (CrM) is an antioxidant and a potential energy source for defective mitochondria, through the phosphocreatine system. It also works as energy shuttle of ATP and phosphates from mitochondrial sites to the cytoplasm. It is commonly used by athletes as a dietary supplement to increase muscle performance in short-duration and high-intensity resistance exercises, at the dosage of 0.3 g/kg daily for 5 to 7 days, followed by maintenance dosing at 0.03 g/kg daily for 4 to 6 weeks<sup>29</sup>.

Patients affected by MM have low creatine levels in muscles and brain. In a randomized, crossover trial on seven patients affected by mitochondrial cytopathies, the administration of CrM (at the oral dosage of 5 g bid for 14 days, followed by 2 g bid for 7 days) significantly improved physical performance and reduced post-exercise lactate. Conversely, no significant effects were observed in lower intensity aerobic activities<sup>30</sup>. Similar results are reported in a small group of children with mitochondrial encephalomyopathies<sup>31</sup>.

### **$\alpha$ -Lipoic acid**

$\alpha$ -lipoic acid is a natural antioxidant, present in mitochondria as a cofactor for pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase. Its role in MM could lie in scavenging ROS generated by defective oxidative phosphorylation, decreasing oxidative stress in mitochondria and cells' membranes. It may also enhance the uptake of phosphocreatine as an alternative energy source to anaerobic glycolysis in defective mito-

**Table II.** Syndromes associated to CoQ deficiencies.

Syndromes	Gene involved	Type of CoQ deficit	Response to CoQ10 supplementation
Neonatal multisystemic disorder, nephropathy, lactic acidosis, Leigh syndrome, neonatal encephalopathy with seizures, MELAS, retinitis pigmentosa	COQ2	Primary	Good response
Encephalomyopathy	COQ4	Primary	Good response
Nephropathy, deafness, seizures	COQ6	Primary	Good Response
Neonatal encephalomyopathy, lactic acidosis, refractory seizures	COQ9	Primary	Poor response
Infantile nephropathy, hepatopathy, mental retardation, lactic acidosis, optic atrophy	PDSS1	Primary	Not known
Leigh syndrome, lactic acidosis and nephropathy	PDSS2	Primary	Poor response
Cerebellar ataxia and atrophy± retardation, lactic acidosis and exercise intolerance	ADCK3	Primary	Poor response
Nephropaty, mild mental retardation	ADCK4	Primary	Good response
Isolated myopathy	ETFDH	Secondary	Good response
Cerebellar ataxia	APTIX	Secondary	Good response

Abbreviations: CoQ: Coenzyme Q10; COQ2: Coenzyme Q2; COQ4: Coenzyme Q4; COQ6: Coenzyme Q6; COQ9: Coenzyme Q9; PDSS1: Prenyl (Decaprenyl) Diphosphate Synthase, Subunit 1; PDSS2: Prenyl (Decaprenyl) Diphosphate Synthase, Subunit 2; ADCK3: aarF domain containing kinase 3 ETFDH: Electron Transfer Flavoprotein Dehydrogenase; APTX: aprataxin.

chondria<sup>32</sup>. Suggested dosage is 50-200 mg/day<sup>33</sup>. A combination therapy of CoQ<sub>10</sub>, CrM, and  $\alpha$ -lipoic acid has been used as a treatment option in a randomized, double-blind, placebo-controlled, crossover study, recruiting sixteen patients with several mitochondrial diseases<sup>34</sup>. Compared to placebo, this cocktail significantly reduced plasma lactate concentration and urinary 8-isoprostane, a marker of oxidative stress. After the treatment, patients showed a minor reduction in peak ankle dorsiflexion strength than that observed after the placebo phase. Moreover, a subgroup of patients with MELAS showed significant improvements in body composition, such as an increase in fat-free mass (FFM) and total body water (TBW) and a decrease in body fat. Authors stressed the positive synergistic effect of these compounds in MD, even if they recognized that not all the patients affected by MD may have the same benefits.

### **Riboflavin**

Riboflavin is a vitamin of group B (B2 vitamin), and it acts as a flavoprotein precursor in C-I and C-II and as a cofactor in several other enzymatic pathways of  $\beta$ -oxidation and Krebs cycle<sup>1</sup>.

In patients with myopathy due to ETFDH mutations, riboflavin associated with CoQ supplementation has been effective in improving clinical and

biochemical parameters (serum CK and lactate)<sup>35</sup>. Non-randomized studies have shown its efficacy in C-I and C-II mitochondrial diseases<sup>36,37</sup>.

The suggested oral dosage is 50-400 mg daily, both in children and in adults<sup>33</sup>.

### **Arginine and citrulline**

Arginine is a nitric oxide (NO) precursor. Its clinical benefit is still debated. Efficacy of intravenous (iv) administration of L-arginine has been proposed in MELAS in the acute phase of the stroke-like episodes<sup>38</sup>. The same group suggested the use of chronic oral arginine supplementation showing a reduction of frequency and severity of stroke-like episodes in patients with MELAS. A daily dose of 150 to 300 mg/kg/day oral arginine divided into three doses was recommended<sup>39</sup>. Arginine efficacy could be related to cerebral vasodilatation and endothelial function, due to an increase in NO availability<sup>40</sup>. However, there are some concerns about the safety of L-arginine, especially for intravenous administration<sup>41</sup>.

Citrulline can also increase NO production in patients with MELAS syndrome, although its clinical effects have not yet been studied. Interestingly, citrulline was associated with a greater increase in NO and cerebral blood flow than arginine supplementation, indicating citrulline as po-

tentially more effective than arginine in patients with MELAS syndrome<sup>40</sup>.

Although these findings may appear promising, they should be confirmed in a long-term randomized controlled trial both for arginine and citrulline.

### **Folinic acid**

The term folate refers to a family of compounds belonging to B vitamins, acting as coenzymes in carbon-transfer reactions. These include folic acid, folinic acid – both present in plants and food – and 5-methyltetrahydrofolate (5-MTHF), the biologically active form. The known metabolic activities of these compounds or their derivatives are the purine synthesis and the methylation of homocysteine to methionine. In mitochondria, folate metabolism regards essentially the synthesis of CO<sub>2</sub> and formate and translation of transfer RNA (tRNA) for protein synthesis<sup>42</sup>. Folate deficiency is quite common in patients affected by MD and it could worsen mitochondrial metabolism<sup>43</sup>. However, folate deficiency seems to be more related to a specific syndrome, the Kearns-Sayre syndrome (KSS) in which there is an impaired ATP-related transport of 5-MTHF through the choroid plexus into cerebrospinal fluid with consequent defects in methylation of myelin protein<sup>44</sup>. In this sense, a systemic folate deficiency may aggravate mitochondrial ATP production and myelin synthesis. For this reason, an external supplementation of folate has been suggested and its efficacy is proven in some case reports<sup>45-47</sup>. Folic acid seems to be ineffective due to a genetic mutation of dihydrofolate reductase (DHFR) methylenetetrahydrofolate reductase (MTHFR), so, the active compound folinic acid is preferred, even if no a standard dosage is to date given<sup>42</sup>.

### **Carnitine**

Carnitine is a cellular compound, predominant in skeletal muscle, heart, and liver, playing a crucial role in energy production. It transports into mitochondria, across the mitochondrial membrane, long-chain fatty acids as acyl carnitine esters<sup>48</sup>. Moreover, it also prevents CoA depletion and facilitates the excess of toxic acyl compounds. For such reason, it is empirically used by some clinicians in the management of MD, although there is not proven efficacy with the exception of primary carnitine deficiency<sup>33</sup>. Recently, some concerns have been raised about its possible role in increasing cardiovascular risk, probably mediated by gut microbiota<sup>49</sup>.

### **Vitamin C and Vitamin K**

Vitamin C and Vitamin K are antioxidants<sup>50,51</sup>; they are also able to bypass mitochondrial C-III deficiencies as electron acceptors. However, clinical evidence of a role in patients with recognized mitochondrial depletion syndromes is lacking. Only in a single case report, the supplementation of both of these vitamins was associated with short-term clinical improvement<sup>52</sup>.

### **Vitamin E**

Tocopherol was proposed for its antioxidant capacity in mitochondrial dysfunctions and in neuroprotection<sup>53</sup>; however, no evidence is available in mitochondrial diseases. It was speculated that tocopherol might have a role only in (LHON), in which a significant deficiency of vitamin E was demonstrated<sup>54</sup>.

All considered, many compounds have been used and tried as nutritional therapy and numerous reports have proposed various cocktails of antioxidants and nutrients. However, in the absence of large, properly controlled trials, the latest Cochrane review concluded that there is currently no clear evidence supporting the use of any intervention in mitochondrial disorders<sup>55</sup>.

Nevertheless, in the recent consensus conference “Nutritional Interventions in Primary Mitochondrial Disorders: Developing an Evidence Base”, an agreement was achieved, using the Delphi consensus methodology, on the following points:

- 1) CoQ10 should be administered to most patients with a diagnosis of mitochondrial disease, and not exclusively for the primary CoQ10 deficiency.
- 2) Alpha-lipoic acid and riboflavin are the only other supplements that obtained a certain degree of agreement.
- 3) Folinic acid may be considered in patients with CNS manifestations especially when is documented a cerebral folate deficiency.
- 5) L-carnitine should only be administered when there is a documented carnitine deficiency.

Moreover, it is suggested to start the treatment with one supplement only and to evaluate the clinical response before adding other supplements, avoiding the “cocktail approach” since the beginning<sup>56</sup>.

## **Conclusions**

Treatment and management of patients with MD remain a major task. Apart from specific disorders such as primary CoQ deficiency and



riboflavin-responsive disorders, in the absence of proven therapies, the most useful approach is supportive care. In this setting, nutritional support has a crucial role. Randomized, controlled clinical trials with large sample sizes are necessary. However, at the basis of any nutritional approach is the evaluation of the nutritional status and the patient's energy needs. Then, in collaboration with the patients and their family, is possible to build an appropriate program which provides for adequate caloric and protein support paying also attention to the food texture and the difficulties in the assumption of the food (i.e., dysphagia, GI problems, etc.). To improve nutritional care in MD, we hence suggest prioritizing nutritional support paying attention to avoid fasting and meet the energy needs.

Every attempt at nutritional intervention should be made knowing patient's disease and focusing on his/her energy and nutrients' requirements. For this reason, clinicians expert in mitochondrial medicine and clinical nutritionists should work together to ameliorate care in these fragile patients.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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