Lead Article

Electron transfer mediators and other metabolites and cofactors in the treatment of mitochondrial dysfunction

Daniele Orsucci, Massimiliano Filosto, Gabriele Siciliano, and Michelangelo Mancuso

Mitochondrial disorders (MDs) are caused by impairment of the mitochondrial electron transport chain (ETC). The ETC is needed for oxidative phosphorylation, which provides the cell with the most efficient energy outcome in terms of ATP production. One of the pathogenic mechanisms of MDs is the accumulation of reactive oxygen species. Mitochondrial dysfunction and oxidative stress appear to also have a strong impact on the pathogenesis of neurodegenerative diseases and cancer. The treatment of MDs is still inadequate. Therapies that have been attempted include ETC cofactors, other metabolites secondarily decreased in MDs, antioxidants, and agents acting on lactic acidosis. However, the role of these dietary supplements in the treatment of the majority of MDs remains unclear. This article reviews the rationale for their use and their role in clinical practice in the context of MDs and other disorders involving mitochondrial dysfunction.

INTRODUCTION

Mitochondria are dynamic and pleomorphic organelles. They evolved from the aerobic bacteria that populated primordial eukaryotic cells approximately 1.5 billion years ago, thus endowing the host cells with oxidative metabolism, which is much more efficient than anaerobic glycolysis.[1] Mitochondria are composed of a smooth outer membrane surrounding an inner membrane of significantly larger surface area that, in turn, surrounds a protein-rich core known as the matrix. They contain from 2 to 10 molecules of DNA, named the mitochondrial DNA (mtDNA). Mitochondria have adapted to their intracellular environment by reducing their genome size to about 16,500 base pairs. This reduction has increased their replication rate and, thus, ensures the transmission of the mitochondrial genome to the two daughter cells. In humans, the mtDNA is transmitted through maternal lineage.[1]

The most crucial task of the mitochondrion is the generation of energy as adenosine triphosphate (ATP), by means of the electron transport chain (ETC). The ETC is needed for oxidative phosphorylation (which provides the cell with the most efficient energetic outcome in terms of ATP production), and consists of five multimeric protein complexes located in the inner mitochondrial membrane.[1] The ETC also requires two small electron carriers, coenzyme Q_{10} (CoQ_{10}, or ubiquinone) and cytochrome *c* (cyt *c*). Electrons are transported along the complexes to molecular oxygen (O_2), finally producing water. At the same time, protons are pumped across the mitochondrial inner membrane, from the matrix to the intermembrane space, by the complexes I, III, and IV. This process creates an electrochemical proton gradient. ATP is produced by the influx of these protons back through the complex V, or ATP synthase (the "rotary motor").[2] This metabolic pathway, represented in Figure 1, is under control of both nuclear (nDNA) and mitochondrial genomes.[1,3] The human mtDNA encodes information for mitochondrial transfer RNAs (tRNAs), for ribosomal RNAs (rRNAs), and for a few of the subunits of the ETC. The rest of the mitochondrial proteins are encoded by genes in the nuclear chromosomes, and finally imported into the mitochondrion.[1]

Affiliations: *D Orsucci*, *G Siciliano*, and *M Mancuso* are with the Department of Neuroscience, Neurological Clinic, University of Pisa, Italy. *M Filosto* is with the Neurological Institute of Brescia, Italy.

Corresponding author: Michelangelo Mancuso, M.D., PhD, Department of Neuroscience, Neurological Clinic, University of Pisa, Via Roma 67, 56126 Pisa, Italy. Phone: +39-050-992440, Fax +39-050-554808, E-mail: mmancuso@inwind.it

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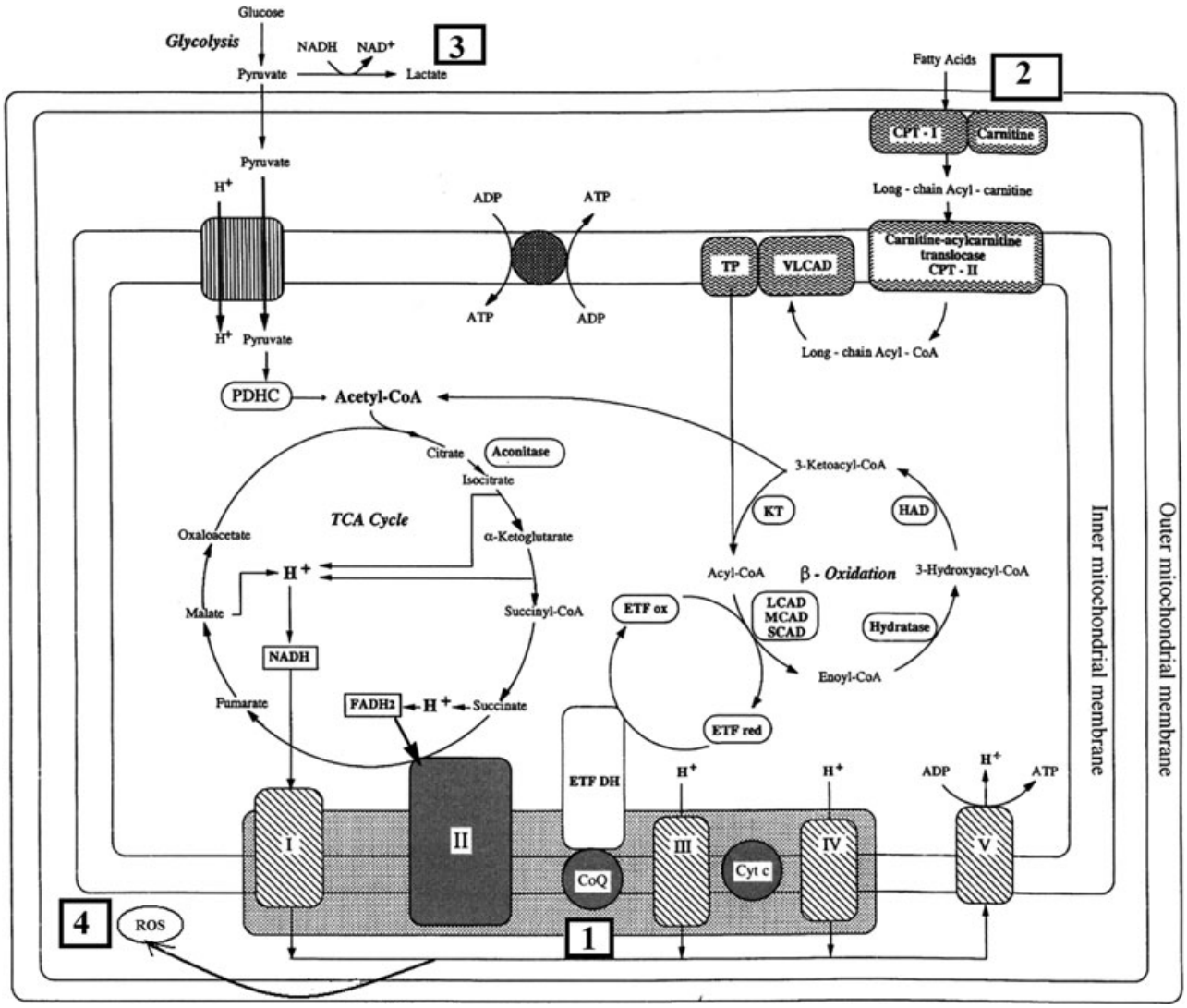

Figure 1 **Mitochondrial metabolism.** Respiratory chain components or complexes encoded exclusively by nuclear genome are solid; complexes containing some subunits encoded by mitochondrial DNA are cross-hatched.
Abbreviations: CPT, carnitine palmitoyltransferase; ETFox, oxidized electron transfer flavoprotein; ETFred, reduced electron transfer flavoprotein; ETF-DH, ETF-coenzyme Q oxidoreductase; HAD, 3-hydroxy-CoA dehydrogenase; KT, 3-ketothiolase; LCAD, long-chain acyl-CoA dehydrogenase; MCAD, medium chain acyl-CoA dehydrogenase; PDHC, pyruvate dehydrogenase complex; ROS, reactive oxygen species; SCAD, short-chain acyl-CoA dehydrogenase; TP, trifunctional protein; VLCAD, very-long-chain acyl-CoA dehydrogenase.
Some therapeutic approaches that have been attempted in mitochondrial dysfunction are shown: [1] Coenzyme Q_{10} administration; [2] carnitine administration; [3] removal of noxious metabolites, such as lactate; [4] antioxidant agents. For further details, see text.
Modified from DiMauro et al. (2004)[74], with permission.

Mitochondria also play a central role in apoptotic cell death, and mitochondrial dysfunction appears to have a certain impact on the pathogenesis of several neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's disease (AD), and Parkinson's disease.[4] A growing body of evidence seems to indicate that oxidative stress, which is increased in damaged mitochondria, is an earlier event associated with mitochondrial dysfunction and neurodegeneration.[4]

The transport of high-energy electrons through the mitochondrial ETC is a necessary step for ATP production, but it is also a source of reactive oxygen species (ROS) production. At the complexes I-III of the respiratory chain, the high-energy electrons can react with O_2 to form superoxide ($O_2^{\bullet -}$).[5] Up to 4–5% of the O_2 consumed by healthy mitochondria is converted into $O_2^{\bullet -}$,[5] and this amount is higher in damaged and aged mitochondria. When the ETC is inhibited, the electrons accumulate in

the early stages of the ETC (complex I and CoQ_{10}), where they are donated directly to O_2 to give an anion $O_2^{\bullet -}$.[5] The accumulation of ROS can potentially damage biomolecules, including lipids, proteins, and nucleic acids. Some tissues, such as brain and skeletal muscle, are much more vulnerable to oxidative stress because of their elevated consumption of O_2.[4] Moreover, compared to other tissues, the brain has a lower activity of antioxidant enzymes such as glutathione peroxidase and catalase, and it contains elevated concentrations of polyunsaturated fatty acids that are highly susceptible to lipid peroxidation.[4] Superoxide can react with nitric oxide (NO), a molecule that, in brain, acts as a neurotransmitter to form peroxynitrite ($ONOO^-$), which is severely damaging for DNA. The accumulation of nDNA and mtDNA damage is thought to be highly deleterious in post-mitotic cells, such as neurons, where DNA cannot be replaced through a cellular division mechanism. Indeed, oxidative base modifications to mtDNA could potentially cause bioenergetic dysfunctions resulting in neuronal death.[6] The cells possess an intricate network of defense mechanisms (mitochondrial manganese superoxide dismutase, glutathione peroxidase, and other molecules) to neutralize excessive accumulation of ROS and, under physiological conditions, are able to cope with the flux of ROS.[4] Oxidative stress describes a condition in which cellular antioxidant defenses are insufficient to keep the levels of ROS below a toxic threshold.

The mtDNA is particularly sensitive to oxidative damage because of its proximity to the inner mitochondrial membrane, where oxidants are formed, and because it is not protected by histones and is inefficiently repaired. Because several of the mtDNA genes encode for subunits of the mitochondrial respiratory chain, oxidative mtDNA damage, if not correctly repaired, could result in mutations and deletions disrupting the function of genes involved in the production of ATP, ultimately leading to mitochondrial dysfunctions, increased production of ROS, and cellular death.[4]

Recent evidence indicates a role for DNA oxidative damage in triggering neuronal apoptosis. Oxidative stress, mtDNA damage, mitochondrial dysfunction, and apoptosis might be interconnected in the cascade leading to neurodegeneration. The current evidence about the role of mitochondria and oxidative stress in neurodegenerative diseases, such as AD and Parkinson's disease, has been extensively discussed elsewhere.[4]

MITOCHONDRIAL DISORDERS

Mitochondrial diseases (MDs) are a group of disorders caused by impairment of the mitochondrial ETC. The effects of mutations that affect the respiratory chain may be multisystemic, with involvement of visual and auditory pathways, heart, central nervous system (CNS), and skeletal muscle. The estimated prevalence of MDs is 1–2 in 10,000.[7,8] MDs are, therefore, one of the most common inherited neuromuscular disorders.

The genetic classification of MDs distinguishes disorders due to defects in mtDNA from those due to defects in nDNA (Table 1).[1,3] The first are inherited according to the rules of mitochondrial genetics (maternal inheritance, heteroplasmy and the threshold effect, and mitotic segregation). Each cell contains multiple copies of mtDNA (polyplasmy), which in normal individuals are identical to one another (homoplasmy). Heteroplasmy refers to the coexistence of two populations of mtDNA, normal and mutated. Mutated mtDNA in a given tissue has to reach a minimum critical number before oxidative metabolism is impaired severely enough to cause dysfunction (threshold effect). Differences in mutational loads surpassing the pathogenic threshold in some tissues but not in others may contribute to the heterogeneity of phenotypes. Because of mitotic segregation, the mutation load can change from one cell generation to the next and, with time, it can either surpass or fall below the pathogenic threshold.[1] Further, the pathogenic threshold varies from tissue to tissue according to the relative dependence of each tissue on oxidative metabolism,[1] i.e., CNS, skeletal muscle, heart, endocrine glands, the retina, the renal tubule, and the auditory sensory cells highly depend on oxidative metabolism.

MDs related to nDNA are caused by mutations in structural components or ancillary proteins of the ETC, by defects of the membrane lipid milieu, of CoQ_{10} biosynthetic genes, and by defects in intergenomic signaling (associated with mtDNA depletion or multiple deletions).[1,3] Moreover, the occurrence of a single large-scale deletion, which is a common cause of progressive external ophthalmoplegia (PEO), is almost sporadic.

The diagnostic process for MDs does not differ from that employed for other neuromuscular diseases; it should start with the patient and family history and a physical and neurologic examination. "Red flags" for MDs are short stature, neurosensory hearing loss, ptosis, ophthalmoplegia, axonal neuropathy, diabetes mellitus, hypertrophic cardiomyopathy, renotubular acidosis, and migraine-like headache.[1] At present, diagnosis requires a complex approach including measurements of serum lactate, electromyography, magnetic resonance spectroscopy, muscle histology and enzymology, and genetic analysis. The typical histological findings are ragged red fibers. A common symptom of MDs is exercise intolerance, because of impaired energy production in skeletal muscle. This leads to increased lactate production, phosphocreatine depletion, and enhanced ROS generation.[9]

Table 1 **Genetic classification of well characterized mitochondrial diseases.**

Molecular mechanisms	Diseases
Disorders of mitochondrial genome	
Sporadic rearrangements	Kearns-Sayre syndrome
	Pearson syndrome
	Sporadic PEO
	Diabetes and deafness
Sporadic point mutations	PEO
	MELAS
	Exercise intolerance
	Isolated myopathy
Maternal-inherited mtDNA point mutations	
Genes encoding structural proteins	Leber hereditary optic neuropathy
	NARP
	Maternal-inherited Leigh syndrome
Genes encoding tRNAs	MELAS
	MERRF
	Cardiomyopathy and myopathy (MIMyCa)
	PEO
	Isolated myopathy
	Diabetes and deafness
	Sensorineural hearing loss
	Hypertrophic cardiomyopathy
	Tubulopathy
Genes encoding rRNAs	Aminoglycosides-induced deafness
	Hypertrophic cardiomyopathy
MD caused by nuclear gene defects	
Defects of genes encoding for structural proteins of the complexes of respiratory chain	Leigh syndrome
	Cardiomyopathy hypertrophic, histiocitoide
	Paraganglioma, pheochromocytomas
	Multisystemic syndromes
Defects of genes encoding factors involved in the assembling complexes of respiratory chain ("Assembly genes")	Leigh syndrome
	Multisystemic syndromes
Defects of genes altering mtDNA stability and integrity (intergenomic communication)	Autosomal PEO
	Early-onset parkinsonism
	Multisystemic syndromes
	MNGIE
	mtDNA depletion syndromes
Coenzyme Q_{10} deficiency	Cerebellar form *coq2*, *coq8*
	Myopathic form *etfdh*
	Encephalomyopathy
	Infantile multisystemic disease *pdss1*, *coq2*
	Leigh syndrome *pdss2*
Defects of the lipid milieu	Barth syndrome
Syndromes due to defects of mitochondrial ribonucleic acid	MLASA
Defects of mitochondrial fission or fusion	Autosomal dominant optic atrophy (ADOA), Charcot-Marie-Tooth (CMT) 2A

Abbreviations: MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; MLASA, mitochondrial myopathy and sideroblastic anemia; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; NARP, neuropathy, ataxia, retinitis pigmentosa; PEO, progressive external ophthalmoplegia.
Modified from Filosto and Mancuso (2007)[3].

Therapeutic approaches

Despite great progress in the molecular understanding of these disorders, the therapy of MDs is still inadequate.[10] Apart from symptomatic therapy, the administration of metabolites and cofactors is the mainstay of real-life therapy and is especially important in disorders due to primary deficiencies of specific compounds, such as CoQ_{10}. There is increasing interest in the administration of ROS scavengers both in primary MDs and in neurodegenerative diseases related to mitochondrial dysfunction.[10] However, there is currently no clear evidence supporting the use of any intervention in MDs,[11] and further research is needed. There have been very few

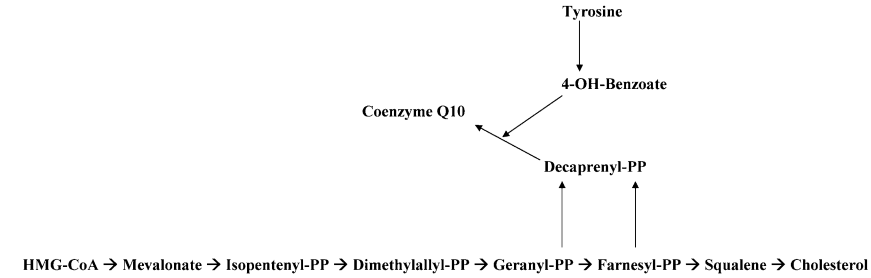

Figure 2 **The mevalonate pathway.** A schematic representation of the mevalonate pathway, the sequence of cellular reactions that leads to farnesyl-PP. Farnesyl-PP is the common substrate for the synthesis of cholesterol, dolichol, and CoQ_{10}, as well as for prenylation of proteins. Coenzyme Q_{10} also contains a benzoate ring originating from tyrosine. After 4-OH-benzoate and decaprenyl-PP are produced, at least seven enzymes (encoded by *COQ2-8* genes) catalyze condensation, methylation, decarboxylation, and hydroxylation reactions needed to synthesize CoQ_{10}. HMG-CoA reductase inhibitors, or statins, block production of mevalonate, a critical intermediary in the cholesterol synthesis pathway. A hypothesized mechanism of statin myopathy involves mitochondrial dysfunction caused by reduced intramuscular CoQ_{10}.
Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; PP, pyrophosphate.

randomized controlled clinical trials for the treatment of MDs. Those that have been performed were of short duration and involved fewer than 20 study participants with heterogeneous phenotypes.[11]

This article reviews the role in clinical practice of ETC cofactors, other metabolites secondarily decreased in MDs, and agents acting on lactic acidosis and antioxidants in the context of MDs and other disorders involving mitochondrial dysfunction. The rationale for their use is also examined.

RESPIRATORY CHAIN COFACTORS

ETC cofactors include nicotinamide (vitamin B_3), thiamine (vitamin B_1), riboflavin (vitamin B_2), succinate, and CoQ_{10}. Nicotinamide is the precursor of NADH, an energized electron carrier that delivers electrons to complex I. Thiamine is a cofactor for pyruvate dehydrogenase. Riboflavin is the precursor of $FADH_2$ and a cofactor for electron transport. Succinate donates electrons directly to complex II. Despite isolated reports of improvement,[11] there is no clear evidence that these dietary supplements improve the symptoms or alter the course of disease for the majority of patients with MD.[11]

An ingenious attempt to bypass a block in complex III of the respiratory chain in a young woman with mitochondrial myopathy and severe exercise intolerance used two artificial electron acceptors (menadiol diphosphate, 40 mg daily, and vitamin C, 4 g daily) whose redox potentials fit the gap created by the cytochrome b dysfunction.[10] The patient initially improved dramatically, as documented by phosphorus-31 magnetic resonance spectroscopy of muscle,[12] but the improvement was not sustained. Other myopathic patients with similar biochemical and molecular defects (complex III deficiency due to mutations in the *cytochrome b* gene of mtDNA) have not responded to this treatment.[10]

Coenzyme Q_{10}

CoQ_{10} is an endogenously synthesized lipid, which shuttles electrons to complex III from complexes I and II and from the oxidation of fatty acids and branched-chain amino acids (via flavin-linked dehydrogenases); CoQ_{10} also has antioxidant properties.[13]

Intracellular synthesis is the major source of CoQ_{10}, although a small proportion is acquired through diet (i.e., oily fish, organ meats such as liver, and whole grains). Its biosynthesis depends on the mevalonate pathway (Figure 2), a sequence of cellular reactions that leads to farnesyl pyrophosphate, the common substrate for the synthesis of cholesterol, dolichol, dolichyl phosphate, and CoQ_{10} (and for the prenylation of proteins, a post-translational modification necessary for the targeting and function of many proteins).[13] The fact that statins block this pathway has prompted the idea that statin-induced CoQ_{10} deficiency is involved in the pathogenesis of statin myopathy (the primary adverse effect limiting their use), which ranges from benign myalgias to rare cases of fatal rhabdomyolysis. Thus, supplementation with CoQ_{10} may be recommended to prevent the myopathic side effects associated with the statins. Evidence for or against this hypothesis were reviewed by Marcoff and Thompson,[14] but the question remains to be answered.

In normal subjects, oral administration of CoQ_{10} improved subjective fatigue sensation and physical performance during fatigue-inducing workload trials.[15]

CoQ_{10} has been widely used to treat MDs, and the multitude of generally positive anecdotal data together with the lack of negative side effects has contributed to its

widespread use in these patients. In studies with eight to 44 patients CoQ_{10} seemed to demonstrate positive trends in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS), Kearns-Sayre syndrome, and myoclonus epilepsy with ragged red fibers (MERRF).[16] Kearns-Sayre syndrome, a disease caused by sporadic large-scale single deletion, is a multisystem disorder defined by the obligate triad of onset before the age of 20 years, external ophthalmoplegia, and pigmentary retinopathy, plus at least one of the following additional features: cardiac conduction block, cerebellar syndrome, and cerebrospinal fluid protein greater than 100 mg/dL. Chen et al.[17] performed a randomized, double-blind cross-over trial on eight patients with MD. Both subjective and objective measures showed a trend towards improvement on treatment, but the global Medical Research Council index score of muscular strength was the only measure reaching statistical significance.[17] There is a need for controlled trials in large cohorts of patients.[11]

Two methodologies useful for detecting the overall level of oxidative damage in MD patients are micronucleus assay followed by fluorescence in situ hybridization (FISH), and comet assay in cultured lymphocytes.[18] An increased number of micronucleated cells is considered a good marker of genotoxic effects. The single-cell gel electrophoresis (comet) assay in lymphocytes can estimate levels of primary and oxidative DNA damage in the body.[19] The DNA damage in patients with MD was decreased by CoQ_{10}, which is also an efficient antioxidant.[19,20]

The rationale for using CoQ_{10} is very powerful when this compound is specifically and markedly decreased because of defective synthesis. Primary CoQ_{10} deficiency is an uncommon heterogeneous condition that has been associated with five major known syndromes: 1) encephalomyopathy (with recurrent myoglobinuria, brain involvement, and ragged red fibers); 2) severe infantile multisystemic disease; 3) cerebellar ataxia; 4) Leigh syndrome (growth retardation, ataxia, and deafness); and 5) isolated myopathy.[21] CoQ_{10} deficiency is an autosomal recessive MD. Infantile mitochondrial encephalomyopathy has been associated with mutations in the first and second subunits of decaprenyl diphosphate synthase (*PDSS1* and *PDSS2*) in the mevalonate pathway.[22,23] Mutations in *PDSS1* seem to lead to a milder phenotype than mutations in subunit 2. Patients with mutations in para-hydroxybenzoate-polyprenyl transferase (*COQ2*), a component of the CoQ_{10} biosynthesis complex that modifies the ring, share early-onset nephrosis and encephalopathy.[22,24] The myopathic form of CoQ_{10} deficiency is caused by mutations in the electron-transferring-flavoprotein dehydrogenase (*ETFDH*) gene.[25] Interestingly, some cases of the ataxic variant of CoQ_{10} deficiency have been linked to a homozygous mutation in the aprataxin (*APTX*) gene, which causes ataxia oculomotor apraxia type 1; the relationship between this protein, involved in DNA repair, and CoQ_{10} homeostasis is still unclear.[26,27] Very recently, CoQ_{10} deficiency with cerebellar ataxia has been associated with mutation in the *CABC1*/*COQ8* gene.[28]

CoQ_{10} deficiency is a treatable condition, so a high grade of "clinical alert" about this diagnosis is essential, especially for pediatricians and infantile neurologists. An early treatment with high-dose CoQ_{10} might radically change the natural history of this group of diseases.[21] Patients with all forms of CoQ_{10} deficiency have shown clinical improvement with oral CoQ_{10} supplementation, but cerebral symptoms are only partially ameliorated (probably because of irreversible structural brain damage before treatment and because of poor penetration of CoQ_{10} across the blood-brain barrier).[29] Patients were given various doses of CoQ_{10} ranging from 90 to 2000 mg daily. The small number of patients precluded any statistical analysis but improvement was reported.[21,29] In several patients, CoQ_{10} supplementation also ameliorated the mitochondrial function (respiratory chain activities, lactic acid values, muscle CoQ_{10} content).[29] The beneficial effects of exogenous CoQ_{10} require high doses and long-term administration. Also, patients with ataxia oculomotor apraxia type 1 may benefit from this treatment.[21]

CoQ_{10} and its analogue, idebenone, have also been widely used in the treatment of other neurodegenerative disorders.[10] Very recently, CoQ_{10} was found to be effective in a Parkinson's disease (PD) mouse model of MPTP toxicity.[30] In PD patients, CoQ_{10} was well tolerated at doses as high as 1200 mg daily, and it significantly slowed disease progression.[31] A recent study reported that nanoparticular CoQ_{10} (300 mg daily) was safe and well tolerated by PD patients, and led to plasma levels similar to 1200 mg/d of standard formulations, although it did not result in symptomatic effects in mid-stage PD.[32] The efficacy of CoQ_{10} in PD remains an open question.[33]

In Huntington's disease (HD), CoQ_{10} slowed the decline in total functional capacity over 30 months.[10] HD is a genetic disease characterized by psychiatric disturbances, progressive cognitive impairment, choreic movements, and death 15 to 20 years after the onset of symptoms. Various lines of evidence demonstrated the involvement of mitochondrial dysfunction in the pathogenesis of HD, but the precise role of mitochondria in the neurodegenerative cascade leading to HD is still unclear. Moreover, CoQ_{10} proved to be safe in 31 subjects with amyotrophic lateral sclerosis (ALS) treated with doses as high as 3,000 mg/day for 8 months,[34] and a clinical trial in the United States involving ALS patients treated with CoQ_{10} is nearing completion.

A very recent short-term, randomized, placebo-controlled trial was performed in progressive

supranuclear palsy (PSP).[35] PSP, the second most common cause of parkinsonism after PD, is characterized by down-gaze palsy with progressive rigidity and imbalance leading to falls. In PSP, complex I appears to be dysfunctional. CoQ_{10} improved cerebral energy metabolism on magnetic resonance spectroscopy studies.[35] Clinically, PSP patients treated with CoQ_{10} improved slightly, but statistically significantly, compared to those who received a placebo.[35]

Other potential indications of CoQ_{10} include migraine,[36–37] hypertension,[38] diabetes, heart failure, and atherosclerosis,[16] although the role of CoQ_{10} in such conditions is still an open question. Although CoQ_{10} is also used for the prevention and treatment of cancer, there is no convincing evidence of efficacy.[16]

No absolute contraindications are known for CoQ_{10}, and adverse effects are rare. Mild dose-related gastrointestinal discomfort is reported in <1% of patients. Potential interactions with warfarin causing decreased international normalized ratio (INR) have been suggested.[16] CoQ_{10} could also have potential hypoglycemic and hypotensive effects. Its various formulations demonstrate variation in bioavailability and dosage consistency, and there is a serious possibility that patients may have been treated suboptimally. It is important to use brands that have passed independent testing for product purity and consistency.[16]

A synthetic shorter chained CoQ_{10} analogue is idebenone. It improved brain and skeletal muscle metabolism in isolated cases of MD, and seemed to enhance the rate and degree of visual recovery in Leber hereditary optic neuropathy.[11] Leber's disease is a maternally inherited condition characterized by acute or subacute bilateral loss of vision, usually in young individuals. Several point mutations in the mitochondrial genome have been identified in patients with the condition.

Most trials performed to date demonstrated that idebenone (5 mg/kg daily) reduced cardiac hypertrophy in Friedreich's ataxia.[39] Friedreich's ataxia is the most common hereditary ataxia among white people, and it is caused by a trinucleotide expansion in the *X25* gene. In this disorder, the genetic abnormality results in the deficiency of frataxin, a protein targeted to the mitochondrion.[40] Although the exact physiological function of frataxin is not known, its involvement in iron-sulphur cluster biogenesis has been suggested. A possible manifestation of this disease is cardiomyopathy. Recently, a randomized, placebo-controlled trial has been conducted on 48 genetically confirmed patients.[41] Treatment with higher doses of idebenone was generally well tolerated and associated with improvement in neurological function and activities of daily living in patients with Friedreich's ataxia.[41] The degree of improvement was correlated with the dose of idebenone, suggesting that higher doses may be necessary to have a beneficial effect on neurological function.[41]

OTHER METABOLITES

Carnitine

In skeletal muscle, carnitine plays an essential role in the translocation of long-chain fatty-acids into the mitochondrial matrix for subsequent β-oxidation, and it plays a vital role in the regulation of both fat and carbohydrate muscle metabolism.[42] During high-intensity exercise, carnitine buffers the excess acetyl groups that are formed; it does this in a reaction catalyzed by carnitine acetyltransferase.[42] The acetyl group delivery seems to be limiting to mitochondrial ATP synthesis at the onset of exercise.[42] Thus, increasing muscle total carnitine content could potentially alleviate the decline in fat oxidation rates routinely observed during high-intensity exercise, and it could concomitantly reduce muscle glycogen utilization.[42] In animal models, subcutaneous injection of l-carnitine suppresses the onset of neuromuscular degeneration and increases the life span of mice with familial ALS due to mutation in *SOD1*.[43]

However, studies in healthy humans failed to show an increase in skeletal muscle carnitine content via either oral or intravenous l-carnitine administration.[42] Muscle carnitine transport seems to be the limiting factor to muscle carnitine accumulation in healthy humans. In one study oral feeding of l-carnitine (3 g) and simple sugars (94 g) increased the whole body retention of carnitine, likely because of an action of insulin on the transporter OCTN2.[44]

Acetyl l-carnitine is more bioavailable, is thought to penetrate the brain better than l-carnitine, and is readily converted to l-carnitine as needed. It boosts mitochondrial ATP production and helps protect mitochondria against oxidative stress; it may also benefit patients with PD.[45] Effective and well-tolerated oral intakes ranged from 1.5 to 3.0 g daily.[46] Other potential indications for acetyl l-carnitine include multiple sclerosis and hypoxic stroke.[47] Further, a meta-analysis of 15 trials concluded that acetyl l-carnitine was beneficial for mild AD.[46]

In MD patients suffering from epilepsy, valproic acid should be used with caution because it inhibits carnitine uptake. Therefore, L-carnitine supplementation is particularly advised in such patients.[10]

Free carnitine tends to be lower than normal in the blood of patients with respiratory chain defects, whereas esterified carnitine tends to be elevated.[10] This shift may reflect a partial impairment of β-oxidation. In MD with secondary carnitine deficiency, replacement therapy (up to 3 g/day) resulted in some improvement in isolated

cases, but objective evidence supporting a therapeutic role is unavailable.[11]

Finally, although primary systemic carnitine deficiency is not a defect of the respiratory chain, we mention it here because of the life-saving effect of replacement therapy.[10] Primary carnitine deficiency is an autosomal recessive disorder due to genetic defects of the plasma membrane carnitine transporter.[48] The most common presentation is progressive childhood dilated cardiomyopathy with massive lipid storage. There is a dramatic response to carnitine supplementation, and indices of cardiac function return to normal within a few months.[49] Thus, it is very important to measure blood carnitine concentration in all children with unexplained cardiomyopathy.[10]

Creatine

Creatine is an amino acid derivative synthesized in the kidneys, liver, and pancreas, with a synthesis rate of about 1–2 g/day.[50] Creatine can also be obtained through the diet, mainly from meat and fish. About 90–95% of the body's creatine is found in skeletal muscle. Of this, approximately two-thirds exist as phosphocreatine (PCr). PCr serves a major role in energy metabolism. When energy demands increase, PCr donates its phosphate to ADP to produce ATP. Because PCr is a limiting factor in maintaining ATP resynthesis during maximal short-term exercise, an increased PCr concentration should theoretically increase the energy reserve for such exercise.[50] It has been reported that the concentration of total creatine in muscle can indeed be increased by oral creatine supplementation.[51] Studies have reported that supplementation with creatine facilitates an increase in anaerobic work capacity and muscle mass when accompanied by resistance training programs in both normal and patient populations.[50] Whereas improvement in the rate of phosphocreatine resynthesis is largely responsible for improvements in acute work capacity, the direct effect of creatine supplementation on skeletal muscle protein synthesis is less clear.[50] Because creatine affects fluid balance, it should be used with attention to fluid needs in hot climates.[50]

Phosphorus-31 magnetic resonance spectroscopy measures fluctuations in PCr and inorganic phosphate (Pi) during muscle exercise. The most probable indicator of MD is the finding of a combined low PCr/Pi ratio at rest and low post-exercise recovery.[52] Creatine monohydrate (CrM) has been tried in six patients with MELAS and one with undefined mitochondrial disease in a randomized controlled study; there was improvement of high-intensity activities but not of lower intensity aerobic exercise.[53] In a subsequent randomized placebo-controlled crossover trial, Klopstock et al.[54] studied the effects of CrM (20 g daily for 4 weeks) in 13 participants with progressive external ophthalmoplegia and three with mitochondrial myopathy. No significant effects of treatment were noted.[54]

Inborn errors of energy metabolism have been identified in three of the main steps in creatine metabolism. Oral CrM has been shown to improve the clinical symptoms in some of these cases.[55] Supplementation has also been shown to have neuroprotective effects in several animal models of neurological diseases, such as HD, PD, and ALS.[55] However, this has not been confirmed in clinical studies in humans.

Clinical trials in patients with Duchenne and Becker's muscular dystrophy have shown improved function, fat-free mass, and some evidence of improved bone health with CrM supplementation.[56] In contrast, the improvements in function in myotonic dystrophy and inherited neuropathies have not been significant.[56] Larger randomized control trials are needed to determine whether creatine supplementation will be of therapeutic benefit to patients with various neuromuscular disorders.[56]

AGENTS ACTING ON LACTIC ACIDOSIS

This category includes dichloroacetate and dimethylglycine. In MD there is an impairment of the respiratory chain, leading to a defective aerobic system; thus, muscle is forced to apply an anaerobic metabolism with enhanced lactate production. Lactic acidosis is a common finding in MD, especially in multisystemic forms.[1] In MELAS, resting plasma lactate level correlates with muscle mtDNA mutation A3243G load.[57] As lactic acid is neurotoxic, it is reasonable to reduce the level of lactic acid. Buffering it with bicarbonate has a transient effect and may also exacerbate cerebral dysfunction.[10]

A more specific lactic acid-lowering agent is dichloroacetate, which acts by inhibiting pyruvate dehydrogenase (PDH) kinase, thus keeping PDH in the dephosphorylated, active form and favoring pyruvate metabolism and lactate oxidation.[58] De Stefano et al.[59] performed a double-blind placebo-controlled study on 11 participants with various MDs. Dichloroacetate produced significant decreases in blood lactate, pyruvate, and alanine at rest and after exercise, and improvements were noted on brain magnetic resonance spectroscopy.[59] Muscle spectroscopy and self-assessed clinical disability were unchanged.[59] No adverse effects were reported.[59] However, a more recent double-blind, placebo-controlled, randomized, crossover trial of dichloroacetate in MELAS patients harboring the A3243G mutation had to be terminated because of peripheral nerve toxicity, which overshadowed any potential beneficial effect in MELAS.[60] Thus, dichloroacetate should not be used over

the long term in patients with MD who are already prone to develop peripheral neuropathy by virtue of their mitochondrial dysfunction.[10]

Interestingly, dichloroacetate may reverse the suppressed mitochondrial apoptosis in cancer and result in suppression of tumor growth.[61] For this reason, dichloroacetate is studied in early-phase cancer clinical trials.[61]

Dimethylglycine is a component of pangamic acid (vitamin B_{15}). There are anecdotal reports of an improvement in patients with congenital lactic acidosis,[11] but one trial using dimethylglycine showed no significant effect.[62]

ANTIOXIDANTS

Some of the most frequently used antioxidant compounds include CoQ_{10} and idebenone (which have been discussed), ascorbic acid, vitamin E, and lipoic acid.

Hargreaves et al.[63] found a deficiency of reduced glutathione in skeletal muscle from patients with MD, possibly as a consequence of diminished ATP availability or increased oxidative stress. The latter may represent a contributing factor in the progressive nature of this group of disorders.[63] In comparison with healthy individuals one group of MD patients showed an increased level of chromosome damage, expressed as frequency of micronucleated lymphocytes. Patients receiving a 2-week therapy with CoQ_{10} showed a statistically significant reduction in the frequency of micronucleated cells after therapy.[20]

There are tight links between mitochondrial dysfunction and oxidative stress (see section above titled "Mitochondria, oxidative stress, and neurodegeneration"). Defects of the ETC have detrimental effects that go beyond impairing ATP production and include altered intracellular calcium buffering, excessive production of ROS, and promoting apoptosis.[64] Increased production of ROS damages cell membranes through lipid peroxidation and further accelerates the high mutation rate of mtDNA, creating a vicious cycle.[10] Evidence of oxidative stress has been provided not only in primary mitochondrial diseases but also in many neurodegenerative disorders, including Friedreich's ataxia, HD, ALS, PD, and AD.[4]

Several ROS scavengers have been utilized in most of the disorders just listed.[10] The main tendency in the scientific community is to divide the potential therapeutic treatments into two different categories: vitamin antioxidants and non-vitamin "cocktails."[65] Among the antioxidant treatments using vitamins, vitamin E, vitamin E analogs, and vitamin C are well characterized. However, there is currently no clear evidence of the efficacy of antioxidants in the prevention or treatment of neurodegenerative diseases.[66,67] More research is needed to identify the role, if any, that vitamin E and other antioxidant agents play in the management of such devastating disorders. More research is also needed to establish the real safety of such compounds. β-carotene supplementation appeared to increase cancer incidence and cancer mortality among smokers,[68] and a recent meta-analysis (which included 68 randomized trials with 232,606 healthy participants and patients with various diseases) reported that treatment with β-carotene, vitamin A, and vitamin E may increase all-cause mortality.[69] Further study of the causes of mortality is needed.[69] Thus, these findings contradict observational studies, claiming that synthetic antioxidant supplements improve health.[69]

Lipoic acid

Oral lipoic acid may enhance PDH activity and has been reported to determine clinical and biochemical improvement in an isolated PEO case.[70] Recently, Rodriguez et al.[71] studied the effect of a combination therapy (CrM, CoQ_{10}, and lipoic acid) on several outcome variables using a randomized, double-blind, placebo-controlled, crossover study design in 17 patients with various MDs. The combination therapy resulted in lower resting plasma lactate and a lowering of oxidative stress, as reflected by a significant reduction in urinary 8-isoprostanes and a directional trend in 8-hydroxy-2′-deoxyguanosine excretion.[71] Further, the combination therapy attenuated the decrease in peak ankle dorsiflexion strength that was observed following the placebo phase.[71] Lipoic acid is found naturally within the mitochondria and is an essential cofactor for PDH and α-ketoglutarate dehydrogenase, and is also a potent antioxidant.[71]

It is interesting to note that when CrM was combined with lipoic acid in healthy volunteers, muscle PCr and total creatine concentrations were significantly higher than when CrM was administered alone.[72]

CONCLUSION

Therapy for MDs remains inadequate and mostly symptomatic, but the rapidly increasing knowledge of their molecular defects and pathogenic mechanisms allows for some cautious optimism about the development of effective treatments in the near future. One of the "cocktails" of choice for the treatment of MDs may be a combination of L-carnitine (1,000 mg three times a day) and CoQ_{10} (at least 300 mg a day), with the rationale of restoring free carnitine levels and exploiting the oxygen radical scavenger properties of CoQ_{10}. Future approaches to these pathologies could include germline therapy and gene therapy.[10] For MDs due to mutations in nuclear genes, the problems are no different from those vexing gene therapy for other mendelian disorders, including choice of appropriate viral or nonviral vectors, delivery to the affected

tissues, and potential immunological reactions. The problems are even more complex for mtDNA-related diseases because of polyplasmy and heteroplasmy and because nobody has yet been able to transfect DNA into mitochondria in a heritable manner.[10] Other potential approaches include neuroprotective strategies; for example, tetracycline seemed to delay ocular motility decline in one isolated PEO case.[73]

Moreover, the role of mitochondrial dysfunction and oxidative stress in the pathogenesis of neurodegenerative diseases is well documented.[4] It will be important to develop a better understanding of the role of oxidative stress and mitochondrial energy metabolism in neurodegeneration, since this may lead to the development of more effective treatment strategies for these devastating disorders.

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