The Elusive Magic Pill: Finding Effective Therapies for Mitochondrial Disorders

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Abstract The incidence of mitochondrial diseases has been estimated at 11.5/100,000 (1:8500) worldwide. In the USA up to 4000 newborns annually are expected to develop a mitochondrial disease. More than 50 million adults in the USA also suffer from diseases in which primary or secondary mitochondrial dysfunction is involved. Mitochondrial dysfunction has been identified in cancer, infertility, diabetes, heart diseases, blindness, deafness, kidney disease, liver disease, stroke, migraine, dwarfism, and resulting from numerous medication toxicities. Mitochondrial dysfunction is also involved in normal aging and age-related neurodegenerative diseases, such as Parkinson and Alzheimer diseases. Yet most treatments available are based on empiric data and clinician experience because of the lack of randomized controlled clinical trials to provide evidence-based treatments for these disorders. Here we explore the current state of research for the treatment of mitochondrial disorders.

Keywords Mitochondria · ATP · mtDNA · nDNA · Respiratory chain · OXPHOS

Introduction

Mitochondria are intracellular organelles found in every human cell that are responsible for generating energy in the form of adenosine triphosphate (ATP). Mitochondria are composed of approximately 1500 proteins, of which approximately 300 are necessary for oxidative phosphorylation and only 13 are

encoded by mitochondrial DNA (mtDNA). Most mitochondrial proteins are encoded by the nuclear DNA (nDNA) and must be transcribed, translated, targeted to mitochondria, imported, and then folded and assembled properly in order to carry out normal functions [1, 2].

There are a total of five enzyme complexes in the inner mitochondrial membrane that carry out oxidative phosphorylation (OXPHOS): 1) nicotinamide adenine dinucleotide (NADH)/coenzyme (Co)Q oxidoreductase (complex I); 2) succinate/CoQ oxidoreductase (complex II); 3) cytochrome C reductase (complex III); 4) cytochrome C oxidase (complex IV); and, 5) ATP synthase (complex V). The transfer of electrons moves from complex I and complex II to CoQ to complex III to complex IV releasing oxygen and water. The protons are pumped across the inner mitochondrial membrane generating an electrochemical gradient that allows protons to flow back from the intermembrane space into the mitochondrial matrix through complex V to make ATP [1, 2].

Complex I has approximately 45 polypeptides: 7 encoded from mtDNA and the others encoded by nuclear DNA. Complex II consists of 5 polypeptides, all encoded by nuclear DNA. Complex III is made up of 11 subunits, only 1 from mtDNA; complex IV has 13 polypeptides, 3 encoded by mtDNA; and complex V contains 17 subunits, of which 2 are encoded by mtDNA [1].

Other than the generation of energy, mitochondria also function in fatty acid and amino acid oxidation, heme and pyrimidine synthesis, calcium homeostasis, and apoptosis [1, 2].

The incidence of mitochondrial diseases was estimated at 11.5/100,000 (1:8500) worldwide in 2000. In 2006 the incidence of respiratory chain defects was predicted to be 1:5000 [3]. In 2008, the incidence of “at-risk” carriers of mtDNA mutations in the UK was estimated at 1:10,000 adults, the equivalent of 1:200 persons [3, 4].

Approximately 4 million children are born each year in the USA with up to 4000 expected to develop a mitochondrial disease. Additionally, more than 50 million adults in

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the USA suffer from diseases in which primary or secondary mitochondrial dysfunction is involved [3, 4]. Mitochondrial dysfunction has been identified in cancer, infertility, diabetes, heart diseases, blindness, deafness, kidney disease, liver disease, stroke, migraine, dwarfism, and resulting from numerous medication toxicities. Mitochondrial dysfunction is also involved in normal aging and age-related neurodegenerative diseases, such as Parkinson and Alzheimer diseases [1, 2].

Mitochondrial disorders should be considered any time a progressive multisystem disorder is suspected and sometimes for isolated symptoms, such as optic atrophy, sensorineural deafness, cardiomyopathy, diabetes, pseudo-obstruction, neuropathy, myopathy, liver disease, early strokes, or seizures [1, 5].

It is now known that disease can occur across the lifespan secondary to the developmental regulation of many mitochondrial proteins. Secondary mitochondrial dysfunction may also be affected by environmental toxins or medication toxicities. Carrier proteins acting as chaperonins and mitochondrial fusion/fission abnormalities have reported to cause mitochondrial diseases [3]. Tissue-specific mtDNA changes make it hard to diagnose mitochondrial diseases using only non-invasive blood or urine studies. Additionally, clinical presentations of some mitochondrial diseases, such as Leber Hereditary Optic Neuropathy (LHON), and sensorineural deafness may be affected by gene–gene interactions [3, 6].

When the mitochondria do not function properly, they cause symptoms usually presenting in the organs with the highest energy needs, such as brain, cranial and/or peripheral nerves, skeletal muscle, heart muscle, endocrine glands, and/or the kidneys [4]. These diseases are highly variable and progressive, leading to significant morbidity and early death. Current treatment is most often supportive and may include vitamin cofactors, nutritional manipulations, and exercise [1, 2]. To date, only small clinical trials have been performed, with primary outcome measures most often focusing on improved muscle strength and/or endurance, biochemical markers of disease when known, and quality of life assessments. In a recent Cochrane review of the treatment for mitochondrial disorders, very few randomized clinical trials could pass scrutiny, and these studies could not be compared well owing to heterogeneous study groups, different dosing regimens, statistical challenges, and a poor understanding of natural history of mitochondrial disease [7].

Mitochondrial Inheritance

About 20 % of mitochondrial diseases are inherited maternally as little or no mtDNA is transferred from the sperm to the fertilized egg. Mitochondrial diseases can also occur sporadically or be inherited in an autosomal dominant or recessive manner. Approximately 70–85 % of mitochondrial diseases are caused by a nuclear gene mutations rather than

a mtDNA mutation [6]. To date, more than 200 mtDNA point mutations or deletions have been associated with mitochondrial diseases primarily affecting respiratory chain function [5]. Since 2006 approximately 100 nDNA mutations that cause mitochondrial disease have been described [5, 7]. Most nuclear gene defects affect the assembly and structure of the respiratory chain protein complexes, the transport of proteins into the mitochondria, or the maintenance of the mtDNA itself [8]. Generally, children seem to have more severe disease related to nDNA mutations that decrease respiratory chain function or impair mtDNA maintenance, whereas many late-onset mitochondrial diseases are related to mtDNA point mutations or deletions that must meet a tissue-specific threshold before disease symptoms manifest.

Treatment

Supportive treatments used to modify respiratory chain dysfunction have traditionally focused on the use of vitamin cofactors or antioxidants, diet manipulation, and/or exercise therapy (Table 1) [8, 9]. Pfeffer et al. [7] identified 12 randomized studies that controlled for bias and had clear outcome measures relevant to mitochondrial disease between 2003 and 2012. Their final analysis identified several weaknesses, including 1) complex and variable phenotypes, even among individuals with the same mitochondrial disorder; 2) difficulties comparing outcome measures when multiple organ systems were affected; 3) the use of multiple endpoints without clearly defined clinical relevance; and 4) the general lack of natural history studies for any group of mitochondrial diseases [7].

Vitamin Cofactors/Antioxidants

Vitamin A

Vitamin A could be beneficial in the management of mitochondrial disorders as it has recently been described that the retinol form is a key regulator of mitochondrial function *in vitro*. Retinol appears to be an essential cofactor of protein kinase C (PKC) and needs to physically bind PKC to form a PKC–retinol complex in order to signal pyruvate dehydrogenase complex that increases flux of pyruvate into the Citric acid cycle.

This work with cell cultures demonstrated that retinol was essential for respiration and ATP synthesis [10].

B Vitamins

The B vitamins, including thiamine (B1), riboflavin (B2), and nicotinamide (B3), have been used alone or in combination to promote electron flux through the proximal

Table 1 Current mitochondrial supplements and status of research

Supplement or modifier	Research focus	Mechanism of action
Ubiquinone/ubiquinol (CoQ)	Primary CoQ deficiencies General mitochondrial dysfunction	Correct primary CoQ deficiency Bypass redox block between complexes I or II and III Antioxidant
Idebenone	General mitochondrial dysfunction with attention on MELAS and LHON	Free radical scavenger Bypass redox block at complex I
Vitamin A (retinol)	Cancer research General mitochondrial function	Forms PKC–retinol complex to signal pyruvate dehydrogenase complex that increases flux of pyruvate into the citric acid cycle essential for respiration and ATP synthesis
Thiamine (B1)	General mitochondrial dysfunction with attention on KSS	Coenzyme of pyruvate dehydrogenase
Riboflavin (B2)	General mitochondrial dysfunction with attention on MELAS and LHON	Provides flavin precursors to complex I and II
Niacin (B3)	General mitochondrial dysfunction with attention on MELAS and complex I	Increase NAD/NADH ⁺ pool
Vitamin C	General mitochondrial dysfunction with attention to MELAS and LHON	Antioxidant Bypass redox block at complex III
Vitamin K3 (menadione)	Treatment of complex III deficiency	Antioxidant Electron acceptors to bypass complex III block
Vitamin E	General mitochondrial dysfunction with attention on MELAS and LHON	Antioxidant
Succinate	General mitochondrial dysfunction with attention on complex I, MELAS and KSS	Donates electrons to complex II
Carnitine	General mitochondrial dysfunction Carnitine deficiency	Antioxidants Promotes excretion of excess organic acids
Creatine	Mitochondrial myopathies	Improve muscle phosphocreatine content Antioxidant
(Alpha) Lipoic acid	General mitochondrial dysfunction with attention to CPEO	Cofactor of PDHC
Dichloroacetate	Management of lactic acidosis in pyruvate dehydrogenase def General mitochondrial dysfunction	Improves PDHC function thereby lowering plasma lactic acid levels
Resveratrol	Aging Obesity General mitochondrial dysfunction	Activates mitochondrial biogenesis Induces mitochondrial fatty acid oxidation Antioxidant
Bezafibrate	General mitochondrial dysfunction	Activates mitochondrial biogenesis Induces mitochondrial fatty acid oxidation
Epi-743	General mitochondrial dysfunction	Antioxidant
Dexramipexole	Secondary mitochondrial injury in neurodegenerative disorders (ALS, PD)	Inhibits calcium-induced permeability Neuroprotective
N-Acetylcysteine	General mitochondrial dysfunction	Glutathione precursor Antioxidant
Calorie restriction	Aging General mitochondrial dysfunction	Promotes mitochondrial biogenesis Decreased reactive oxygen species formation
High fat/ketogenic diet	Complex I deficiency Pyruvate dehydrogenase deficiency	Bypass block at complex I Promotes mitochondrial biogenesis
L-Arginine	MELAS	Improves nitric oxide production thereby promoting endothelial relaxation
L-Citrulline	MELAS	Improves nitric oxide production thereby promoting endothelial relaxation
BCAA	Aging Obesity/diabetes General mitochondrial dysfunction	Promotes mitochondrial biogenesis Antioxidant Improved endurance
Endurance exercise	General mitochondrial dysfunction	Promotes mitochondrial biogenesis

CoQ coenzyme Q; *BCAA* branched chain amino acids; *MELAS* mitochondrial encephalopathy lactic acidosis stroke-like episodes; *LHON* Leber hereditary optic neuropathy; *KSS* Kearne–Sayre syndrome; *CPEO* chronic progressive external ophthalmoplegia; *ALS* amyotrophic lateral sclerosis; *PD* Parkinson disease; *PKC* Parkinson disease; *NAD/NADH* nicotinamide adenine dinucleotide; *PDHC* pyruvate dehydrogenase complex

respiratory chain as nicotinamide is a precursor of NADH, thiamine is a cofactor of pyruvate dehydrogenase, and riboflavin is the precursor of flavin adenine dinucleotide. To date, no studies support the effectiveness of these vitamin cofactors [7, 9].

Vitamin C and Vitamin K

Vitamin C and vitamin K3 (Menadione) have been used for both their antioxidant effects and as electron acceptors to bypass complex III deficiencies. In a single case report of a patient with complex III deficiency manifested by a myopathy, clinical improvement on both these supplements was documented by ³¹P-magnetic resonance spectroscopy findings, although the improvement was short-term [9]. Since then, vitamin K3, specifically, has fallen out of favor as it has been found to inhibit the function of DNA polymerase γ (POLG) by as much as 50 % of normal function. Although vitamins K1 and K2 were also studied they had no impact on POLG activity at the same concentrations. Menadi-one showed selective, dose-dependent inhibition of POLG [11]. As POLG is the only polymerase in mammalian mitochondria and it plays a key role in mitochondrial DNA replication, this supplement may not be appropriate to use in patients with recognized mitochondrial depletion syndromes.

CoQ

CoQ shuttles electrons from complexes I and II to complex III [1, 2, 9]. The biosynthesis of CoQ depends on the mevalonate pathway and is the end result of cholesterol biosynthesis. It is the most commonly recommended mitochondrial supplement used to treat mitochondrial diseases and primary CoQ disorders [9]. CoQ is used as a powerful antioxidant, a respiratory chain electron carrier, and to treat disorders of primary CoQ deficiency [1, 9]. To date, clinical trials using CoQ have not demonstrated clinically significant findings aside from the primary CoQ disorders [2]. Currently, there is an ongoing randomized, controlled trial using CoQ in patients with primary mitochondrial disease (<http://www.clinicaltrials.gov/>).

Idebenone

A synthetic water-soluble form of CoQ, Idebenone, crosses the blood–brain barrier and has been used in therapeutic trials in patients with Friedreich Ataxia [2, 9]. This autosomal recessive disease is caused by a trinucleotide repeat in frataxin, a protein critical for iron–sulfur (FeS) subunits of complexes I, II, and III. Initial studies suggested a beneficial effect of Idebenone in the treatment of Friedreich ataxia-related cardiomyopathy [2, 9].

Idebenone has also been used for LHON in a randomized controlled trial with promising results in the treatment group (46 % visual recovery) compared with the untreated group (32 %), with emphasis on early treatment [12].

Carnitine

Carnitine homeostasis in humans is maintained by a combination of dietary intake from red meat and dairy products, endogenous biosynthesis from lysine and methionine, and renal reabsorption. In skeletal and cardiac muscle, carnitine is vital to the import of long-chain fatty acids into the mitochondrial matrix for subsequent fatty acid oxidation [1, 9]. Though carnitine supplementation is Food and Drugs Association-approved, considered safe and effective for a large number of patients with chronic renal disease undergoing renal dialysis, multiple patients with inborn errors of organic acid and energy metabolism, there have not been, to date, randomized controlled studies demonstrating clear clinical improvement in patients with respiratory chain disorders [2, 13].

Creatine

Creatine is a guanidino compound produced endogenously by the liver, kidney, and pancreas that requires the amino acids arginine and glycine to be synthesized [2, 9]. Methionine is also used to supply a methyl group to the overall structure. Creatine can be taken up by the brain, heart, and skeletal muscle utilizing a sodium-dependent transporter. Two randomized studies of creatine monohydrate supplementation $\pm$ lactulose demonstrated no clinical significance in treatment outcomes [2].

N-acetylcysteine

In respiratory chain defects, mitochondria were shown to be highly sensitive to oxidative stress. For that reason, N-acetylcysteine, the N-acetyl derivative of the amino acid cysteine, as it increases the glutathione pool and subsequently antioxidant defenses, might be an interesting therapeutic option [2, 9].

Alpha Lipoic Acid

Alpha lipoic acid is an essential cofactor of the E2 component of inner mitochondrial α -ketoacid dehydrogenase enzymes, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and branched chain α -ketoacid dehydrogenase complexes for energy production and the regulation of carbohydrate and protein metabolism [14, 15]. Pyruvate dehydrogenase catalyzes the oxidative carboxylation of pyruvate and plays a critical role in carbohydrate metabolism and

bioenergetics between anaerobic and aerobic energy metabolism. It is the entry point of carbohydrates into the citric acid cycle (CAC).

Lipoic acid is synthesized and almost entirely covalently bound to the E2 component of three α -ketodehydrogenase complexes and the glycine cleavage system in humans. Although dietary intake is not essential, when lipoic acid is supplemented it is readily absorbed and functions as a redox modulator and antioxidant [14, 15]. Rodriguez et al. [15] studied 16 participants with various mitochondrial disorders and 3 with biochemical evidence, suggesting a mitochondrial disorder. The active cocktail comprised 3 g creatine monohydrate with 2 g of dextrose, 300 mg alpha-lipoic acid, and 120 mg CoQ [15]. To date, no single studies of lipoic acid in patients with mitochondrial diseases has occurred.

Dichloroacetate

Dichloroacetate has been studied in small numbers of patients with mitochondrial myopathies, chronic progressive external ophthalmoplegia, Kearne–Sayre syndrome, Leigh syndrome, mitochondrial encephalopathy lactic acidosis and stroke-like episodes (MELAS), congenital lactic acidosis, and 1 patient with a mtDNA depletion syndrome [16–19]. Most of the studies have included children and adults with multiple mitochondrial disorders, making results difficult to interpret. Study designs have been double blind, placebo-controlled crossover trials with washout periods of various lengths between study arms [16–19]. Statistically significant decreases in blood lactate, pyruvate, and alanine at rest and after exercise and improvements on brain MRS, including a reduction of the brain lactate/creatine ratio, increase in brain choline/ creatine ratio, and statistically significant increase in the N-acetyl aspartate (NAA)/creatine ratio, have been reported in some, but not all, studies [16, 17]. A single study including only adults with the common MELAS mutation 3243A>G mtDNA was discontinued owing to peripheral nerve toxicity. Of note, no significant differences were noted in the outcome measures of this group [18].

Resveratrol

Resveratrol, a naturally-occurring polyphenol and SIRT1 activator extracted from grape skins, induces mitochondrial biogenesis, improves aerobic capacity through deacetylation and activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), and corrects fatty acid oxidation defects *in vivo* [9, 20]. Resveratrol diminished neurodegeneration in the hippocampus, prevented learning impairments and decreased PGC-1 α acetylation. Resveratrol also exerted neuroprotective effects on midbrain dopaminergic neurons exposed to several types of insults,

suggesting potential benefits in the management of Parkinson's disease [9, 20]. The effect of resveratrol on the SIRT1 pathway that modulates a number of factors affecting fatty acid oxidation, mitochondrial biogenesis, and neuroprotection from some human neurodegenerative disorders makes this a promising future intervention for patients with mitochondrial diseases.

Amino Acid Supplements

The pathogenesis of the stroke-like episodes that characterize MELAS syndrome remain unclear; however, vasoconstriction is likely involved, as well as altered brain vascular endothelial function [21, 22]. L-arginine administration during the acute phase and prophylactically appears effective. L-arginine is a nitric oxide synthase substrate that stimulates nitric oxide synthase to generate nitric oxide (NO) and L-citrulline, alleviating vasoconstriction by maintaining the NO-mediated vasodilation [23]. Additionally, L-citrulline raised the NO levels higher than L-arginine alone, though the authors did recommend randomized controlled studies in other mitochondrial disorders where stroke-like episodes also occur [23].

Diet Manipulation

High-fat diet may benefit some patients with mitochondrial disorders, such as pyruvate dehydrogenase or complex I deficiencies. This effect may be the ability of fatty acid oxidation to feed electrons through electron transfer flavoprotein bypassing complex I [9]. It has also been reported that rats fed a high-fat diet had an increase in muscle mitochondrial biogenesis resulting in increased mtDNA copy number, increases in mitochondrial enzyme levels, and increased mitochondrial fatty acid oxidation [9, 24, 25]. The ability of fatty acids to stimulate mitochondrial biogenesis may explain the beneficial effects of a high-fat diet in pyruvate dehydrogenase or complex I deficiencies.

Mild-to-moderate (20–40 %) calorie restriction without over-restriction of any particular food group has been shown to extend the mean lifespan of mice, rats, and monkeys. Initial studies suggested this effect could be attributed to a decreased metabolic rate and decreased oxygen consumption with a decrease in the generation of reactive oxygen species. More recent studies suggest that calorie restriction activated mitochondrial biogenesis and improved OXPHOS [9]. Sirtuin 2 (Sir2)-related proteins are nicotinamide adenine dinucleotide-dependent protein deacetylases that appear to mediate the lifespan extension in yeast and eukaryote models [26]. SIRT1, a SIRT2 ortholog, is activated by calorie restriction and increases in mitochondrial biogenesis in multiple mouse tissues, including brain, liver, kidney, heart, and adipose tissue by activating the complex

nicotinamide phosphoribosyltransferase pathway [9, 26–28]. The fasting-induced regulation of *SIRT1* activity is likely hypothalamus specific [27, 28]. Calorie restriction appears to increase mitochondrial autophagy that leads to an increase in healthy mitochondria and a decrease of unhealthy mitochondria in skeletal muscle [28].

Nutritional modulation has a major effect on mitochondria and therefore holds considerable promise as a therapeutic intervention. Treatments used for patients with inherited OXPHOS disorders have generally given disappointing results, with the exception of CoQ therapy in primary CoQ deficiencies [2, 9]. However, a high-fat diet, such as a modified Atkins and/or ketogenic diet, remain promising therapeutic options for some mitochondrial diseases. Newer pharmacological interventions that activate the *SIRT1*/PGC1 α pathway also deserve future clinical study.

Exercise

In recent studies, exercise training has shown promising results as a treatment for exercise intolerance in patients with mitochondrial disease. Exercise has been found to stimulate PGC-1 α and promotes mitochondrial biogenesis [29]. Resistance training, such as weight-lifting, has shown a shift towards wild type heteroplasmy in the muscle satellite cells in patients with larger mtDNA deletions [30]. Exercise has also been shown to improve the symptoms of exercise intolerance in this patient population [31]. Endurance training, via aerobic exercise, has shown dramatic results in the *POLG* mutator mouse, a mouse model of aging [32]. When these mice were exercised from birth for 5 months, they were phenotypically identical to wild type mice, had no signs of multisystem pathology, as seen in their litter mates (alopecia, sarcopenia), and had a normal lifespan. Exercise promises to be an effective therapeutic intervention in patients with both primary and secondary mitochondrial disease. Although future clinical trials need to be performed, clinicians should be prescribing a supervised exercise program to patients affected by mitochondrial disease, as it has been found to be safe and effective as a therapy [33, 34].

Future Considerations

PGC1 α and SIRT1 activators

PGC-1 α upregulation stimulates OXPHOS in cells harboring mtDNA mutations and may therefore provide therapeutic benefits in patients with mtDNA disorders [9]. PGC-1 α overexpression has also been found to delay symptom onset and to increase the lifespan in a mouse model of muscle cyclooxygenase deficiency generated by muscle-specific inactivation of *COX10*. In mouse models, activation of the peroxisome proliferator-

activated receptor (PPAR)/peroxisome proliferator-activated receptor- γ coactivator-1 α pathway has been shown to induce mitochondrial biogenesis leading to a delayed onset of myopathy, as well as a prolonged lifespan [35]. Bezafibrate, a known PPAR agonist, had a similar effect. Bezafibrate has also been shown to increase several mitochondrial functions, including enzyme activities and protein levels in human fibroblasts with respiratory chain deficiencies [36]. Therefore, modulation of the PPAR/PGC-1 α pathway may hold promise for the treatment of mitochondrial disorders. Similarly, despite the absence of therapeutic trials or data from cultured cells or animal models, *SIRT1* axis modulation using the most potent natural *SIRT1* activator, resveratrol, may prove useful in patients with mitochondrial diseases [36].

Triheptanoin (C7)

The impairment of ATP production in mitochondrial disease is due to failure of the respiratory chain, but can also be attributed to other metabolic pathways occurring in the mitochondria. Secondary impairments may exist in fatty acid oxidation owing to a depletion of CAC intermediates or the physical association of mitochondrial fatty acid oxidation and oxidative phosphorylation subunits [37]. Patients with primary mitochondrial disease often develop symptomatic and biochemical evidence of a secondary defect in the CAC and fatty acid oxidation [38]. Further evidence of this association arrived when a cause of complex I deficiency was reported to be due to mutations in *ACAD9*, a long chain fatty acid enzyme, and was also reported in an infant with mitochondrial disease who was diagnosed with targeted whole exome sequencing [39, 40]. Supplementation with medium chain triglycerides [predominantly composed of octanoate (C8) triglyceride] has been suggested to help mitochondrial disease by bypassing long-chain fatty acid oxidation and producing acetyl-CoA as substrate for the CAC. However, it does not replete any other potentially reduced CAC intermediates. Therefore, anaplerotic therapy (supplementation with compounds that lead to the production of CAC intermediates) is an attractive possibility to improve energy production and alleviate symptoms such as hypoglycemia, myopathy, and cardiomyopathy. One potential anaplerotic supplement for use in these disorders is oral triheptanoin (a C7 triacylglyceride). Complete mitochondrial β -oxidation of heptanoate yields two acetyl-CoA molecules and one propionyl-CoA. Propionyl-CoA can subsequently be converted to succinate, a key CAC intermediate. A compassionate use study using triheptanoin for patients with long chain fatty acid oxidation disorders has shown that triheptanoin is superior than medium chain triglyceride oil with outcome measures that include decreased hospitalizations for rhabdomyolysis, improved quality of

life, and a decrease in muscle pain and hypoglycemia. Therefore, anaplerotic therapy is an attractive possibility to improve energy production and alleviate symptoms in primary mitochondrial disease due to these deficits in alternative pathways, and a future clinical trial is underway.

Epi-743

Epi-743 is a para-benzoquinone analog developed to have improved pharmacologic properties and therapeutic efficacy than either CoQ or Idebenone. Epi-743 has a unique redox potential secondary to its lipid side chain structure making it more potent than either CoQ or Idebenone and affording improved protection to cells subjected to oxidative stress related to mitochondrial disease [41]. To date, two open-label clinical trials have been completed using Epi-743 in multiple mitochondrial diseases. The first study included 13 children and 1 adult with molecularly confirmed polymerase γ (POLG1) deficiency, Leigh syndrome, MELAS, mtDNA deletion syndrome, and Friedreich ataxia—all of which were at risk for end-of-life care. Safety and efficacy measures included biochemical, neurological, quality-of-life, and brain redox assessments using technetium-99 m-hexamethylpropyleneamine oxime single photon emission computed tomography radionuclide imaging [41]. Twelve of the 13 patients treated with EPI-743 survived with various levels of clinical improvement and/or improved quality of life. EPI-743 had modified disease progression in >90 % of patients in this open-label study, as assessed by clinical, quality-of-life, and noninvasive brain imaging parameters [41]. In the second open-label trial, EPI-743 successfully stopped disease progression and reversed vision loss in 4 of 5 patients with LHON [42]. In 2012, Martinelli et al. [43] reported another open-label study on 10 patients with Leigh's syndrome who received oral Epi-743 three times daily and all had reversal of disease progression, improvement on movement disorder rating scale, and improved brain glutathione levels.

Dexramipexole

Dexramipexole [DEX; KNS-760704; ((6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine)] is an investigational drug that has been used to treat oxidative stress in liver cells of patients with amyotrophic lateral sclerosis [44]. Oxidative stress or cellular injury can result in secondary mitochondrial dysfunction, which has been linked to many chronic neurodegenerative disorders, including amyotrophic lateral sclerosis and Parkinson's disease. Mitochondrial dysfunction can lead to increased conductance mitochondrial membrane currents, and bioenergetics efficiency. Inefficient energy production puts cells at risk of death if energy demands exceed energy production. DEX and cyclosporine A inhibited increases calcium-induced ion conductance in rat brain mitochondria, as well as that caused by treatment with a proteasome inhibitor [44]. Cyclosporine A inhibited calcium-induced permeability transition in liver-derived mitochondria. In cultured cortical neurons, DEX significantly decreased oxygen consumption and maintained or increased the production of ATP [44]. DEX was found to be protective against proteasome inhibitor cytotoxicity [44]. This study suggests that DEX increases the oxidative phosphorylation efficiency and therefore may be useful as therapy for other types of mitochondrial diseases.

Conclusions

There are no proven therapies for primary mitochondrial disorders that cause respiratory chain deficiencies, owing, in part, to the lack of randomized, controlled clinical trials and small sample sizes. Initiatives such as the North American Mitochondrial Disease Consortium show promise for the future of mitochondrial therapeutics by developing patient registries that should improve access to clinical trials for both researchers and patients. Additionally, the North

Table 2 Food and Drugs Administration (FDA) and National Institutes of Health resources supporting rare disease research

Initiative	Access information
FDA	http://pharma.about.com/od/FDA/a/2012-Renewal-Of-The-Prescription-Drug-User-Fee-Act-Pdufa.htm http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/default.htm
Office of Rare Disease Research	http://rarediseases.info.nih.gov/
North American Mitochondrial Disease Consortium	http://rarediseasesnetwork.epi.usf.edu/namdc/
Office of Dietary Supplements	http://ods.od.nih.gov/
National Center for Advancing Translational Sciences	http://www.ncats.nih.gov/
Therapeutics for Rare and Neglected Diseases	http://www.ncats.nih.gov/research/rare-diseases/trnd/trnd.html
Clinical trials	http://www.clinicaltrials.gov/

American Mitochondrial Disease Consortium can facilitate natural history studies in homogenous populations of patients with specific mitochondrial diseases, as well as defining clinically relevant endpoints in order to develop a broad range of therapeutic options for patients with these progressive disorders [3]. Future research for effective therapies to treat mitochondrial diseases need to include natural history, as well as therapeutic trials in animal models of mitochondrial diseases that can provide critical information regarding mechanisms of action and safety prior to human trials. To that end, the Food and Drugs Administration and several National Institutes of Health initiatives are supporting new research for rare diseases, including disorders impacting on respiratory chain function (Table 2).

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

References

1. Koene S, Smeitink J (eds): Mitochondrial Medicine a Clinical Guideline, 1st ed. 2011. Khondrion, Nijmegen.
2. Calvo SE, Mootha VK. The mitochondrial proteome and human disease. *Annu Rev Genomics Hum Genet* 2010;11:25–44.
3. DiMauro S. A history of mitochondrial diseases. *J Inherit Metab Dis* 2011;34:247–248.
4. Elliot H, Samules DC, Eden JA, et al. Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet* 2008;83:254–260.
5. Johns DR. Mitochondrial DNA and disease. *N Engl J Med* 1995;333:638–44.
6. Thorburn DR, Chow CW, Kirby DM. Respiratory chain enzyme analysis in muscle and liver. *Mitochondrion* 2004;4:363–375.
7. Pfeiffer G, Majamaa K, Turnbull DM, Thorburn D, Chinnery PF. Treatment of mitochondrial disorders. *Cochrane Database Syst Rev* 2012. doi:10.1002/14651858.CD004426.pub3
8. DiMauro S and Schon EA. Mitochondrial disorders in the nervous system. *Annu Rev Neurosci* 2008;31:91–123.
9. Schiff M, Benit P, Coulilaly A, Loublier S, El-Khoury R, Rustin P. Mitochondrial response to controlled nutrition in health and disease. *Nutrition Rev* 2011;69:65–75.
10. Acin-Perez R, Hoyos B, Zhao F, et al. Control of oxidative phosphorylation by vitamin A illuminates a fundamental role in mitochondrial energy homeostasis. *FASEB J* 2010;24: 627–636.
11. Matsubara K, Kayashima T, Mori Masaharu, Yoshida H, Mizushima Y. Inhibitory effects of vitamin K3 on DNA polymerase and angiogenesis. *Int J Mol Med* 2008;22:381–387.
12. Klopstock T, Yu-Wai-Man P, Dimitriadis K, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. *Brain* 2011;134:2677–2686.
13. Nasser M, Javaheri H, Fedorowicz Z, Noorani Z. Carnitine supplementation for inborn errors of metabolism. *Cochrane Database Syst Rev* 2012. doi:10.1002/14651858.CD006659.pub3
14. Packer L, Cadenas E. Lipoic acid: energy metabolism and redox regulation of transcription and cell signaling. *J Clin Biochem Nutr* 2011 48: 26–32.
15. Rodriguez MC, MacDonald JR, Mahoney DJ, Parise G, Beal MF, Tarnopolsky MA. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. *Muscle Nerve* 2007;35:235–242.
16. DeStefano N, Matthews PM, Ford B, Genge A, Karpati G, Arnold DL. Short-term dichloroacetate treatment improves indices of cerebral metabolism in patients with mitochondrial disorders. *Neurology* 1995;45:1193–1198.
17. Duncan GE, Perkins LA, Theriaque DW, Neiberger RE, Stacpoole PW. Dichloroacetate therapy attenuates the blood lactate response to submaximal exercise in patients with defects in mitochondrial energy metabolism. *J Clin Endocrinol Metab* 2004 8:1733–1738.
18. Vissing J, Gansted U, Quistorff B. Exercise intolerance in mitochondrial myopathy is not related to lactic acidosis. *Ann Neurol* 2001;49:672–676.
19. Stacpoole PW, Kerr DS, Barnes C, Bunch ST, Carney PR, Fennell EM, et al. Controlled clinical trial of dichloroacetate for treatment of congenital lactic acidosis in children. *Pediatrics* 2006;117:1519–1531.
20. Bastin J, Lopes-Costa A, Djouadi F. Exposure to resveratrol triggers pharmacological correction of fatty acid utilization in human fatty acid oxidation-deficient fibroblasts. *Hum Mol Genet* 2011;20:2048–2057.
21. Koga Y, Akita N, Junko N et al. Endothelial dysfunction in MELAS improved by L-arginine supplementation. *Neurology* 2006;66:1766–1769.
22. Koga Y, Povalko N, Nishioka J, Katayama K, Kakimoto N, Matsuishi T. MELAS and L-arginine therapy: pathophysiology of stroke-like episodes. *Ann NY Acad Sci* 2010;1201:104–110.
23. El-Hattab AW, Emrick LT, Craigen WJ, Scaglia F. Citrulline and arginine utility in treating nitric oxide deficiency in mitochondrial disorders. *Mol Genet Metab* 2012;107:247–252.
24. Kang HC, Lee YM, Kim HD, Lee JS, Slama A. Safe and effective use of the ketogenic diet in children with epilepsy and mitochondrial respiratory chain complex defects. *Epilepsia* 2007;48:82–88.
25. Kossoff EH, Zupec-Kania BA, Rho JM, MD. Ketogenic diets: an update for child neurologists. *J Child Neurol* 2009;24:979–988.
26. Lin SJ, Ford E, Haigis M, Liszt G, Guarente L. Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev* 2004;18:12–16.
27. Yang H, Yang T, Baur JA, et al. Nutrient-sensitive mitochondrial NAD⁺ levels dictate cell survival. *Cell* 2007;130:1095–1107.
28. Imai S, Guarente L. Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. *Trends Pharmacol Sci* 2010;31:212–220.
29. Safdar A, Little JP, Stokl AJ, Hettinga BP, Akhtar M, Tarnopolsky MA. Exercise increases mitochondrial PGC-1 α content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J Biol Chem* 2011;286:10605–10617.
30. Murphy JL, Blakely EL, Schaefer AM, He L, Wyrick P, Haller RG, et al. Resistance training in patients with single, large-scale deletions of mitochondrial DNA. Mitochondrial Research Group, Newcastle University, Framlington Place, Newcastle upon Tyne, UK. *Brain* 2008;131:2832–2840.
31. Taivassalo T, Gardner JL, Taylor RW, Schaefer AM, Newman J, Barron MJ, et al. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. *Brain* 2006;129:3391–3401.
32. Safdar A, Bourgeois JM, Ogborn DI, Little JP, Hettinga BP, Akhtar M, et al. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. *Proc Natl Acad Sci U S A* 2011;108:4135–4140.
33. Jeppesen TD, Schwartz M, Olsen DB, et al. Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy. *Brain* 2006;129:3402–3412.
34. Taivassalo T, Haller RG. Exercise and training in mitochondrial myopathies. *Med Sci Sports Exerc* 2005;37:2094–2101.
35. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, et al. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-Activated protein kinase. *J Biol Chem* 2008;283:20015–20026.

36. Yatsuga S, Suomalainen A. Effect of bezafibrate treatment on late-onset mitochondrial myopathy in mice. *Hum Mol Genet* 2012;21:526–535.
37. Wang Y, Mohsen AW, Mihalik SJ, Goetzman ES, Vockley J. Evidence for physical association of mitochondrial fatty acid oxidation and oxidative phosphorylation complexes. *J Biol Chem* 2010;285:29834–29841.
38. Enns GM, Bennett MJ, Hoppel CL, Goodman SI, Weisiger K, Ohnstad C, et al. Mitochondrial respiratory chain complex I deficiency with clinical and biochemical features of long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 2000;136:1–4.
39. Haack TB, Danhauser K, Haberberger B, Hoser J, Strecker V, Boehm D, et al. Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. *Nat Genet* 2010;42:1131–1134.
40. Calvo SE, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, et al. Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci Transl Med* 2012; 25:118:118ra10.
41. Enns GM, Kinsman SL, Perlman SL, Spicer KM, Abdenur JE, Cohen BH, et al. Initial experience in the treatment of inherited mitochondrial disease with EPI-743. *Mol Genet Metab* 2012;105(1):91–102.
42. Sadun AA, Chicani CF, Ross-Cisneros FN, Barboni P, Thoolen M, Shrader WD, et al. Effect of EPI-743 on the clinical course of the mitochondrial disease leber hereditary optic neuropathy. *Arch Neurol* 2012;69:331–338.
43. Martinelli D, Catteruccia M, Piemonte F, Pastore A, Tozzi G, Dionisi-Vici C, et al. EPI-743 reverses the progression of the pediatric mitochondrial disease—denetically defined Leigh syndrome. *Mol Genet Metab* 2012;107:383–388.
44. Alavian KN, Dworetzky SI, Bonanni L, Zhanga P, Sacchetti S, Mariggio MA, et al. Effects of dexamipexole on brain mitochondrial conductances and cellular bioenergetic efficiency. *Brain Res* 2012;1446:1–11.