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Review Article

Treatments for mitochondrial dysfunction associated with autism spectrum disorders

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Abstract. Mitochondrial disease (MD) and dysfunction are associated with autism spectrum disorder (ASD) and most likely affect a substantial number of children with ASD. The mitochondrion is the powerhouse of the body's cells which supports and is supported by many metabolic systems, so mitochondrial dysfunction can have widespread consequences on cellular metabolism, especially in high energy cells like the brain, gastrointestinal tract and immune system, and especially during critical periods of high energy demand like childhood. This article reviews the treatments for MD as applied to children with ASD. We discuss supportive measures which aim at preventing further damage from occurring due to malfunctioning mitochondria, treatment with high dose vitamins that can support metabolism in light of dysfunctional mitochondria, dietary changes that can be useful in mitochondrial disease, and secondary organ systems to investigate due to mitochondrial dysfunction. We also discuss several treatments that have been reported to be of benefit in children with ASD which are also treatments that are standard of care for MD. This review provides a guide for appropriate treatments for children with ASD/MD and children with ASD that have mitochondrial dysfunction.

Keywords: Autism spectrum disorder, mitochondrial disease, oxidative stress, treatment

1. Introduction

Recent studies suggest that autism spectrum disorder (ASD) is associated with abnormal function of the mitochondria, at least in a subset of children. Approximately 5% of individuals with ASD have strictly defined mitochondrial disease (MD) while a larger number of individuals with ASD, possibly up to 30%, have abnormal biomarkers indicating dysfunction of the mitochondria that may or may not be considered classic MD [1]. A recent study has demonstrated that biomarkers of mitochondrial dysfunction are consistently elevated and valid in up to 50% of children with ASD [2]. A high prevalence of mitochondrial dysfunction in ASD is supported by other studies. For example, when electron transport chain (ETC) function in lymphocytes was compared between ASD and typically developing (TD) controls 80% of the children with ASD clearly demonstrated lower than normal ETC function [3]. Thus, these studies suggest that the mitochondria may be dysfunctional in children with ASD and that such dysfunction might be slightly different than what is considered classic MD.

The mitochondrion is the powerhouse of the body's cells. It is primarily responsible for producing cellular energy. Many metabolic systems feed their final biochemical products into mitochondrial pathways and/or derive their biochemical substrates from mitochondrial pathways; thus, mitochondrial dysfunction can affect both non-mitochondrial energy and non-energy producing metabolic systems. Mitochondria are a major

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Table 1
Factors to avoid in mitochondrial disease

Factors associated with regression	Substances/medications associated with mitochondrial toxicity
Infection, particularly viral	Acetaminophen
Inflammation	Non-steroidal anti-inflammatory drugs
Fever	Antipsychotics
Dehydration	Antidepressants
Prolonged fasting	Antiepileptic medications
Extreme heat	Anesthesia
Extreme cold	Heavy metals
	Insecticides
	Cigarette smoke

source of reactive oxygen species (ROS) and dysfunctional mitochondria can create a high level of ROS that can further damage the mitochondria and other important cellular components. Moreover, the mitochondria have other important regulatory roles in the cell, including calcium buffering and mediating programmed cell death (also known as apoptosis). Thus, it is essential for the mitochondria to function optimally in order for neurodevelopment to progress appropriately.

In this review article, we discuss treatments for MD and how they overlap with treatment for children with ASD with and without MD. To this end, we divide this article into two parts. First, we discuss the general management of MD, including a discussion of the secondary effects of mitochondrial dysfunction. Second, we discuss common treatments for ASD which can also positively influence mitochondrial function. Through this review we hope to outline important treatments for children with ASD/MD and demonstrate how some available treatments for ASD already address mitochondrial dysfunction.

2. General management of mitochondrial disease

Since there is no known cure for MD, standard-of-care treatment for MD is primarily supportive [4] and relies on three approaches: (a) precautions to prevent metabolic decompensation (b); vitamin supplements to support mitochondrial function; and (c) modification of the diet to optimize mitochondrial function. Additionally, investigation and treatment of medical disorders associated with MD can sometimes result in significant functional improvement. Unfortunately, many treatment recommendations for MD are not based on objective studies but are rather based on expert opinion. Few high-quality clinical trials have been performed with such studies demonstrating mixed results with small samples [5]. Here we summarize the available evidence in order to provide a sufficient overview of treatment of MD.

2.1. Preventing metabolic decompensation

There are several factors that are associated with developmental regression and mitochondrial toxicity in individuals with MD (Table 1). Although limited empirical studies exist, it is widely believed that infections can result in metabolic decompensation in children with underlying metabolic disease such as MD [6]. One study demonstrated that infection precipitated neurodevelopment regression within two weeks of a fever in 72% of patients with MD and history of episodic symptomatology. Interestingly, symptoms of neurodevelopmental regression were delayed three to seven days from the peak symptoms of infection [7]. The connection between infection/inflammation and neurodevelopmental regression may be particularly troublesome in children with ASD and classic MD. In a case series of 28 children with ASD and classic MD, Shoffner et al. [8] found that 61% of these children manifested neurodevelopmental regression into ASD and that of these children, regression occurred within 2 weeks of an episode of fever greater than 101° F in 71%. Interestingly, in 33% of these cases, the fever was associated with a routine vaccination. Other cases of autistic regression in a child with MD following a febrile episode induced by vaccination have been reported [9].

Unfortunately, it is not clear whether there is a specific type of MD associated with fever and neurodevelopmental regression, thereby limiting our ability to pinpoint children at risk. Many practitioners suggest vaccination as a way to prevent severe illnesses and point to the fact that, for the large majority of individuals with MD, vaccination does not cause neurodegenerative events [10]. However, vaccine safety studies have not been conducted specifically on children with MD, so objective data is lacking. Some protocols for vaccination suggest pretreating children with antipyretics/analgesics prior to vaccination. However, at least one study has suggested that treatment with ac-

etaminophen for fever and pain during MMR vaccination is associated with autistic regression [11,12]. This notion is consistent with the fact that acetaminophen is known to deplete glutathione, resulting in increased levels of oxidative stress [13], and induction of mitochondrial dysfunction [14]. Thus, there is no simple solution for limiting fever induced regression in children with underlying MD, and it is unclear whether it is the fever, the underlying inflammatory process, or other factors that results in neurodevelopmental regression. Clearly further studies are needed to clarify the role of illness, fever, vaccination, inflammation and iatrogenic medications as risk factors for neurodevelopmental regression in children with MD.

Dehydration and/or prolonged fasting can result in metabolic decompensation in children with MD. Dysfunctional mitochondria use fuels inefficiently. That is, much of the fuel is wasted and not turned into energy. Thus, prolonged fasts will deplete the body of necessary fuels (food). In some patients, an overnight fast is enough to cause stress on the mitochondria. Dehydration also results in physiological stress on the body and should be avoided for several reasons. Dysfunctional mitochondria result in the buildup of metabolites in the blood, such as lactic acid, that can be potentially toxic. Dehydration will increase the concentration and reduce the clearance of these toxins from the body. In addition, dehydration reduces perfusion of the body, thereby reducing the delivery of nutrients to cells, making cells that are already fragile more vulnerable. Exposure to extreme heat and cold is also best to avoid as many individuals with MD have dysautonomia, preventing them from adequately regulating body temperature when faced with exogenous extreme temperatures [15].

Certain common medications, such as acetaminophen, several non-steroidal anti-inflammatory drugs including aspirin and naproxen, antipsychotics including risperidone, quetiapine, clozapine, and olanzapine, and antidepressants including amitriptyline, citalopram, and fluoxetine can inhibit mitochondrial function [16]. Additionally, certain antiepileptic medication can be potentially detrimental to mitochondrial function. Valproate can cause serious adverse effects and even death in individuals with POLG1 mutations and myoclonic epilepsy with ragged red fibers syndrome [17]. Other AEDs such as phenobarbital, carbamazepine, phenytoin, oxcarbazepine, ethosuximide, zonisamide, topiramate, gabapentin and vigabatrin can also compromise mitochondrial function [17,18]. Specific precautions are required for surgery and anesthetics should be avoided; an anesthesiologist familiar with MD should be consulted if anesthesia is necessary [19]. Certain environmental toxins can also depress mitochondrial function. For example, common toxins inhibit mitochondrial function including heavy metals, insecticides and cigarette smoke [20–23].

2.2. Vitamin supplementation

Table 2 lists vitamins that may be helpful with improving mitochondrial function and gives suggested dosing schedules. High doses of B vitamins are the standard of care for individuals with MD. Such treatment is believed to be efficacious for several reasons. First, many of the enzymes systems in the mitochondria require various B vitamins. Since mitochondria can increase in number to compensate for dysfunctional mitochondria, higher vitamin requirements may be necessary for these extra mitochondria. Dysfunctional mitochondrial enzymes most likely undergo increased turnover thus increasing the vitamin requirements. In addition, metabolic systems that support mitochondrial function such as redox regulation systems require B vitamins to operate efficiently. Since dysfunctional mitochondria can overload these systems, B vitamins requirements are increased to support these systems. Lastly, certain mitochondrial disorders, such as thiamine responsive pyruvate dehydrogenase complex deficiency, respond to specific B vitamins.

Co-enzyme Q10 (CoQ10) is a fatty-soluble vitamin that is an essential component of the ETC. It is known as ubiquinone when oxidized and ubiquinol when fully reduced. Dosing of CoQ10 varies widely with some mitochondrial experts recommending very high doses, up to 30 mg/kg/day of ubiquinone [10]. Animal studies suggest that even high doses are non-toxic [24]. Various formulations of CoQ10 such as lipid microspheres and liposomal preparations have better bioavailability than standard formulations of co-enzyme Q10, providing the same affect at 1/2th to 1/5th the dose. CoQ10 is concentrated within the inner mitochondrial membrane associated with the ETC but is also found in other membranous organelles such as the endoplasmic reticulum, peroxisome, lysosome and vesicles. CoQ10 carries electrons to complex III from complex I and II within the ETC as well as from electron transfer flavoprotein. CoQ10 is an excellent antioxidant which has a role in regenerating vitamin E as well as protecting lipids, proteins and DNA from oxidation. CoQ10 has been shown to be protective of mitochondrial toxicity in animal models of Parkinson's disease [25] and dia-

Table 2
Recommend doses of vitamin supplements

Vitamin	Dose
Electron Transport Chain Support	
Co-enzyme Q10: Ubiquinol	5–30 mg/kg/day divided in 2 doses per day
Co-enzyme Q10: Ubiquinone	10–30 mg/kg/day divided in 2 doses per day
Energy Storage and Transportation	
Creatine monohydrate	0.1 g/kg/day divided in 1–2 doses per day
Fatty Acid Oxidation Support	
L-carnitine	30–100 mg/kg/day divided in 2–3 doses per day
Acetyl-L-carnitine	250–1000 mg/day divided in 2 doses per day
Biotin (B7)	5–10 mg/day given once per day
B-Vitamins	
Thiamine (B1)	50–100 mg/day given once per day
Riboflavin (B2)	100–400 mg/day given once per day
Niacin (B3)	50–100 mg/day given once per day
Pyridoxine (B6)	200 mg/day given once per day
Antioxidants	
Acetyl-L-carnitine	250–1000 mg/day divided in 2 doses per day
Vitamin E	200–400 IU/day given once per day
Vitamin C	100–500 mg/day given once per day
alpha-lipoic acid	50–200 mg/day given once per day
Oxidative Stress Support	
Methylcobalamin (B12)	5–1000 mcg/day given once per day
Folinic Acid / leucovorin (B9)	400–800 ug/day given once per day
5-methytetrahydrofolate (B9)	400–800 ug/day given once per day
N-acetyl-L-cysteine (NAC)	10–70 mg/kg/day divided in 1–3 doses

betes [26]. CoQ10 deficiency can be a primary disorder of mitochondrial metabolism with a variable presentation [27] but has also been associated with many cases of mitochondrial disease [28] and non-mitochondrial disease, including various forms of ataxia [29–31], food intolerance and allergies [32], and autism [33]. Supplementation in the context of deficiency can result in dramatic improvements [27,28]. Two years of CoQ10 therapy appeared to improve symptoms in a subgroup of patients with Friedreich's ataxia who demonstrated low CoQ10 levels at baseline [31]. CoQ10 therapy at 120–150 mg/day was found to improve biochemical markers of mitochondrial disease as well as clinical symptoms in an open-label trial of five patients with Kearns-Sayre syndrome [34]. There have been two double-blind placebo-controlled trials of CoQ10 for MD. CoQ10 along with creatine monohydrate and lipoic acid resulted in improvement in markers of mitochondrial metabolism and oxidative stress and attenuated the decline in strength in a randomized, double-blind, placebo-controlled, crossover study design in patients with mitochondrial cytopathies (including three patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes, three with chronic progressive external ophthalmoplegia, one with Kearns-Sayre syndrome, and nine with a variety of other mitochondrial diseases) [35]. In another double-blind placebo-controlled short-term trial, 30 patients with mitochondrial cytopathy received 1200 mg/day of CoQ10 for 60 days. Significant improvements were found in several biochemical measures of mitochondrial function and exercise capacity but not in clinically relevant variables [36].

L-carnitine is essential for fatty acid transportation into the mitochondria. Many mitochondrial disorders, especially those that affect the ETC, can inhibit beta-oxidation, the process by which fatty acids are broken down. Elevations in non-metabolized fatty acids lead to toxicity. Formation of non-metabolizable acyl-coenzyme A is also an essential step in the biotransformation of xenobiotics such as pivaloyl antibiotics, valproate and ifosfamide. L-carnitine is an acceptor of non-metabolizable acyl groups, allowing such fatty-acids to be cleared in the urine. Clearance of these substances by L-carnitine can result in an L-carnitine deficiency. L-carnitine also interacts with cardiolipin, an essential component of the mitochondrial membrane, to protect the mitochondria from toxicity [37]. L-carnitine has been shown to improve glucose homeostasis and insulin sensitivity [38] and can prevent free fatty acid induced lipotoxicity through reversal of mitochondrial dysfunction [39]. Acetyl-L-carnitine, the acetylated form of L-carnitine, is a natural constituent of the inner mitochondrial membrane. Acetyl-L-carnitine can

be metabolized into L-carnitine and acetyl-CoA outside of the mitochondrial. Both L-carnitine and acetyl-L-carnitine are neuroprotective [40,41]. Thus, supplementation with L-carnitine and/or acetyl-L-carnitine is believed to be essential for mitochondrial health in the context of mitochondrial dysfunction.

Creatine and phosphocreatine comprise an important cellular energy buffering and transportation system. Creatine has been shown to decrease cortical glutamate in Huntington's disease, a disease known to involved mitochondrial dysfunction [42], enhance mitochondrial function in an animal model of Duchenne muscular dystrophy (DMD) [43], improve high-energy phosphate turnover in the healthy human brain [44], and provide a significant genoprotective activity on mitochondrial DNA [45,46]. Creatine supplementation has also been shown to improve working memory and intelligence in a double-blind, placebo-controlled, cross-over study in young healthy adults [47]. However, the results from trials on clinical populations are mixed. In randomized, placebo-controlled trials, creatine supplementation improved cellular energetic and muscle strength in 33 patients with DMD [48], increased strength in 30 patients with DMD [49] and in 12 patients with various muscular dystrophies [50], but was not beneficial in 50 boys with DMD [51]. It was not found to be beneficial in a randomized double-blind, placebo controlled trial of 104 patients with amyotrophic lateral sclerosis [52]. For patients with MD, creatine supplementation has been shown to improve skeletal muscle power in a case series of five patients with MD (two with Kearns-Sayre syndrome, one with neuropathy ataxia, and retinitis pigmentosa syndrome, and two with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) [53], increase the strength of high-intensity anaerobic and aerobic type activities in seven patients with mitochondrial cytopathies in a randomized cross-over study [54], but was not beneficial in five patients with chronic progressive external ophthalmoplegia or Kearns-Sayre syndrome due to single large-scale mitochondrial DNA [55]. Since creatine monohydrate is generally tolerated with minimal adverse effects, many practitioners include it as part of the mitochondrial vitamin supplementation.

The carriers that are essential for delivering electrons to the ETC, NADH and $FADH_2$, are derived from niacin (vitamin B3) and riboflavin (vitamin B2), respectively. Coenzyme A which is combined with pyruvate to produce Acetyl-CoA, the first compound in the tricarboxylic acid (TCA) cycle, is derived from pantothenic acid (vitamin B5). Other enzymes in and related to the TCA cycle and ETC require additional B vitamins and have heme containing cofactors. For example, the pyruvate dehydrogenase complex requires lipoic acid and thiamin (vitamin B1). Heme production requires several vitamins and minerals, including iron, copper, zinc as well as riboflavin and pyridoxine (vitamin B6) and other enzymes require magnesium, manganese, cysteine and sulfur.

Biotin is an important cofactor for several mitochondrial enzymes, especially those that process fatty acids. Antioxidants useful for MD include vitamin E, vitamin C and alpha-lipoic acid. Other compounds like N-acetyl-L-cysteine, folate and cobalamin are important precursors for glutathione production. In addition, some vitamins serve as antioxidants, which may slow the progression of MD caused by high amounts of reactive oxygen species. Glutathione is also important, especially since it is produced in the cytosol and imported into mitochondria. Supplementation with antioxidants has been reported to improved glutathione levels and clinical functioning in patients with MD [56].

2.3. Diet modification

There have been no controlled clinical trials on diet manipulation in individuals with MD. Some patients respond to frequent meals high in complex carbohydrates. For some patients, an overnight fast can destabilize mitochondrial function. Such patients can be treated with complex carbohydrates such as corn starch before bedtime, while some can be awakened in the middle of the night for a snack, while others may require a feeding tube to receive feeding overnight. Other patients respond to low carbohydrate diets. For example, the ketogenic diet may be an effective treatment in MD [57], particularly individuals with MD and epilepsy [58]. In an open-label study, treatment with the ketogenic diet along with a vitamin cocktail was shown to improve brain energy metabolites in 12 patients with MD [59]. The ketogenic diet should be initiated and monitored by a practitioner familiar with the diet as it can exacerbate metabolic disorders by causing acidosis in certain cases. Some patients respond to medium chain triglyceride oil supplementation since these fats do not require carnitine to be transported into the mitochondria.

2.4. Secondary effects of mitochondrial disease

Patients affected by mitochondrial dysfunction can manifest abnormalities in multiple organ systems, par-

ticularly tissues that require significant energy, such as the nervous, endocrine, gastrointestinal, cardiovascular, visual and immune systems. Thus, such organ systems should be screened for dysfunction. Seizures and subclinical electrical discharges are relatively common in mitochondrial disorders, so practitioners should have a high index of suspicion for these abnormalities. Cerebral folate deficiency has been reported in both mitochondrial disorders and ASD, including one study where 50% of patients with MD had CFD [60]. This disorder can be easily treated with folinic acid, so it should be strongly considered in individuals with MD.

2.5. Caveats to treatment

In general, milder MD responds better to treatment than more severe MD and treatment initiated sooner in the course of the disorder will probably be more effective than treatment initiated after MD has been longstanding. However, the success rate of treatment is extremely variable for several reasons. First, the efficacy of mitochondrial treatment, even for well-known MDs, has not been well studied. Second, the mitochondrial dysfunction identified in ASD has not been well characterized and treatment for mitochondrial dysfunction in ASD has not been well studied. Third, the benefit of treatment may not be obvious as treatment may simply prevent progression of symptoms rather than reverse symptoms. Finally, any benefit from treatment may take several months or longer to observe.

Most vitamins are well tolerated, even at high doses. However, pyridoxine (vitamin B6) has been suggested to result in peripheral neuropathy at high doses. Some children with ASD may have behavioral side-effects from some vitamins. Thus, it is important to start vitamins one at a time so that any side-effects can be linked to a particular vitamin. Children should be carefully monitored when the ketogenic diet is started as the diet can worsen metabolic acidosis associated with MD.

3. Autism spectrum disorder treatments which improve mitochondrial function

Several studies have suggested that treatment with mitochondrial cofactor supplementation, including antioxidants, coenzyme Q10, carnitine, and B-vitamins may improve mitochondrial function and behavior in some children with ASD [1]. L-carnitine may be particularly helpful in children with ASD since carnitine deficiency has been implicated in ASD [61,62] and some studies have reported improvements with the use of carnitine in ASD [9,63–67]. One double blind, placebo controlled study reported improvements in children with ASD using L-carnitine (50 mg/kg/day), including hand muscle strength and cognition [68]. A second double-blind, placebo-controlled study of L-carnitine (100 mg/kg/day) reported significant improvements over six months of treatment in ASD symptoms compared to placebo [69]. Two double-blind, placebo controlled studies using a multivitamin containing B vitamins, antioxidants, vitamin E, and coenzyme Q10 reported various improvements in ASD symptoms compared to placebo [70,71]. Treatments for oxidative stress have also been shown to be of some benefit for children with ASD. For example, methylcobalamin and folinic acid have been reported to significantly increase glutathione concentrations in children with ASD and appear to improve certain autistic behaviors [72, 73]. A recent double-blind placebo-controlled study has demonstrated that N-acetyl-L-cysteine improves irritability in children with ASD compared to placebo [74]. Several other antioxidants [75], including vitamin C [76] and carnosine [77] have also been reported to significantly improve autistic behaviors. One study reported improvements in ASD symptoms using NADH and D-ribose [78]. Finally, one study reported improvements in ASD symptoms in 18 of 30 children with the use of a ketogenic diet [79].

Table 3
Resources for parents and patients with mitochondrial disease

MitoAction Website	www.mitoaction.org
United Mitochondrial Disease Foundation	www.umdf.org
Mitochondrial Autism and Autism Like Mito	Facebook group

4. Discussion

Although the exact prevalence of MD in ASD is not certain at this time, it is clear that MD and mitochondrial dysfunction are associated with ASD and recent recommendations suggest that every child with ASD should be screened for MD [1]. The mitochondrion is the powerhouse of the body's cells which supports and is supported by many metabolic systems, so mitochondrial dysfunction can have widespread consequences on cellular metabolism, especially in high energy cells like the brain, gastrointestinal tract and immune system, and especially during periods of high energy demands such as childhood. Thus, it is important to carefully support mitochondrial systems. This manuscript provides a brief overview of the factors that can nega-

tively influence mitochondrial function and treatments that might be of use in cases of MD. Notably, several treatments that have been reported to be of benefit in children with ASD are treatments that are standard of care for MD. This review provides a guide for appropriate treatments for children with ASD/MD and children with ASD who also have mitochondrial dysfunction. Table 3 provides a short list of resources for both professionals and parents in order to keep updated with new developments in mitochondrial disease associated with ASD.

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