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MITOCHONDRIAL DYSFUNCTION AND AUTISM SPECTRUM DISORDERS: A SIMPLIFIED APPROACH

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INTRODUCTION

Recently, evidence has accumulated that some children with autism spectrum disorders (ASDs) have mitochondrial disease (also known as mitochondrial disorders) or mitochondrial dysfunction. Mitochondrial dysfunction generally refers to mitochondria that are impaired in function but not severely impaired enough to fulfill the criteria necessary for the diagnosis of mitochondrial disease. In essence, mitochondrial disease can be thought of as a severe form of mitochondrial dysfunction, and mitochondrial dysfunction can be thought of as a less severe form of mitochondrial disease. If mitochondrial dysfunction can be represented by an engine that is sputtering, mitochondrial disease would be represented by an engine that is constantly in the repair shop.

The evidence for mitochondrial dysfunction in ASD has expanded over the last several years based on multiple published papers on this topic.[1-5] This article reviews the role of mitochondria in health and disease, the proper functioning of mitochondria, possible causes of mitochondrial dysfunction in ASD, laboratory testing and criteria that can help identify mitochondrial dysfunction, and potential treatments. Before beginning any workup for mitochondrial dysfunction and before performing laboratory testing or starting any treatment (including over-the-counter nutritional supplements), please consult with your or your child's physician.

THE ROLE OF MITOCHONDRIA

In the simplest terms, mitochondria are the powerhouses of the cell, generating energy from the breakdown of food. Figure 1 depicts a mitochondrion and shows the pathways involved when mitochondria break down food and use oxygen to create ATP (the energy source for the body, analogous to gasoline for a car).

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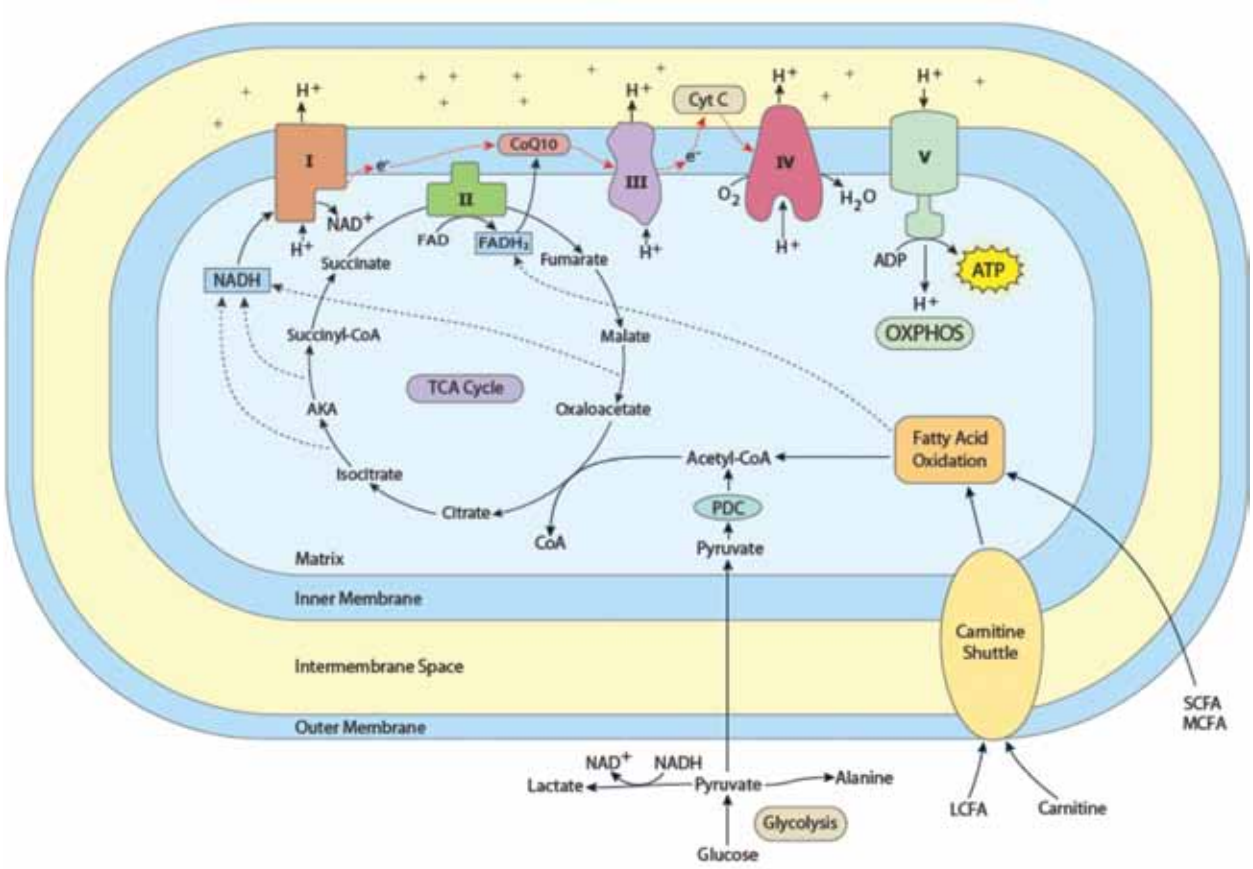
Figure 1: Mitochondrial structure and pathways



MITOCHONDRIAL FUNCTION

As seen in Figure 1, the structure of the mitochondrion consists of outer and inner membranes, with a space (the intermembrane space) in between. The matrix is the innermost part of the mitochondria where many biochemical reactions occur, including the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle or citric acid cycle. The inner mitochondrial membrane contains five complexes (known as complexes I through V) that make up the electron transport chain. On the bottom of the figure, you can see glucose, which is eventually broken down into pyruvate through the process of glycolysis. Pyruvate is then transported into the mitochondria and eventually is broken down into acetyl-CoA, which enters the TCA cycle. In the case of mitochondrial dysfunction, pyruvate transportation can be slowed, and, therefore, pyruvate can convert into lactate (also known as lactic acid) and alanine, leading to elevations in these markers.

Fatty acid metabolism is shown on the bottom right-hand corner of the figure. Short chain fatty acids (SCFA) and medium chain fatty acids (MCFA) can diffuse directly into the mitochondria, whereas long chain fatty acids (LCFA) are transported into the mitochondria by attaching to carnitine, which shuttles these fatty acids across the inner and outer mitochondrial membranes. Once inside the mitochondria, the fatty acids, like pyruvate, are broken down and converted into acetyl-CoA, which feeds into the TCA cycle. However, some of the electrons released from burning fatty acids (fatty acid oxidation) can feed into complex II through FADH2, bypassing complex I in the process (which may partially explain why a ketogenic diet, which involves a high intake of fats, might be helpful for treating mitochondrial dysfunction).[6]

The three dotted lines with arrows coming off of the TCA cycle are electrons (negatively charged particles) that are transferred through NADH into complex I. Complex I then transfers these electrons (depicted by the red dotted line) to coenzyme Q10 (CoQ10) which, in turn, transfers the electrons to complex III. When the electrons pass through complex I, NADH is converted to NAD+. Hydrogen protons (positively charged hydrogen particles, H+) are pumped from the matrix (the innermost part of the mitochondria) through the inner membrane and into the intermembrane space, where they build up and form an electrochemical gradient. The electrons that passed to complex III are now transferred by cytochrome C (Cyt C) to complex IV. This process also pumps more hydrogen protons into the intermembrane space through complexes III and IV. During this process, oxygen is converted into water in complex IV (this is how camels generate water from fat). The hydrogen protons in the intermembrane space then diffuse back into the matrix through complex V (ATP synthase), and this generates ATP through a process known as oxidative phosphorylation.

If the electron transport chain does not work properly (is "blocked"), metabolites begin to "back up" and elevations can then occur in TCA cycle metabolites, fatty acids, pyruvate, lactate (lactic acid) and alanine. Elevations in these metabolites are laboratory markers of mitochondrial dysfunction.[4] Generally, the higher the elevations and the more metabolites affected, the more likely that mitochondrial dysfunction exists.

CLINICAL HISTORY AND LABORATORY TESTING

To evaluate possible mitochondrial dysfunction, it is important to examine the patient's clinical history. Sometimes there will be a family history of mitochondrial disease. Other clinical history that is

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often observed in mitochondrial dysfunction includes developmental regression (loss of previously acquired skills), seizures, fatigue or lethargy, ataxia (lack of coordination of muscle movements), motor delays, gastrointestinal (GI) abnormalities (such as reflux, constipation, dysmotility, diarrhea and inflammation), and cardiomyopathy (significant heart problems). After a clinical history and examination of the patient, laboratory testing can also be helpful (ideally performed in the morning after fasting for 8-10 hours). The lab tests in question are typically covered by insurance and often can be performed by LabCorp or Quest Diagostics. It is important to have an experienced phlebotomist who is familiar with these tests because keeping the tourniquet on during the blood draw or struggling during the blood draw can cause false elevations in some laboratory tests.

The labs include:

- Lactate (lactic acid)
- Pyruvate
- Carnitine (free and total)
- Acylcarnitine panel (fatty acids attached to carnitine)
- *Quantitative* plasma amino acids
- Ubiquinone (also known as coenzyme Q10)
- Ammonia
- Creatine kinase (CK)
- AST and ALT
- CO2 and glucose

If the labs are abnormal, they may need to be repeated for confirmation. If the labs are normal but mitochondrial dysfunction is still suspected, then repeating the labs when the child is sick or under stress might help unmask and identify mitochondrial dysfunction. (Illness or stress will generally place mitochondria under more stress and increase dysfunction.) Our recent publication on mitochondrial dysfunction in children with ASD (available free online) provides more details.[4]

CRITERIA FOR MITOCHONDRIAL DISORDER

To help identify mitochondrial disorders, some investigators have developed certain criteria. The use of a criterion helps physicians and researchers to systematically evaluate and identify mitochondrial problems in individuals. Figure 2 (next page) reviews one such criterion for mitochondrial disorders (the Morava criteria).[7] This criterion has 3 sections, focusing on clinical signs and symptoms, metabolic and imaging studies, and morphology. A total of 4 points are possible in each category. A total score of 8-12 is consistent with definite mitochondrial disorder, and a total score of 5-7 is consistent with probable mitochondrial disorder. This tool can be utilized to help determine if further workup for mitochondrial dysfunction is needed.

MITOCHONDRIAL DYSFUNCTION AND ASD

Children with ASD are more likely to have deficits in their ability to produce cellular energy than are typically developing children.[3] Cumulative damage and oxidative stress in mitochondria might influence both the onset and severity of autism, suggesting a significant link between autism and mitochondrial problems. Some individuals with mitochondrial disease will have a genetic cause (termed primary mitochondrial disease). However, in our recent systematic review and meta-analysis,[4] most children (79%) who had ASD and mitochondrial disease did not have a genetic reason that could explain their mitochondrial dysfunction. Therefore, the mitochondrial problems reported in these children may have been due to a biochemical abnormality (termed secondary mitochondrial disease).

SECONDARY MITOCHONDRIAL DYSFUNCTION AND ASD

Several studies have documented a significantly lower mean glutathione (GSH) concentration[8-10] and a lower mitochondrial GSH reserve[11] in children with ASD compared to controls. GSH is the major antioxidant in humans, and GSH depletion is associated with impaired mitochondrial function[12] as well as increased free radical production.[13] An example of a free radical is an oxygen molecule that has an unpaired electron (this free radical is called a "reactive oxygen species"), which can then remove an electron from enzymes and DNA/RNA and cause damage. This damage is termed oxidative stress. Antioxidants can donate an electron to the free radical to allow all of its electrons to be paired, which quenches the free radical and prevents oxidative stress.

Of note, increased free radicals can impair mitochondrial function[14] and may be particularly significant in individuals with ASD since the latter have been shown, as a group, to be under higher oxidative stress and have reduced levels of antioxidants compared to typically developing children.[8, 11, 15, 16] Furthermore, GSH protects mitochondria against the adverse effects of TNF-α,[13] a proinflammatory cytokine that can inhibit mitochondrial function.[17, 18] This might be particularly important since some studies have reported higher TNF-α in lymphocytes,[19] cerebral spinal fluid,[20] and brains[21] of

GSH protects mitochondria against the adverse effects of TNF-α, a proinflammatory cytokine that can inhibit mitochondrial function. This might be particularly important since some studies have reported higher TNF-α in lymphocytes, cerebral spinal fluid, and brains of individuals with ASD compared to controls.

Figure 2
Morava et al., 2006 Criteria for Mitochondrial Disorder†

Section I: Clinical signs and symptoms *(1-2 points per sign/symptom as indicated. 4 points max this section)*

(a) Muscular presentation

(2 points max this category) **Points** I(a)

- ☐ Ophthalmoplegia *(2 points)*
- ☐ Facies myopathica
- ☐ Exercise intolerance
- ☐ Muscle weakness
- ☐ Rhabdomyolysis
- ☐ Abnormal EMG

(b) CNS presentation

(2 points max this category) **Points** I(b)

- ☐ Developmental delay
- ☐ Loss of skills
- ☐ Stroke-like episodes
- ☐ Migraine
- ☐ Seizures
- ☐ Myoclonus
- ☐ Cortical blindness
- ☐ Pyramidal signs
- ☐ Extrapyramidal signs
- ☐ Brain stem involvement

(c) Multisystem disease

(3 points max this category) **Points** I(c)

- ☐ Hematology
- ☐ GI tract
- ☐ Endocrine/growth
- ☐ Heart
- ☐ Kidney
- ☐ Vision
- ☐ Hearing
- ☐ Neuropathy
- ☐ Recurrent/familial

I(a) + I(b) + I(c)

(4 points max this section) **Total Points Section I:** I

Section II: Metabolic/imaging studies *(1-2 points per sign/symptom as indicated. 4 points max this section)*

- ☐ Elevated lactate *(2 points)*
- ☐ Elevated lactate/pyruvate ratio
- ☐ Elevated alanine *(2 points)*
- ☐ Elevated CSF lactate *(2 points)*
- ☐ Elevated CSF protein
- ☐ Elevated CSF alanine *(2 points)*
- ☐ Urinary tricarbon acid excretion *(2 points)*
- ☐ Ethylmalonic aciduria
- ☐ Stroke-like picture MRI
- ☐ Leigh syndrome/MRI *(2 points)*
- ☐ Elevated lactate/MRS

(4 points max this section) **Total Points Section II:** II

Section III: Morphology (muscle biopsy) *(1-4 points per sign/symptom as indicated. 4 points max this section)*

- ☐ Ragged red/blue fibers *(4 points)*
- ☐ Cox-negative fibers *(4 points)*
- ☐ Reduced COX staining *(4 points)*
- ☐ Reduced SDH staining
- ☐ SDH positive blood vessels *(2 points)*
- ☐ Abnormal mitochondria/EM *(2 points)*

(4 points max this section) **Total Points Section III:** III

SCORE: **Please consult your physician for help with interpretation**

I + II + III

Total Score

- **1** Mitochondrial disorder unlikely
- **2-4** Possible mitochondrial disorder
- **5-7** Probable mitochondrial disorder
- **8-12** Definite mitochondrial disorder

†**Reference:** Morava, E., L. van den Heuvel, F. Hol, M.C. de Vries, M. Hogeveen, R.J. Rodenburg, et al. (2006). Mitochondrial disease criteria: diagnostic applications in children. *Neurology, 67*, 1823-6.

These findings suggest that mitochondria from children with ASD may be more vulnerable to damage from environmental toxicants than mitochondria from typically developing children. In this context, exposures to environmental toxicants could contribute to secondary mitochondrial dysfunction in some children with ASD.

individuals with ASD compared to controls.

Additionally, in one study, exposure to ethylmercury (thimerosal) led to a larger increase in free radical generation and a greater reduction in the ratio of reduced GSH to oxidized GSH in ASD cells compared to control cells.[11] These findings suggest that mitochondria from children with ASD may be more vulnerable to damage from environmental toxicants than mitochondria from typically developing children.[11] In this context, exposures to environmental toxicants could contribute to secondary mitochondrial dysfunction in some children with ASD.[5, 22] For example, *in vitro* exposure to diesel exhaust particles (DEP) has been shown to inhibit mitochondrial function,[23] and elevated environmental concentrations of DEP have been associated with ASD.[24] Other environmental toxicants that inhibit mitochondrial function and have been associated with ASD include mercury,[24-28] lead,[29-32] cadmium,[24, 33] PCBs,[34, 35] and pesticides.[36-39]

Finally, *Clostridia*, an anaerobic, spore-forming Gram-positive rod bacterium, is known to produce propionic acid,[40] and a derivative of propionic acid recovered in the urine of ASD individuals has been reported as a marker of *Clostridia*.[41] A recent rat model of ASD demonstrated that the administration of propionic acid induced mitochondrial dysfunction and led to certain behavioral and biochemical features of ASD such as repetitive behaviors, social interaction problems, hyperactivity, oxidative stress, lowered GSH levels and altered carnitine levels.[40, 42-44] Furthermore, significantly elevated concentrations of *Clostridia* in the GI tract have been reported in some ASD children compared to controls,[45-47] with improvements noted with vancomycin treatment in some children.[48, 49] Therefore, *Clostridia* may be a contributor to mitochondrial dysfunction in some children with ASD.

TREATMENTS FOR MITOCHONDRIAL DYSFUNCTION IN ASD

Several studies have reported that nutritional supplements and/or antioxidants may be beneficial in some children with ASD who have mitochondrial dysfunction. Six studies have reported various improvements (including language and coordination) with the use of carnitine in children with ASD and mitochondrial disease.[1,8,50-53] Recently, another study reported improvements in children with ASD using carnitine compared to placebo, although it was not reported if the children had concomitant mitochondrial dysfunction.[54] Along with carnitine, some investigators report clinical improvements with coenzyme Q10[1,53,55] and high doses of B vitamins, including thiamine or riboflavin.[1, 50, 53] Cerebral folate deficiency (CFD) has been described in one child with ASD and mitochondrial disease.[56] In some studies, treatment with folinic acid and a milk-free diet has been reported to result in significant improvements in ASD symptoms in children with CFD.[57, 58]

Treatment of mitochondrial dysfunction also consists of specific precautions to avoid prolonged fasting, implement dietary recommendations (ensuring an adequate number of calories),[59] avoid certain medications,[60] adopt anesthesia precautions for surgery, and avoid infections[61] (if possible). Additional treatment recommendations pertain to antipyretic (fever) therapy, intravenous hydration, and nutritional supplements during acute illnesses. Furthermore, because hypoxia can impair mitochondrial function,[62] increasing oxygen delivery to dysfunctional mitochondria through hyperbaric oxygen therapy (HBOT) might aid in improving mitochondrial function.[63-67] In one animal study, HBOT was reported to activate mitochondrial DNA transcription and replication, and increase the biogenesis of mitochondria in the brains of animals.[68] Clinically, some patients treated by Dr. Rossignol who have ASD and mitochondrial disease have improved with the use of HBOT provided at low atmospheric pressure (1.3 to 1.5 atmospheres), including one child whose improvements in mitochondrial function were documented by repeat muscle biopsy. However, increasing oxygen delivery to mitochondria can increase oxidative stress; the HBOT pressure should therefore be carefully monitored under the guidance of an experienced physician, and generally low levels of pressure (1.3 to 1.5 atmospheres) and lower oxygen concentrations (~24%) should be used initially.[69, 70] Further studies examining these treatments for mitochondrial dysfunction in ASD are needed.

MITOCHONDRIAL DYSFUNCTION AND ASD: IMPLICATIONS FOR THE FUTURE

There is much still to be learned regarding the biology of mitochondrial disease and ASD. Evidence has rapidly accumulated that clearly supports an association between these two seemingly different disorders. Although some children with ASD have a genetic cause for mitochondrial dysfunction, many will have a secondary cause. It appears that at least a subpopulation of children with ASD has mitochondrial disease as the core biological lesion contributing to their ASD and associated comorbidities. Further studies in the field of mitochondrial medicine may one day help unlock the mysteries that define ASD.

Clinically, some patients treated by Dr. Rossignol who have ASD and mitochondrial disease have improved with the use of HBOT provided at low atmospheric pressure (1.3 to 1.5 atmospheres), including one child whose improvements in mitochondrial function were documented by repeat muscle biopsy.

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