Mitochondrial Dysfunction in Autism

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Defeat Autism Now! Fall Conference
October 9, 2009
Disclosures:
I have received funding for two studies on hyperbaric treatment in children with autism from the International Hyperbarics Association but I have no commercial or financial relationships with chamber manufacturers.

With all treatments and recommendations, please consult with your child’s physician before implementation.

The use of every treatment in individuals with autism is “off-label” except for risperidone for the treatment of irritability.
Autism: Pathophysiology

- Cerebral hypoperfusion
- Inflammation
  - Cerebral
  - Gastrointestinal
- Dysbiosis
- Mitochondrial dysfunction
- Oxidative stress
- Impaired glutathione production
- Environmental toxicant exposures
Autism: a disorder of brain bioenergetic metabolism?

Autism: a mitochondrial disorder?

J. LOMBARD

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Mitochondrial Disease (MD) and Regression

- Illness
- Fever
- Surgery
- Anesthesia
- **Fasting**
- Dehydration
- High altitude / hypoxia
- Stressors
- Increased oxidative stress
Mitochondria

• Descendent of an ancestral purple, non-sulfur, photosynthetic bacteria
• Generate **ATP** (energy)
• Has its own DNA (genome)
• Contain 5 compartments
• Play a role in programmed cell death
• Involved in porphyrin / heme production
• Mitochondria in the liver are important in ammonia detoxification
Mitochondria

- ATP production in the mitochondria through the electron transport chain (ETC) from aerobic metabolism creates about 13-fold more ATP from glucose than produced from anaerobic metabolism.
- An electrochemical and pH gradient is produced across the inner membrane.
- Mitochondria are primary source of ROS by electron leak from the electron transport chain; 1-2% of oxygen normally produces free radicals.
Proteins → Amino Acids → Carnitine → AcetylCoA → Citric acid cycle → NADH → ATP 

Polysaccharides → Monosaccharides → AcetylCoA → Citric acid cycle → NADH → ATP 

Fats → Fatty Acids → AcetylCoA → Citric acid cycle → NADH → ATP
Mitochondria

- Increasing inspired $PO_2$ increases ROS generation
- Major protector against oxidative stress and mtDNA damage in mitochondria is glutathione (GSH)
- Mitochondria cannot produce GSH, but it is made in the cytoplasm
- Accumulation of mtDNA mutations thought to be involved in aging
Respiratory Chain

\[ \text{O}_2 \rightarrow \text{SOD} \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{Catalase} \rightarrow \text{H}_2\text{O} \]

\[ \text{OH} \rightarrow \text{mtDNA} \]

Mitochondrial Permeability Transition

Lipid Peroxidation
Antioxidant

Oxygen

8 electrons
Glutathione deficiency leads to mitochondrial damage in brain
(buthionine sulfoximine/glutathione ester/turnover/hydrogen peroxide/animal model)

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Contributed by Alton Meister, December 5, 1990

Mini review

Oxidative stress: Role of mitochondria and protection by glutathione

Fernandez-Checa et al., 1998  BioFactors 8:7-11
Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia

Atkuri et al., 2009 Proc Natl Acad Sci USA, in press
Mitochondria: 5 Compartments

- Outer membrane
- Intermembrane space
- Inner membrane
- Cristae: invaginations of inner membrane
- Matrix
Electron Transport Chain (ETC)

- Five complexes, located in inner mitochondrial membrane
- Mitochondrial oxidative phosphorylation occurs here; 2 main functions:
  - Generates **ATP** from electrons (from food) through ATP synthase (complex V)
  - Generates **heat**
- Electrons that leak out of the ETC produce **free radicals**: reactive oxygen species (ROS)
ETC

• If the ETC is damaged or inhibited, then ROS production may be increased
• ETC can be blocked or impaired (“leaky”) and this increases ROS
  – Genetic defects in nDNA and mtDNA
  – Toxins (e.g., cyanide)
  – Medications
Complex I

Intermembrane Space

CoQ10

Cytochrome C

Complex II

Inner Membrane

Complex III

Matrix

Complex IV

ATP Synthase (Complex V)
Mitochondrial Disease (MD)

- Primary *mitochondrial disease* typically refers to genetic defects leading to mitochondria dysfunction (MtD)
- Secondary mitochondrial disease *(dysfunction)* refers to impaired functioning of mitochondria
- Organs with highest aerobic demand are most affected *(CNS, heart, and skeletal muscle)*, but any organ can be affected including GI and endocrine
MD

- Can present at any age
- Consider family history
- No reliable biomarkers exists
- Under-diagnosed
- Think of MD when 3 or more organ systems are involved without a unifying diagnosis
- Consider specialist referral
MtD and synaptic function

- Synaptic neurotransmitter release decreased by 50% when mitochondrial dysfunction exists.
- Neurons firing at high rates affected the most: GABAergic interneurons.
- Decreases in GABA release can contribute to seizures and regression.
Primary MD

- Genetic defects associated with MD: can be in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA)
- mtDNA mutations estimated at 1 per 5,000; recent study of 3,168 neonatal cord blood samples: 1 in 200 [Elliott, 2008]
- mtDNA haplotype associated with longevity
- Over 150 mtDNA point mutations and 100 mtDNA deletions have been identified
Dual genome: MD

- nDNA encodes for ~850 genes involved in forming structural subunits of mitochondria
- Most common cause of MD is genetic defects in nDNA (often autosomal recessive): classical Mendelian inheritance, accounts for 75-90% of primary MD [Haas, 2008]
- With increasing age, genetic basis for MD is often mtDNA: maternal inheritance
Primary MD in ASD

• Inverted duplicated 15q11-q13 [Filipek, 2003]
• Rare familial associated with SIDS
• SLC22A5: encodes for sodium-dependent carnitine co-transporter OCTN2, causes **carnitine deficiency**
• A3243G: MELAS [Pons, 2004; Serajee, 2006]
• Defects in L-type calcium channel
• mtDNA G8363A mutation [Graf, 2000]
Secondary MD

• Medications: valproic acid (depletes carnitine), salicylates, antiretroviral HIV meds

• **Estrogen** increases mitochondrial efficiency [Stirone, 2005]

• Decreased metabolic reserve
  – Oxidative stress
  – Lowered glutathione

• Hypoxia
Secondary MD: Toxins

- Heavy metals (mercury, lead, arsenic, cadmium, aluminum)
- Pesticides
- Diesel exhaust
- Propionic acid from clostridia

- Toxins leading to secondary MD rarely (never ?) discussed in mito literature.
Symptoms / Signs of MtD

- “Any symptom in any organ at any age” [Munnich, 1996]
- Developmental delay
- **Hypotonia** (low muscle tone)
- Constipation / GI dysmotility
- Slow cognitive processing speed
- Fatigue [Weissman, 2008]
- Seizures
- Oxidative stress
Labs: MtD (blood)

- Basic chemistry (CO$_2$, anion gap)
- Liver enzymes (AST, ALT)
- Ammonia
- Creatine kinase
- Lactic acid and pyruvate
- Plasma amino acids: alanine (compared to lysine), glycine, proline, sacrosine, tyrosine
- Fasting plasma acylcarnitine analysis
Labs: MtD (urine)

- Urinary organic acid testing
- TCA cycle intermediates
- Ethylmalonate
- 3-methyl-glutaconate
- 2-ketoglutarate
- Dicarboxylic acids
- Urinary oxidized RNA (8-OG)
Other tests: MtD

MRS
- High lactate peak
- Low $N$-acetyl-L-aspartate (NAA)
- Low choline
- High succinate

Biopsy
- Skin (fibroblast)
- Muscle
- Liver
- Cardiac
<table>
<thead>
<tr>
<th>Depletes brain ATP  [Kosenko, 1994]</th>
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<tbody>
<tr>
<td>Toxic to brain cells  [Cagnon, 2007]</td>
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<tr>
<td>Potential causes:</td>
</tr>
<tr>
<td>High protein intake</td>
</tr>
<tr>
<td>Dysbiosis (e.g., <em>Clostridia</em> species)</td>
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<tr>
<td>Mitochondrial dysfunction</td>
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<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Alpha-ketoglutarate (AKA) 300 mg bid</td>
</tr>
<tr>
<td>Yucca root: 250-500 mg bid</td>
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</tbody>
</table>
Diseases with MtD

- Parkinson’s disease
- Alzheimer’s disease
- Huntington’s disease
- Multiple Sclerosis
- Amyotrophic lateral sclerosis (ALS)
- Friedreich ataxia
- Rett syndrome
- Production of ROS correlates well with disease progression
Mitochondrial Dysfunction in Autistic Patients with 15q Inverted Duplication

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Mitochondrial dysfunction in autism spectrum disorders: a population-based study

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Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment

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Abstract: Classical mitochondrial diseases occur in a subset of individuals with autism and are usually caused by genetic anomalies or mitochondrial respiratory pathway deficits. However, in many cases of autism, there is evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial disease. MtD appears to be more common in autism and presents with less severe signs and symptoms. It is not associated with discernable mitochondrial pathology in muscle biopsy specimens despite objective evidence of lowered mitochondrial functioning. Exposure to environmental toxins is the likely etiology for MtD in autism. This dysfunction then contributes to a number of diagnostic symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. MtD and oxidative stress may also explain the high male to female ratio found in autism due to increased male vulnerability to these dysfunctions. Biomarkers for mitochondrial dysfunction have been identified, but seem widely under-utilized despite available therapeutic interventions. Nutritional supplementation to decrease oxidative stress along with factors to improve reduced glutathione, as well as hyperbaric oxygen therapy (HBOT) represent supported and rationale approaches. The underlying pathophysiology and autistic symptoms of affected individuals would be expected to either improve or cease worsening once effective treatment for MtD is implemented.
We will review information on alterations of structure of mitochondrial DNA and abnormal mitochondrial function in autism and indications that interactions of the nuclear and mitochondrial genomes may play a role in autism pathogenesis. We include data on two sets of monozygotic twins. Collectively these data provide additional evidence of nuclear and mitochondrial genome imbalance in autism and evidence of specific candidate genes in autism.
We report a family with a heterogeneous group of neurologic disorders associated with the mitochondrial DNA G8363A transfer ribonucleic acid (RNA)Lys mutation. The phenotype of one child in the family was consistent with autism. During his second year of life, he lost previously acquired language skills and developed marked hyperactivity with toe-walking, abnormal reciprocal social interaction, stereotyped mannerisms, restricted interests, self-injurious behavior, and seizures.

Graf et al., 2000  J Child Neurol 15(6):357-61
A 19-month-old girl was born after a normal full-term pregnancy. Her development was progressing well, with normal receptive and expressive language and use of prelinguistic gestures. Imaginary play and social reciprocity were typical for age. She used at least 20 words and could point to five body parts on command. Within 48 hours after immunizations to diphtheria, tetanus, and pertussis; *Haemophilus influenzae B*; measles, mumps, and rubella; polio; and varicella (Varivax), the patient developed a fever to 38.9 °C, inconsolable crying, irritability, and lethargy and refused to walk. Four days later, the patient was waking up multiple times in the night, and could no longer normally climb stairs. Instead, she crawled up and down the stairs.

Poling et al., 2006  J Child Neurol 21:170-2
The autistic group had decreased levels of phosphocreatine and esterified ends (alpha ATP + alpha ADP + dinucleotides + diphosphosugars) compared to the controls. This pilot study provides tentative evidence of alterations in brain energy and phospholipid metabolism in autism that correlate with the neuropsychologic and language deficits.

Minshew et al., 1993 Biol Psychiatry 33:762-73
Preliminary results show lower levels of NAA cerebellum in autistic children (p = 0.043). Lactate was detected in the frontal lobe in one autistic boy, but was not detected any of the other autistic subjects or siblings. Plasma lactate levels were higher in the 15 autistic children compared to 15 children with epilepsy (p = 0.0003).

Chugani et al., 1999  Prog Neuropsychopharmacol Biol Psychiatry 23(4):635-41
MtD: autism

- 343,718 children screened for ASD prevalence in Portugal
- 120 children with ASD identified
- 18 children had previously identified medical condition, no blood drawn
- Out of remaining 102, blood drawn for lactic acid in 69
- Lactic acid high in 14 (20.3%)
- Out of these 14, 11 received deltoid muscle biopsies
- 5/11 were +, had mitochondrial disease (5/69 = 7.2% of the children with ASD)

Oliveira et al., 2007  Dev Med Child Neuro 49:726-33
36/210 (17.2%) had elevated lactic acid levels. Out of these 36, 20 were assessed for MD, and 7/20 (35%) had MD (7/36 = 19.4%).
30 children with autism, 30 TD

Autism group had lower carnitine and higher lactate (both p<0.05)

77% had high lactate

Children with severe autism (on CARS) had lower carnitine and higher lactate compared to less severe (both p<0.05)

Negative correlation between carnitine and lactate
Mitochondrial Disease in Autism Spectrum Disorder Patients: A Cohort Analysis

- 25 children with initial ASD, later MD
- 64% developmental delay, 76% fatigue, 84% with GI problems (reflux, constipation)
- 32%: more than 5 std dev later in walking
- Lactate high in 76%, alanine in 36%, AST or ALT in 52%
- 40% with unusual regression: repeated regressions, loss of gross motor function, or regression after age 3
- Complex I deficiency in 64%, Complex III in 20%, 2 with rare mtDNA mutations

Weissman et al., 2008  PLoS ONE 3(11):e3815
• 12 children with **autism and hypotonia, seizures, and developmental delay**
• Other disorders ruled out.
• Measured mtDNA, ETC, mito structure
  – 5 children with mtDNA deletions
  – **7/8** children with Complex I, III, IV, or V def.
  – 3 out of 4 mito ultrastructural abnormalities

Filiano et al., 2002  J Child Neurol 17(6):435-9
ATPase: involved in cellular potential
Converts ATP to ADP

<table>
<thead>
<tr>
<th>Groups</th>
<th>HMG CoA reductase (ratio of HMG CoA/mevalonate)</th>
<th>Digoxin (ng/dl)</th>
<th>Dolichol (µg/dl)</th>
<th>Ubiquinone (µg/dl)</th>
<th>Na⁺-K⁺ ATPase (µg/p/mg protein)</th>
<th>Magnesium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>1.15 ± 0.12</td>
<td>12.80 ± 1.09</td>
<td>39.1 ± 2.36</td>
<td>144.2 ± 8.65</td>
<td>5.04 ± 0.221</td>
<td>2.40 ± 0.24</td>
</tr>
<tr>
<td>2. Autism</td>
<td>0.510 ± 0.06**</td>
<td>30.90 ± 1.41**</td>
<td>120.9 ± 9.65**</td>
<td>91.40 ± 5.92**</td>
<td>1.51 ± 0.142**</td>
<td>1.04 ± 0.12**</td>
</tr>
</tbody>
</table>

**p less than .01.
The results indicated that the GSH/GSSG redox ratio was decreased and percentage oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. Acute exposure to physiological levels of nitric oxide decreased mitochondrial membrane potential to a greater extent in the autism LCLs. These results suggest that the autism LCLs exhibit a reduced glutathione reserve capacity in both cytosol and mitochondria that may compromise antioxidant defense and detoxification capacity under prooxidant conditions.
Novel plasma phospholipid biomarkers of autism: Mitochondrial dysfunction as a putative causative mechanism

Élodie Pastural\textsuperscript{a}, Shawn Ritchie\textsuperscript{a}, Yingshen Lu\textsuperscript{a}, Wei Jin\textsuperscript{a}, Amir Kavianpour\textsuperscript{a}, Khine Khine Su-Myat\textsuperscript{a}, Doug Heath\textsuperscript{a}, Paul L. Wood\textsuperscript{a}, Maura Fisk\textsuperscript{b}, Dayan B. Goodenowe\textsuperscript{a,*}

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### Diagram 1: Peripheral Mitochondrial Stress

- **Peripheral mitochondrial stress**
  - Elevated liver synthesis of DHA/VLCFA
  - Transport to the brain
  - Microglial activation
  - Selective CNS neurodegeneration
  - Microglial activation

### Diagram 2: Increased Extracellular Glutamate

- **In neurons and astrocytes**
  - Increased extracellular glutamate
  - Increased mitochondrial \(\beta\)-oxidation
  - Decreased palmitate mitochondrial \(\beta\)-oxidation
  - Increased palmitate peroxisomal \(\beta\)-oxidation
  - Increased VLCFA, DHA, plasmalogen levels
  - Membrane oxidation
  - Decreased GSH, methionine and cysteine
  - Decreased mitochondrial turnover of glutathione
Chances of Mito Dysfunction in ASD?

- Epidemiological studies: 4-7% mito disease, 20% mito dysfunction
- If low GSH is a marker of MtD, then may be as high as 70%
- If you suspect mito dysfunction, and especially if lactic acid is elevated, 45-65% chance of mitochondrial defect upon muscle biopsy
Treatments: MtD

- CoEnzyme Q10: 5-10 mg/kg/day
- Idebenone: 45-360 mg/day
- Acetyl-L-Carnitine: 50-100 mg/kg/day
- L-Carnitine (Carnitor)
- Thiamine (B1): 15 mg/kg/day
- Pyridoxine (B6): 5-15 mg/kg/day
- Riboflavin (B2): 15 mg/kg/day
- Pantothenic acid (B5): 15 mg/kg/day
- Vitamin E: 15 IU/kg/day
Treatments: MtD

• Vitamin C: 25 mg/kg/day
• Alpha-lipoic acid: 15 mg/kg/day
• Vitamin K3: 5-80 mg/day
• Folate: 1-10 mg/day
• Creatine monohydrate: 5-10 g/day
• B12, selenium, succinate, Ginkgo biloba
• D-ribose: 0.5-1 gram bid
• Antioxidants
• Chelation / HBOT
Carnitine

- Co-factor that helps transport long chain fatty acids into mitochondria for beta-oxidation
- Antioxidant that neutralizes free radicals, including those produced by ETC
- Supplementation in high doses can lead to GI side effects
Values of **free and total carnitine** (p < 0.001), and pyruvate (p = 0.006) were significantly reduced while **ammonia and alanine** levels were considerably elevated (p < 0.001) in our autistic subjects. The relative carnitine deficiency in these patients, accompanied by slight elevations in lactate and significant elevations in alanine and ammonia levels, is **suggestive of mild mitochondrial dysfunction**. It is hypothesized that a mitochondrial defect may be the origin of the carnitine deficiency in these autistic children.

Filipek et al., 2004  J Autism Dev Disord 34(6):615-23
Compared with the Rett syndrome controls, treatment with L-carnitine led to significant improvements in sleep efficiency ($P=0.027$), especially in the subjects with a baseline sleep efficiency less than 90%, energy level ($P<0.005$) and communication skills ($P=0.004$). In addition, before and after comparisons of the treatment group showed improvements in expressive speech ($P=0.011$).
Efficacy of carnitine in the treatment of children with attention-deficit hyperactivity disorder

Randomized, double-blind, placebo-controlled double-crossover trial. In 13/24 boys receiving carnitine, **home behavior improved** as assessed with the CBCL total score (P<0.02). In 13/24 boys, **school behavior improved** as assessed with the Conners teacher-rating score (P<0.05). Treatment with carnitine significantly decreased the attention problems and aggressive behavior in boys with ADHD.
Mitochondrial biogenesis

• When energy needs of a cell are high, mitochondria divide
• Increased ROS triggers mtDNA proliferation; nDNA can also trigger increased mitochondrial division
• If mitochondrion cannot maintain ATP production, then undergoes apoptosis
• Cells normally remove old mitochondria (autophagy) and synthesize new mitochondria (biogenesis)
days. The finding that O$_2$ activates regional mitochondrial DNA transcription, replication, and mitochondrial biogenesis in the hippocampus may have important implications for maintaining neuronal viability after brain injury. © 2005 IBRO.
Hyperbaric oxygen and normobaric oxygen both attenuated brain injury, restored the levels of adenosine triphosphate and phosphocreatine, decreased the levels of the glycolytic intermediates, and increased the utilization of energy. These results suggest that oxygen treatment during the initial period of recovery from a hypoxia-ischemic insult is able to attenuate energy deficits in the brain, which ultimately leads to a reduction in brain injury.

Mito Websites

- www.mitosoc.org: diagnosis
- www.mitomap.org: mtDNA mutations
- www.umdf.org: United Mitochondrial Disease Foundation
- www.cdc.gov/ncbddd/autism/mitochondrial.htm