Secondary Mitochondrial Dysfunction in Neurodevelopmental Disorders: Origins, Significance and Treatment

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Disclaimer

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Mitochondrial Dysfunction
Mitochondrial Disease

- Relatively new field
- First diseases described in 1988
  - Wallace, Leber’s hereditary optic neuropathy, published in Science
  - Holt, Mitochondrial Myopathy, published in Nature
- Usually defined by extremely clinical symptoms with a progressive course
  - High energy dependent tissues
  - Neurological Disease
  - Gastrointestinal Disease
  - Immune Dysfunction
- Not just powerhouse, involved in
  - programmed (apoptotic) cell death
  - Oxygen Radical Regulation
Mitochondrial Dysfunction

a) ER-mitochondrion contact sites

- Ins(1,4,5)P3
- Ca2+
- VAPB
- ER-MEMES complex
- MAMs
- MFN2
- 10-50 nm
- Tenfold local increase in Ca2+
- PTPPI51

b) MCU complex

- OMM
- IMS
- MCUb
- MICU2
- MICU1
- EMRE
- MCU
- MCUR1
- Matrix

Scaffold for MCU assembly?
Regulation of ΔΨ?

ER

Mitochondrion

d) mNCX and mHCX

- OMM
- NCX3
- VDAC
- IMS
- NCLX
- 1 Ca2+
- 1 Ca2+
- LETM1
- IMMs
- 3 or 4 Na+

NCLX: electrogenic
LETM1: electroneutral

c) Respiratory complex

- OMM
- IMS
- H+
- H+
- IMM
- H+
- V
- H+
- H+
- IV
- ΔΨ = -150 mV to -180 mV
- Matrix

H+
Mitochondrial Dysfunction

Diagram showing the urea cycle and related pathways involving amino acids and intermediates like glutamine, ammonia, and ATP.
Mitochondrial Dysfunction

1. Antioxidants in mitochondria
   - ETC Complex I and III
   - \( \text{Cu/ZnSOD} \)
   - \( \text{MnSOD} \)
   - SIRT3
   - AC
   - \( \text{Gpx} \)
   - MCAT
   - GSH
   - GSSG
   - NAD\(^+\)
   - NADPH
   - Pentose Phosphate Pathway

2. SIRT3 FOXOs
   - Antioxidants
   - Protein deglutathionylation
   - UCP2
   - Mitochondria membrane potential decrease
   - ATP synthesis
   - Cytokine production
   - ROS
   - Harmful lipids removal
   - Post translational modification of mitochondrial proteins
   - mtDNA mutation
Now believed to that Mitochondrial Dysfunction is Important in Many Diseases
Primary Mitochondrial Disease and Secondary Mitochondrial Dysfunction: Importance of Distinction for Diagnosis and Treatment

Dmitriy M. Niyazov\textsuperscript{a}  Stephan G. Kahler\textsuperscript{b}  Richard E. Frye\textsuperscript{b}

![Mitochondrial Dysfunction Flowchart]

- Clinical diagnosis of mitochondrial dysfunction
  - Oxphos etiology
    - Oxphos gene defect
      - PMD
        - Treat mitochondrial dysfunction
    - Unknown etiology
      - Unknown gene defect
      - PMD or SMD
        - Reanalyze WES/WGS every year, collaborate with others, use resources such as ClinVar, NIH Rare Diseases program, PhenoDB, GeneMatcher, etc.
        - Treat mitochondrial dysfunction
  - Non-oxphos etiology
    - Non-oxphos gene defect
    - Non-oxphos enzyme defect
      - SMD
        - Treat specific non-oxphos disorder
        - Treat mitochondrial dysfunction

\textsuperscript{a} Barrow Neurological Institute, \textsuperscript{b} Phoenix Children's Hospital

Mol Syndromol
DOI: 10.1159/000446586
Mitochondrial Dysfunction in Autism
Discrepancy between prevalence of diagnosed mitochondrial disease and prevalence of biomarkers of mitochondrial disease likely be due to criteria used to define mitochondrial disease.

### Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

DA Rossignol and RE Frye

Mol Psych 2012, 17:290-314

<table>
<thead>
<tr>
<th>General ASD population</th>
<th>Studies</th>
<th>Total N</th>
<th>Overall prevalence</th>
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<tr>
<td>Mitochondrial disease in ASD</td>
<td>3</td>
<td>536</td>
<td>5.0% (3.2%, 6.9%)</td>
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<tr>
<td>Elevated lactate</td>
<td>6</td>
<td>479</td>
<td>31.1% (27.0%, 35.3%)</td>
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<td>Elevated pyruvate</td>
<td>2</td>
<td>110</td>
<td>13.6% (7.2%, 20.1%)</td>
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<td>Elevated lactate/pyruvate ratio</td>
<td>1</td>
<td>192</td>
<td>27.6% (21.2%, 33.9%)</td>
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<td>36</td>
<td>8.3% (0.0%, 20.1%)</td>
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<td>Low total carnitine</td>
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<td>90.0% (81.0%, 99.0%)</td>
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<td>Elevated creatine kinase</td>
<td>1</td>
<td>47</td>
<td>46.8% (32.4%, 61.2%)</td>
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<td>Elevated ammonia</td>
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<td>Elevated AST</td>
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<td>45.6% (37.5%, 53.7%)</td>
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<td>1</td>
<td>87</td>
<td>7.0% (0.5%, 13.5%)</td>
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### Biomarker Analysis

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<th>Biomarker</th>
<th>Number of studies</th>
<th>ASD</th>
<th>Control</th>
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<td>Mean (95% CI)</td>
<td>Total N</td>
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<td>Lactate (mM l⁻¹)</td>
<td>5</td>
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<td>1.73 (1.61, 1.88)</td>
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<td>0.12 (0.11, 0.14)</td>
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<td>Carnitine (mg ml⁻¹)</td>
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<td>30</td>
<td>3.83 (3.44, 4.31)</td>
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<tr>
<td>Ubiquinone</td>
<td>1</td>
<td>15</td>
<td>91.4 (81.9, 103.0)</td>
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</table>
Mitochondrial Dysfunction

Mitochondrial Dysfunction in Autism

- Lymphocytes from 10 children with autism and 10 age and gender matched controls
- 80% demonstrated abnormal function in at least one electron transport chain complex
  - 60% complex I abnormality
  - 40% complex V abnormality
  - 50% multiple complexes
- 20% demonstrated abnormalities in cytB, a mitochondrial DNA gene
ASD children with mitochondrial disease have more medical abnormalities than idiopathic ASD children.

Only 23% of ASD children with mitochondrial disease have mitochondrial DNA abnormalities.

<table>
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<tr>
<th></th>
<th>ASD/MD</th>
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<th>General MD</th>
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<td></td>
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<td>N</td>
<td>%</td>
<td>$\chi^2$</td>
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<td>Male</td>
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Mol Psych 2012, 17:290-314
## Autism Associated With the Mitochondrial DNA G8363A Transfer RNA<sup>Lys</sup> Mutation

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<td>•  Irritable bowel</td>
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<td>•  Cognitive regression</td>
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<td>•  Autism</td>
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<td>Percent in blood</td>
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<td>ND</td>
<td>ND</td>
<td>Normal</td>
<td>↑ Lipid</td>
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= absent; ++ = mild; +++ = moderate; +++ = severe; ND = not determined; ↑ = increased, ↓ = decreased; COX = citrate synthase corrected respiratory chain complex activity; COX– = absence of cytochrome c oxidase staining; PCR = polymerase chain reaction.

Autistic disorder with complex IV overactivity: A new mitochondrial syndrome


Richard E. Frye⁷, and Robert K. Naviaux⁸
Altered calcium homeostasis in autism-spectrum disorders: evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1
Bioenergetic variation is related to autism symptomatology

Leanna Delhey¹,², Ekim Nur Kilinc¹,², Li Yin³, John Slattery¹,², Marie Tippett¹,², Rebecca Wynne¹,², Shannon Rose¹,², Stephen Kahler¹,², Shirish Damle⁴, Agustin Legido⁴, Michael J. Goldenthal⁴, Richard E. Frye¹,²

Metab Brain Dis
DOI 10.1007/s11011-017-0087-0

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<th>Citrate Synthase</th>
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<td></td>
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<td>b</td>
<td>c</td>
</tr>
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<td>nmol/min/mg protein</td>
<td>% Citrate Synthase activity</td>
<td>% Citrate Synthase activity</td>
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Graphs a, b, and c show the relationship between Citrate Synthase, Complex I Activity, and Complex IV Activity, respectively, across different values.
Mitochondrial Dysfunction

(a) Communication Subscale (Scaled Score) vs. Complex IV Activity (nmol/min/mg protein)

(b) Daily Living Skills Subscale (Scaled Score) vs. Complex IV Activity (nmol/min/mg protein)

(c) Social Skills Subscale (Scaled Score) vs. Complex IV Activity (nmol/min/mg protein)

(d) Motor Skills Subscale (Scaled Score) vs. Complex IV Activity (nmol/min/mg protein)
Mitochondrial Dysfunction

- 95–99% of ASD do not have MD
- 1–5% of ASD have MD; 10–20% of MD have an ASD endophenotype

- Classical Autism: 1:250
- Definite Mitochondrial Disease: 1:4000 Children
- Probable Mitochondrial Disease: 1:2000
- Possible Mitochondrial Disease: 1:1000

Not Classical Mitochondrial Disease (MD): ≥ 198:200
Seahorse Bioscience Extracellular Flux Analyzer

- Poly D lysine coated plates
- 110k cells/well
- Plated 1hr prior to assay
- Seahorse DMEM
  - 11mM glucose
  - 2mM glutamax
  - 1mM pyruvate
- DMNQ added directly to cells in plate
- Each plate with an AD/Control LCL pair
Mitochondrial Dysfunction

![Diagram showing OCR (pMol/min) over time with markers for Basal Respiration, ATP-Linked Respiration, Proton Leak, Maximal Respiratory Capacity, Reserve Capacity, and Non-mitochondrial respiration. Key points include Oligomycin, FCCP, Antimycin A/Rotenone, and various phases of respiration and capacity.](Diagram Image)
Mitochondrial Dysfunction

A. ATP-linked OCR

B. Proton Leak OCR

C. Maximal OCR

D. Reserve Capacity

** indicates statistical significance.
Cluster analysis reveals 2 significantly different subgroups.

- AD-N (n=17)
- AD-A (n=8)
Mitochondrial Dysfunction

**Figure B**

Bar graph showing relative UCP2 content in AD-N and AD-A samples. The graph indicates a significant difference marked by an asterisk (*).

**Figure A**

A table with two columns labeled AD-A and AD-N, and rows labeled 1, 3, 4, 7, 25, 18, 20, 21, 22, and 23. The table is followed by a representative gel image showing UCP2 and total protein stain (band approx. 45kDa).
Mitochondrial and redox abnormalities in autism lymphoblastoid cells: a sibling control study

Shannon Rose, Sirish C. Bennuri, Rebecca Wynne, Stepan Melnyk, S. Jill James, and Richard E. Frye

[Graphs showing ATP-linked Respiration and Reserve Capacity]
Mitochondrial Dysfunction in Autism

Mechanisms of Molecular Dysregulation
Mitochondrial Dysfunction

Differentiate Typical Development from Autism

Differentiate ASD from Sibling Controls
↓↓ miR-320a (PTEN)

Typically Developing Sibling Control

Differentiate ASD from Both Control Groups
↑ C, ↓S miR-92a (PTEN, CamKinase II)
↓ miR-181a (PTEN, T-Cell Fx)
↓ miR-181b (PTEN, T-Cell Fx)
↑ miR-191 (PTEN, NEURL4)

ASD

Typically Developing Nonrelated Control

Differentiate ASD from Unrelated Controls
↑ miR-7a (IL6, IL10)
↓ miR-21 (IL1b, IL10)
↑ miR-26a (IL6, PTEN)
↓ miR-92a (PTEN, CamKinase II)
↑ miR-146b (IL6)

Regulation of Mitochondrial Function

ASD Abnormal Mitochondrial Function

Differentiate Mitochondrial Subgroups
↑↑ miR-30d (CELSR3, SCN2A)
↑↑ miR-181b (PTEN, T-Cell Fx)
↓ miR-874 (MSR3, FMR1, HIF1AN)

ASD Normal Mitochondrial Function
Mitochondrial Dysfunction in Autism

Effects of the Environment
Mitochondrial Dysfunction in Autism

**Mitochondrial Dysfunction**
- Environmental Toxicants
- Genetic Defects
- Increased Free Radicals

**AD-A Bioenergetic Response**
- ↑ ATP turnover
- ↑↑ UCP2
- ↑ Proton leak
- ↑ Maximal capacity
- ↑ Reserve capacity

**Maladaptive Response**
- High ATP Demand
  - ↑↑ ATP turnover
  - ↑↑ Proton leak
  - ↓↓ Reserve capacity

**Reserve capacity depleted**
- Damaged membranes
- Damaged ETC
- **Cell Death**

**AD-N Bioenergetic Response**
- Small ↑ Proton leak
- Small ↑ UCP2
- Small ↓ Reserve capacity

**Severe Oxidative Insult**
- High ATP Demand
  - ↑ ATP turnover
  - ↑ Proton leak
  - ↓ Reserve capacity

**De novo Production of Glutathione**
- ATP
- gamma-L-glutamyl-L-cysteine
- L-glutamate + L-cysteine
Mitochondrial Dysfunction in Autism

Prolonged 1uM DMNQ Exposure

Acute DMNQ Exposure 0, 5, 10, 15uM

Seahorse Assay

96 hrs

1 hr

(D)

Reserve for Controls

OCR (pmol/min)

0 5 10 15

DMNQ (uM)

No ROS

Prolonged ROS

(L)

Reserve for AD-A

OCR (pmol/min)

0 5 10 15

DMNQ (uM)

No ROS

Prolonged ROS

Prolonged 1uM DMNQ Exposure

Acute DMNQ Exposure 0, 5, 10, 15uM

Seahorse Assay

96 hrs

1 hr

(D)

Reserve for Controls

OCR (pmol/min)

0 5 10 15

DMNQ (uM)

No ROS

Prolonged ROS

(L)

Reserve for AD-A

OCR (pmol/min)

0 5 10 15

DMNQ (uM)

No ROS

Prolonged ROS
Mitochondrial Dysfunction in Autism

TCAH Exposure
0 mM, 0.1 mM, 0.5 mM, 1.0 mM

DMNQ Exposure
0 uM, 5 uM, 10 uM

Seahorse Assay

96 hrs / / 1 hr

(A) Reserve Capacity at DMNQ 0

(B) Reserve Capacity at DMNQ 5

(C) Reserve Capacity at DMNQ 10

% Change from Baseline OCR (pmol/min)

96 hr TCAH (mM)

Control - AD-N - AD-A

% Change from Baseline OCR (pmol/min)

96 hr TCAH (mM)

% Change from Baseline OCR (pmol/min)

96 hr TCAH (mM)

Control - AD-N - AD-A

* ** †
25% of Children with Autism also show abnormal Reserve Capacity

This pattern of abnormal Reserve Capacity is distinct from children with classic mitochondrial disease.
Changes in Mitochondrial Function in Childhood is Associated with Exposure to Air Pollution (PM2.5) during Gestation
Mitochondrial Dysfunction in Autism

Effects of the Gut Microbiome
**Mitochondrial Dysfunction**

**THE HUMAN MICROBIOME**

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.

- **600+ SPECIES** in the mouth, pharynx and respiratory system include:
  - Streptococcus viridans
  - Neisseria sicca
  - Candida albicans
  - Streptococcus salivarius

- **1,000 SPECIES** in the skin include:
  - Pityrosporum ovale
  - Staphylococcus epidermidis
  - Corynebacterium jeikeium
  - Trichosporon
  - Staphylococcus haemolyticus

- **500-1,000 SPECIES** in the intestines include:
  - Lactobacillus casei
  - Lactobacillus reuteri
  - Lactobacillus gasseri
  - Escherichia coli
  - Bacteroides fragilis
  - Bacteroides thetaiotaomicron
  - Lactobacillus rhamnosus
  - Clostridium difficile

- **25 SPECIES** in the stomach include:
  - Helicobacter pylori
  - Streptococcus thermophilus

- **60 SPECIES** in the urogenital tract include:
  - Ureaplasma parvum
  - Corynebacterium aurimucosum

Sources: National Institutes of Health, Scientific American, Human Microbiome Project.
The microbiota influences physiology by short chain fatty acids.
Mitochondrial Dysfunction

Passive/active uptake to gut and CNS (monocarboxylate transporters (fatty acids, ketones)

Fatty Acid G coupled protein receptor activation
Neurotransmitter synthesis (catecholamine, 5HT) and release
Increased intracellular calcium

Gut motility and inflammation
Malabsorption
Tight/Gap Junction impairment barrier dysfunction
(immune and enteric nervous system effects)

Short Chain Fatty Acid Bacterial Fermentation Products

Neuroinflammation/neurodevelopment
Cortical dysplasia

Gap Junction closure
Electrotonic coupling,
Neuronal Migration
Impaired synaptic pruning

Altered gene expression
(Histone deacetylase inhibition)
CREB activation (memory)
Epigenetic effects
(More pronounced at critical neurodevelopmental Windows)

Autism-like behavior
Repetitive, Antisocial, Object fixation,
Anxiety-like behavior, Perseveration
Seizure disorder, Dystonia, Tics,
Sensory processing

Mitochondria
Altered TCA cycle
Phospholipid alterations
Oxidative stress
Reduced glutathione, Carnitine deficiency
Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders

Derrick F. MacFabe, MD*
Mitochondrial Dysfunction

**Autistic Children**

- C18:2OH
- C18:1OH
- C18:1
- C18
- C16:1OH
- C16:1
- C16OH
- C16
- C14:2
- C14:1OH
- C14:1
- C14OH
- C14
- C12OH
- C12:1
- C12
- C10:1
- C10
- C8DC
- C8:1
- C8
- C6OH
- C6DC
- C6
- C5OH
- C5:1
- C5DC
- C5
- C4OH
- C4DC
- C4
- C3
- C2

**Upper Limit of Normal**

**Rodents**

- C18:1
- C18:0
- C17:0
- C16:1
- C16:0
- C15:0
- C14:2
- C14:1
- C14:0
- C13:0
- C12:0
- C9:0
- C8:0
- C6:0
- C5:0
- C4:0
- C3:0
- C2:0

- Propionic Acid
- Vehicle

* *p<=0.05*
Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder

RE Frye¹, S Melnyk¹ and DF MacFabe²

- 213 ASD patients screened with acyl-carnitine biomarkers
- 74 (35%) with >=3 fasting acyl-carnitine elevations
- Acyl-carnitine abnormalities were confirmed in 48%
- Corrected prevalence of 17% of ASD children screened.
Mitochondrial Dysfunction

![Mitochondrial Metabolism Diagram]

- Lactate → Glycolysis → Pyruvate → Fatty Acids → 3-hydroxy-3-methylglutaryl-CoA → Citrate Synthase → Citrate → Aconitate
- Malate Dehydrogenase → Malate → Fumarase → Fumarate → Succinyl-CoA Synthetase → Succinyl-CoA → Succinate
- Oxaloacetate → Citrate Synthase → Citrate → Aconitate
- NADH
- FADH
- Propionate-CoA Transferase → Propionate-CoA → Propionyl-CoA → Isoleucine, Valine, Odd-Chain Fatty Acids, α-Ketobutyric acid
- Biotin
- Methylmalonyl-CoA Mutase
- Methylmalonyl-CoA
- B12
Mitochondrial Dysfunction

Tricarboxylic Acid Cycle

- Lactate → Pyruvate
- Fatty Acids
- Acetyl-CoA
- Oxalacetate
- Citrate
- α-ketoglutarate
- Fumarate
- Succinate ← Succinyl-CoA
- NADH
- FADH₂

Electron Transport Chain

- Complex I
- Complex II
- Complex III
- Complex IV
- Complex V
Decreased Complex I Activity

(A) Muscle Electron Transport Chain Complex Activity

(B) Muscle Electron Transport Chain Complex Activity Corrected by Citrate Synthase Activity

(C) Muscle Electron Transport Chain Complex Activity Corrected by Maximal Complex Function
Modulation of mitochondrial function by the microbiome metabolite propionic acid in autism and control cell lines

RE Frye\textsuperscript{1,2}, S Rose\textsuperscript{1,2}, J Chacko\textsuperscript{1}, R Wynne\textsuperscript{1,2}, SC Bennuri\textsuperscript{1,2}, JC Slattery\textsuperscript{1,2}, M Tippett\textsuperscript{1,2}, L Delhey\textsuperscript{1,2}, S Melnyk\textsuperscript{1,2}, SG Kahler\textsuperscript{1,2} and DF MacFabe\textsuperscript{3}
Mitochondrial Dysfunction

**Graphs showing ATP Linked Respiration, Proton Leak Respiration, Maximal Reserve Capacity, and Reserve Capacity for 24hr and 48hr PPA (mM).**

- **Graph a:** ATP Linked Respiration for 24hr PPA (mM)
- **Graph b:** Proton Leak Respiration for 24hr PPA (mM)
- **Graph c:** Maximal Reserve Capacity for 24hr PPA (mM)
- **Graph d:** Reserve Capacity for 24hr PPA (mM)
- **Graph e:** ATP Linked Respiration for 48hr PPA (mM)
- **Graph f:** Proton Leak Respiration for 48hr PPA (mM)
- **Graph g:** Maximal Reserve Capacity for 48hr PPA (mM)
- **Graph h:** Reserve Capacity for 48hr PPA (mM)
Mitochondrial Dysfunction
Butyrate enhances mitochondrial function during oxidative stress in cell lines from boys with autism

Mitochondrial Dysfunction

Predominant Fuel

(A) Fatty Acids
- FADH₂ → Complex II → Complex III → Complex IV → Complex V → ADP → ATP
- Glucose
- NADH → Complex I

(B) Glucose
- Butyrate
- Pyruvate
- Glycolysis
- Acetyl-CoA
- Oxaloacetate
- Citrate Synthase → Citrate
- Aconitase
- Isocitrate → Isocitrate Dehydrogenase
- α-ketoglutarate
- Succinic Dehydrogenase
- Succinate
- Fumarase
- Fumarate
- Malate Dehydrogenase
- Malate
- Succinyl-CoA Synthetase
- Succinyl-CoA
- Propionate-CoA Transferase
- Propionyl-CoA
- Propionyl-CoA Carboxylase
- Methylmalonyl-CoA Mutase
- B12

Fatty Acid Oxidation

Fatty Acids
Mitochondrial Dysfunction

(A) ATP Linked Respiration
(B) Proton Leak Respiration
(C) Maximum Resp Capacity
(D) Reserve Capacity

* p≤0.05
** p≤0.01
*** p≤0.001
**** p≤0.0001
Mitochondrial Dysfunction

(A) ATP Linked Respiration
(B) Proton Leak Respiration
(C) Maximal Reserve Capacity
(D) Reserve Capacity

(E) ATP Linked Respiration
(F) Proton Leak Respiration
(G) Maximal Reserve Capacity
(H) Reserve Capacity

24hr BUT (mM)
48hr BUT (mM)
Mitochondrial Dysfunction

(A) ATP Linked Respiration
(B) Proton Leak Respiration
(C) Maximal Reserve Capacity
(D) Reserve Capacity

(E) ATP Linked Respiration
(F) Proton Leak Respiration
(G) Maximal Reserve Capacity
(H) Reserve Capacity

24hr BUT (mM)

48hr BUT (mM)
Mitochondrial Dysfunction

**Graphs Showing Gene Expression Changes**

- **MFN2 (Fusion)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p<0.001

- **OPA1 (Fusion)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p=0.001

- **DRP1 (Fission)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p<0.001

- **FIS1 (Fission)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p<0.001

- **MFF (Fission)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration

- **BINP3 (Apoptosis)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration

- **PINK1 (Mitophagy)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p<0.001

- **PTEN (Mitophagy)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p<0.001

- **LC3 (Mitophagy)**: 0 mM, 0.1 mM, 1.0 mM Butyrate Concentration
  - p<0.001
Mitochondrial Dysfunction

**UCP2 (Oxidative Stress)**
- p<0.001

**SOD2 (Oxidative Stress)**
- p<0.001

**NRF2 (Oxidative Stress)**
- p<0.001

**ANT2 (Oxidative Stress)**

**mTOR (Metabolic Reg)**
- p<0.001

**AMPK (Metabolic Reg)**
- p<0.001

**SIRT1 (Metabolic Reg)**

**SIRT3 (Metabolic Reg)**
- p<0.001

**HIF1α (Cellular Stress)**
- p<0.001

**PGC1α (Cellular Stress)**
- p<0.001

**CREB1 (Behavior)**
- p<0.001

**CamKinase II (Behavior)**
- p<0.001
Mitochondrial dysfunction in the gastrointestinal mucosa of children with autism: A blinded case-control study

Shannon Rose¹, Sirish C. Bennuri¹, Katherine F. Murray², Timothy Buie³, Harland Winter², Richard Eugene Frye¹*

¹ Autism Research Program, Arkansas Children's Research Institute, Little Rock, Arkansas, United States of America, ² Department of Pediatric Gastroenterology and Nutrition, MassGeneral Hospital for Children, Boston, Massachusetts, United States of America, ³ Department of Gastroenterology, Boston Children's Hospital, Boston, Massachusetts, United States of America

* REFrye@uams.edu
Mitochondrial Dysfunction

(A) Healthy Colon

- Dysbiotic Bacteria
- Commensal Bacteria

- Immune Modulation
- Vitamin Production
- Metabolic Regulation

(B) Autism Colon

- Inflammation
- Oxidative Stress
- Mitochondrial Dysfunction

Biopsy
Cecum
Rectum
Sigmoid Colon
Descending Colon
Transverse Colon
Ascending Colon
Microbiome
Mitochondrial Dysfunction

- Dysbiotic Bacteria
- Commensal Bacteria
- Xenobiotic Agent

Autism Colon

- Transverse Colon
- Descending Colon
- Cecum
- Sigmoid Colon
- Rectum

- Mitochondrial Dysfunction
- Oxidative Stress
- Buytrate

- Complex I, III, IV, V

Symptoms
Examples of Genetic Disorder
Examples of Genetic Disorder

Down Syndrome
Mitochondrial Dysfunction

Shamim I. Ahmad, Editor

Neurodegenerative Diseases

OXIDATIVE STRESS AND MITOCOCHONDRIAL DYSFUNCTION IN DOWN SYNDROME

Giovanni Pagano* and Giuseppe Castello
CROM, Cancer Research Center, Mercogliano, Italy
*Corresponding Author: Giovanni Pagano—Email: gbpagano@tin.it
## Mitochondrial Dysfunction

### Table 1. Reported changes in oxidative stress parameters in cells, tissues or body fluids from DS patients

<table>
<thead>
<tr>
<th>Cells/Tissues/Body Fluids</th>
<th>Endpoints</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foetal Brain</td>
<td>↑ SOD-1; ↔ glutathione peroxidase (GPx); ↑ MDA</td>
<td>5</td>
</tr>
<tr>
<td>Brain</td>
<td>↑ reactive carbonyls and carbonyl reductase;</td>
<td>9</td>
</tr>
<tr>
<td>Amniotic Fluid</td>
<td>↑↑ Isoprostanes</td>
<td>10</td>
</tr>
<tr>
<td>Erythrocytes and Neutrophils</td>
<td>↑↑ ratio SOD-1:(GPx + CAT); ↑ MDA and lipofuscin</td>
<td>12-16</td>
</tr>
<tr>
<td>Leukocytes, Whole Blood and Plasma</td>
<td>Age-dependent ↑ 8-hydroxy-2′-deoxyguanosine</td>
<td>17-18</td>
</tr>
<tr>
<td>Plasma</td>
<td>Age-related ↑↓ GSSG:GSH; ↑ Plasma Glx levels in young patients; ↑ Plasma uric acid and ascorbic acid; ↔ Vitamin E</td>
<td>19</td>
</tr>
<tr>
<td>Plasma and Urine</td>
<td>↑ Uric acid and allantoin; ↓ hypoxanthine and xanthine</td>
<td>19</td>
</tr>
<tr>
<td>Plasma</td>
<td>↓ Plasma melatonin and urinary kynurenine; urinary kynurenic acid and anthranilic acid</td>
<td>20</td>
</tr>
<tr>
<td>Plasma</td>
<td>↑ Citrulline:arginine and neopterin in demented patients; ↑ NO production</td>
<td>21</td>
</tr>
<tr>
<td>Serum</td>
<td>↑ Uric acid</td>
<td>22</td>
</tr>
<tr>
<td>Urine</td>
<td>↑ 8-OHdG and MDA</td>
<td>23</td>
</tr>
<tr>
<td>Urine</td>
<td>↑ Isoprostane 8,12-iso-iPF2alpha-VI</td>
<td>24-25</td>
</tr>
<tr>
<td>Amniotic Fluid</td>
<td>Dysregulation of oxidative stress response genes; phopholipids, ion transport molecules, heart, muscle, structural proteins, and DNA damage repair genes</td>
<td>25</td>
</tr>
</tbody>
</table>
### Table 2. Main mitochondrial anomalies/dysfunctions reported in cells from DS patients or from trisomy 16 mice

<table>
<thead>
<tr>
<th>Cells/Organisms</th>
<th>Endpoints</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets from DS patients</td>
<td>↓ Monoamine oxidase, cytochrome oxidase and isocitrate dehydrogenase</td>
<td>39</td>
</tr>
<tr>
<td>Trisomy 16 cerebellar neurons</td>
<td>↑ Levels of microtubules, abnormally shaped mitochondria and dense bundles of abnormal filaments</td>
<td>40</td>
</tr>
<tr>
<td>Brain of mouse trisomy 16</td>
<td>↑ $O_2^-$ formation; ↓ respiration with the Complex I substrates malate and glutamate but not with the Complex II substrate succinate; ↓ the 20 kDa subunit of Complex I; ↓ pyruvate dehydrogenase levels</td>
<td>41-43</td>
</tr>
<tr>
<td>Astrocyte and neuronal cultures from foetal DS brain</td>
<td>Alterations in the processing of amyloid beta precursor protein (AbetaPP); impaired mitochondrial function in DS astrocytes</td>
<td>44</td>
</tr>
<tr>
<td>Fibroblasts from DS patients</td>
<td>Impaired repair of oxidative damage to mtDNA</td>
<td>45</td>
</tr>
<tr>
<td>Heart of DS fetuses</td>
<td>Oligonucleotide microarrays: downregulation of genes encoding mitochondrial enzymes and upregulation of genes encoding extracellular matrix proteins</td>
<td>46</td>
</tr>
<tr>
<td>PBMC from DS children</td>
<td>↑ Lucigenin-derived chemiluminescence; ↓ $\Delta\Psi(m)$</td>
<td>47</td>
</tr>
</tbody>
</table>
Mitochondrial Dysfunction
Mitochondrial Dysfunction

Genome-wide expression studies in Autism spectrum disorder, Rett syndrome, and Down syndrome

Carla Lintas, Roberto Sacco, Antonio M. Persico *

Laboratory of Molecular Psychiatry and Neurogenetics, University “Campus Bio-Medico”, Rome, Italy
Department of Experimental Neurosciences, I.R.C.C.S. “Fondazione Santa Lucia”, Rome, Italy

**DOWN SYNDROME**

- Overexpression of trisomic genes
- Dysregulation of some non-trisomic genes

**AUTISM**

- Dysreactive immune process and microglial activation
- Enhanced intracellular Ca²⁺ spikes
- Abnormal energy metabolism and ROS production
- Prenatally: altered cell proliferation and migration
- Abnormal neuronal wiring
- Functional dysconnection in association cortices
- Lack of integration in information processing

**RETT SYNDROME**

- MECP2 mutation
- Postnatally: mitochondrial dysfunction and oxidative stress
- Abnormal synaptic functioning
- Dendritic damage

---

*Neurobiology of Disease 45 (2012) 57–68*
Examples of Genetic Disorder

Phelan-McDermid Syndrome
Mitochondrial Dysfunction may explain symptom variation in Phelan-McDermid Syndrome

Richard E. Frye, Devin Cox, John Slattery, Marie Tippett, Stephen Kahler, Doreen Granpeesheh, Shirish Damle, Agustin Legido & Michael J. Goldenthal

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Enzyme Name and Function</th>
<th>Disease Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO2</td>
<td>22q13.2</td>
<td>• Mitochondrial aconitase</td>
<td>• infantile cerebellar-retinal degeneration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Second enzymes in the tricarboxylic acid cycle</td>
<td></td>
</tr>
<tr>
<td>NDUFA6</td>
<td>22q13.2</td>
<td>• Nicotinamide adenine dinucleotide-ubiquinone oxidoreductase 1 alpha subcomplex 6</td>
<td>• Subunits of electron transport chain complex 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Subunits of electron transport chain complex 1</td>
<td></td>
</tr>
<tr>
<td>TRMU</td>
<td>22q13.31</td>
<td>• Transfer ribonucleic acid 5-methylaminomethyl-2-thioimidylate methyltransferase</td>
<td>• Aminoglycoside-induced and nonsyndromic deafness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Modification of mitochondrial transfer ribonucleic acid</td>
<td>• Acute infantile liver failure</td>
</tr>
<tr>
<td>SCO2</td>
<td>22q13.33</td>
<td>• Homolog of S. Cerevisiae</td>
<td>• Fatal infantile cardioencephalomyopathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Assembly of electron transport chain complex IV</td>
<td>• Spontaneous abortion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cytochrome c oxidase deficiency</td>
<td>• Autism</td>
</tr>
<tr>
<td>TYMP</td>
<td>22q13.33</td>
<td>• Thymidine phosphorylase</td>
<td>• Mitochondrial deoxyribonucleic acid depletion syndrome-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Deoxynucleotide metabolism</td>
<td>• Mitochondrial neurogastrointestinal encephalopathy</td>
</tr>
<tr>
<td>CPT1B</td>
<td>22q13.33</td>
<td>• Mitochondrial carnitine palmitoyltransfer</td>
<td>• Heterozygous deletions can result in embryonic death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Transports long-chain fatty acyl-CoA from the cytoplasm into the mitochondrial</td>
<td>• or fatality after cold-challenge in mice</td>
</tr>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Mitochondrial Dysfunction may explain symptom variation in Phelan-McDermid Syndrome

Richard E. Frye\textsuperscript{1}, Devin Cox\textsuperscript{2}, John Slattery\textsuperscript{1}, Marie Tippett\textsuperscript{1}, Stephen Kahler\textsuperscript{1}, Doreen Granpeesheh\textsuperscript{3}, Shirish Damle\textsuperscript{4}, Agustin Legido\textsuperscript{4} & Michael J. Goldenthal\textsuperscript{4}
Mitochondrial Dysfunction may explain symptom variation in Phelan-McDermid Syndrome

Richard E. Frye¹, Devin Cox², John Slattery¹, Marie Tippett¹, Stephen Kahler¹, Doreen Granpeesheh³, Shirish Damle⁴, Agustin Legido⁴ & Michael J. Goldenthal⁴

<table>
<thead>
<tr>
<th>Development</th>
<th>Complex I Underactivity</th>
<th>Complex I Overactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD</td>
<td>44% (4/9)</td>
<td>75% (3/4)</td>
</tr>
<tr>
<td>Regression</td>
<td>63% (5/8)</td>
<td>100% (4/4)</td>
</tr>
<tr>
<td>Loss of Language</td>
<td>80% (4/5)</td>
<td>75% (3/4)</td>
</tr>
<tr>
<td>Loss of Social Skills</td>
<td>40% (2/5)</td>
<td>100% (4/4)</td>
</tr>
<tr>
<td>Loss of Gross Motor Skills</td>
<td>60% (3/5)</td>
<td>0% (0/4)</td>
</tr>
<tr>
<td>Loss of Fine Motor Skills</td>
<td>80% (4/5)</td>
<td>25% (1/4)</td>
</tr>
<tr>
<td>Proximal Trigger</td>
<td>80% (4/5)</td>
<td>0% (0/4)</td>
</tr>
<tr>
<td>ASD Regression Typical Age</td>
<td>20% (1/5)</td>
<td>75% (3/4)</td>
</tr>
<tr>
<td>Mean (SD) Age at Regression</td>
<td>63 m (29 m)</td>
<td>27 m (16 m)</td>
</tr>
<tr>
<td>Multiple Regressions</td>
<td>60% (3/5)</td>
<td>25% (1/4)</td>
</tr>
</tbody>
</table>
Examples of Genetic Disorder

WDR45
De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood.
Mitochondrial Dysfunction

Potential Treatments
**Table 3. Agents commonly used to treat PMD and SMD**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Dose</th>
<th>Adverse effects</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron transport chain support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoQ10 (reduced): ubiquinol</td>
<td>5–30 mg/kg/day, 1–2x/day</td>
<td>appetite loss, nausea, diarrhea at high doses</td>
<td>energy carrier between complex I and III, and complex II and III</td>
</tr>
<tr>
<td>CoQ10 (oxidized): ubiquinone</td>
<td>10–30 mg/kg/day, 1–2x/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electron carrier support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacin (B3)</td>
<td>50–100 mg given daily</td>
<td>flushing reaction</td>
<td>nicotinamide adenine dinucleotide (NAD) precursor</td>
</tr>
<tr>
<td>Riboflavin (B2)</td>
<td>100–400 mg given daily</td>
<td>nausea at high doses</td>
<td>flavin adenine dinucleotide (FAD) precursor</td>
</tr>
<tr>
<td>Energy Storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine monohydrate</td>
<td>100 mg/kg/day; 1–2x/day</td>
<td>increased urination</td>
<td>high-energy phosphate buffer precursor to phosphocreatine</td>
</tr>
<tr>
<td>Fatty acid oxidation support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-carnitine or acetyl-L-carnitine</td>
<td>30–120 mg/kg/day, 1–2x/day</td>
<td>stool loose/fishy smell</td>
<td>carrier of long-chain fatty acids</td>
</tr>
<tr>
<td>Biotin (B7)</td>
<td>5–10 mg/day given daily</td>
<td>none</td>
<td>cofactor for carboxylase enzymes</td>
</tr>
<tr>
<td>Mitochondrial enzyme cofactors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine (B1)</td>
<td>50–100 mg given daily</td>
<td>none</td>
<td>cofactor for citric acid cycle enzymes</td>
</tr>
<tr>
<td>Pantothenic acid (B5)</td>
<td>5–1,200 mg/day, 1–3x/day</td>
<td>diarrhea at high doses</td>
<td>precursor to coenzyme A</td>
</tr>
<tr>
<td>Pyridoxine (B6)</td>
<td>200 mg given daily</td>
<td>headache, paresthesia, nausea, headache at high doses</td>
<td>cofactor for over 100 enzymes</td>
</tr>
<tr>
<td>Biotin (B7)</td>
<td>as above</td>
<td>none</td>
<td>cofactor for carboxylase enzymes</td>
</tr>
<tr>
<td>Alpha-lipoic acid</td>
<td>50–200 mg given daily</td>
<td>headache, paresthesia, rash, muscle cramps</td>
<td>cofactor for citric acid cycle enzymes</td>
</tr>
<tr>
<td>Antioxidants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoQ10</td>
<td>as above</td>
<td>as above</td>
<td>targets ETC oxidative stress</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>as above</td>
<td>as above</td>
<td>scavenger of organic acids</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>200–400 IU given daily</td>
<td>bleeding at high doses</td>
<td>protects cell membranes</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>100–500 mg given daily</td>
<td>diarrhea at high doses</td>
<td>protects iron and copper</td>
</tr>
<tr>
<td>Redox metabolism support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylcobalamin (B12)</td>
<td>5–2,000 µg every 1–3 days</td>
<td>hyperactivity, sleep disruption</td>
<td>supports methylation and folate cycles, and glutathione production</td>
</tr>
<tr>
<td>Reduced folate (B9)</td>
<td>folinic acid 400–800 µg/day</td>
<td>none</td>
<td>supports methylation and folate cycles</td>
</tr>
<tr>
<td>N-acetyl-L-cysteine (NAC)</td>
<td>10–70 mg/kg/day, 1–3x/day</td>
<td>diarrhea at high doses</td>
<td>precursor to glutathione</td>
</tr>
<tr>
<td>Zinc</td>
<td>10–40 mg daily</td>
<td>suppresses iron and copper absorption</td>
<td>supports superoxide dismutase</td>
</tr>
<tr>
<td>Central folate support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folinic acid/leucovorin calcium (B9)</td>
<td>0.5–4 mg/kg/day, 1–3x/day</td>
<td>hyperactivity</td>
<td>supports adequate folate levels in the brain</td>
</tr>
</tbody>
</table>
The Effect of Mitochondrial Supplements on Mitochondrial Activity in Children with Autism Spectrum Disorder

Leanna M. Delhey ¹,², Ekim Nur Kilinc ¹, Li Yin ³, John C. Slattery ¹,², Marie L. Tippett ¹,², Shannon Rose ¹,², Sirish C. Bennuri ¹,², Stephen G. Kahler ¹,², Shirish Damle ⁴, Agustin Legido ⁴, Michael J. Goldenthal ⁴ and Richard E. Frye ¹,²,*

Table 3. Means (Standard Error) of Normalized Complex I activity on and off supplements by Mitochondrial Disease group. Supplements that are confirmed to be significant in the stepwise regression are bolded and italicized.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>No Mitochondrial Disease</th>
<th>Mitochondrial Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Off Supplement</td>
<td>On Supplement</td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>0.1 (0.20)</td>
<td>1.2 (0.48)</td>
</tr>
<tr>
<td>Folate</td>
<td>0.2 (0.21)</td>
<td>0.7 (0.5)</td>
</tr>
</tbody>
</table>
The Effect of Mitochondrial Supplements on Mitochondrial Activity in Children with Autism Spectrum Disorder


Table 4. Means (Standard Error) of Normalized Citrate Synthase activity on and off supplements by Mitochondrial Disease group. Supplements that are confirmed to be significant in the stepwise regression are bolded and italicized.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>No Mitochondrial Disease</th>
<th>Mitochondrial Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Off Supplement</td>
<td>On Supplement</td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>0.8 (0.17)</td>
<td>1.2 (0.40)</td>
</tr>
<tr>
<td>Folate</td>
<td>0.9 (0.18)</td>
<td>0.8 (0.40)</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>0.9 (0.17)</td>
<td>0.8 (0.42)</td>
</tr>
</tbody>
</table>
The Effect of Mitochondrial Supplements on Mitochondrial Activity in Children with Autism Spectrum Disorder

Leanna M. Delhey¹,², Ekim Nur Kilinc¹, Li Yin³, John C. Slattery¹,², Marie L. Tippett¹,², Shannon Rose¹,², Sirish C. Bennuri¹,², Stephen G. Kahler¹,², Shirish Damle⁴, Agustin Legido⁴, Michael J. Goldenthal⁴ and Richard E. Frye¹,²,*

![Graph showing the relationship between Complex I and IV activity](image)

**Figure 1.** The relationship between Normalized Complex I and IV activity. Folate supplementation is associated with a significantly greater slope in the relationship between complex activities.
The Effect of Mitochondrial Supplements on Mitochondrial Activity in Children with Autism Spectrum Disorder

Leanna M. Delhey¹,², Ekim Nur Kilinc¹, Li Yin³, John C. Slattery¹,², Marie L. Tippett¹,², Shannon Rose¹,², Sirish C. Bennuri¹,², Stephen G. Kahler¹,², Shirish Damle⁴, Agustin Legido⁴, Michael J. Goldenthal⁴ and Richard E. Frye¹,²,*

Figure 2. The relationship between normalized Complex I and Citrate Synthase activity. (a) Folate and (b) B12 supplementation are associated with a significantly greater slope in the relationship between Complex I and Citrate Synthase.
Effect of a Combination of Carnitine, Coenzyme Q10 and Alpha-Lipoic Acid (MitoCocktail) on Mitochondrial Function and Neurobehavioral Performance in Children with Autism Spectrum Disorder


Objective: To determine if patients with ASD and mt dysfunction would improve clinically and/or biochemically on a combination of carnitine, coenzyme Q10 and alpha-lipoic acid (MitoCocktail) in an open-label pilot trial with a baseline-treatment-baseline design.

Results: Mean buccal complex I/IV activity ratio was significantly (p<0.02) reduced during MitoCocktail treatment compared to baseline. All subjects showed at least one specific sign of metabolic improvement, which waned 3 months post-treatment in 7 of the 11 participants. Of the 11 total or subscale scores considered, all showed change in means from Time 1 to Time 2. Statistically significant changes were observed for the Unusual Behavior subscale from the ASRS (p<0.006), the Lethargy subscale from the ABC (p<0.01), and the Inappropriate Speech subscale from the ABC (p<0.02). From Time 2 to Time 3, scores worsened on each of these three subscales with statistically significant changes on Lethargy (p<0.01) and Inappropriate Speech subscales (p<0.007).
Effect of a vitamin/mineral supplement on children and adults with autism

James B Adams¹*, Tapan Audhya², Sharon McDonough-Means³, Robert A Rubin⁴, David Quig⁵, Elizabeth Geis¹, Eva Gehn¹, Melissa Loresto¹, Jessica Mitchell⁶, Sharon Atwood¹, Suzanne Barnhouse¹ and Wondra Lee¹
Mitochondrial Dysfunction in Autism

The graph shows the percent levels of various molecules in the mitochondria, comparing control, pre-treatment, and post-treatment groups.

- **ATP**: The control group is around 90%, the pre-treatment group is slightly higher, and the post-treatment group shows a significant increase.
- **NADH**: Similar trends are observed with NADH, with the post-treatment group showing a notable increase.
- **NADPH**: The post-treatment group also shows a significant rise in NADPH.
- **CoQ10**: The control and pre-treatment groups show relatively lower levels, but the post-treatment group experiences a dramatic increase.

Significance levels are indicated by asterisks: ** *** denotes a statistically significant difference.
Mitochondrial Dysfunction in Autism

Parental Global Impressions - Revised

Charts showing comparisons between Placebo and Supplement groups across various domains such as Expressive Language, Rec. Language, Play, Cognition, GI, Sleep, Sociability, Hyperactivity, Tantruming, Eye Contact, Overall, and Average.
Green tea EGCG plus fish oil omega-3 dietary supplements rescue mitochondrial dysfunctions and are safe in a Down's syndrome child.
Treatments for biomedical abnormalities associated with autism spectrum disorder

(Frye and Rossignol, 2014)
Identification and Treatment of Pathophysiological Comorbidities of Autism Spectrum Disorder to Achieve Optimal Outcomes

Richard E. Frye¹,² and Daniel A. Rossignol³

¹Arkansas Children’s Research Institute, Little Rock, AR, USA. ²Division of Neurology, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA. ³Rossignol Medical Center, Irvine CA, USA.

ABSTRACT: Despite the fact that the prevalence of autism spectrum disorder (ASD) continues to rise, no effective medical treatments have become standard of care. In this paper we review some of the pathophysiological abnormalities associated with ASD and their potential associated treatments. Overall, there is evidence for some children with ASD being affected by seizure and epilepsy, neurotransmitter dysfunction, sleep disorders, metabolic abnormalities, including abnormalities in folate, cobalamin, tetrahydrobiopterin, carnitine, redox and mitochondrial metabolism, and immune and gastrointestinal disorders. Although evidence for an association between these pathophysiological abnormalities and ASD exists, the exact relationship to the etiology of ASD and its associated symptoms remains to be further defined in many cases. Despite these limitations, treatments targeting some of these pathophysiological abnormalities have been studied in some cases with high-quality studies, whereas treatments for other pathophysiological abnormalities have not been well studied in many cases. There are some areas of more promising treatments specific for ASD including neurotransmitter abnormalities, particularly imbalances in glutamate and acetylcholine, sleep onset disorder (with behavioral therapy and melatonin), and metabolic abnormalities in folate, cobalamin, tetrahydrobiopterin, carnitine, and redox pathways. There is some evidence for treatments of epilepsy and seizures, mitochondrial and immune disorders, and gastrointestinal abnormalities, particularly imbalances in the enteric microbiome, but further clinical studies are needed in these areas to better define treatments specific to children with ASD. Clearly, there are some promising areas of ASD research that could lead to novel treatments that could become standard of care in the future, but more research is needed to better define subgroups of children with ASD who are affected by specific pathophysiological abnormalities and the optimal treatments for these abnormalities.

KEYWORDS: autism spectrum disorders, carnitine, cobalamin, epilepsy, folate, genetic disorders, mitochondrial dysfunction, review
Questions