Mitochondrial Dysfunction in ASD: Diagnosis, Analysis and Treatment

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While Dr. Rossignol has attempted to make the information in this presentation as accurate as possible, the information is provided without any express or implied warranty. The purpose of this lecture is to provide information about different conditions or treatments that affect individuals with autism and other conditions. Please be advised that Dr. Rossignol is not giving medical advice and that circumstances may dictate different treatments. If the issues that are discussed in this lecture affect you or your loved ones, seek professional advice. All of the reviewed treatments in this lecture are considered off-label and not FDA-approved. Before beginning any treatment, please consult with your or your child's physician.

Metabolic Abnormalities: ASD

- Cerebral Folate Deficiency
- Mitochondrial Dysfunction
- Oxidative stress
- Impaired methylation / sulphation
- Inflammation
- Seizures
- Hypothyroidism: ASD and ADHD
- Deficiencies: iron (ASD and ADHD)

Mitochondria

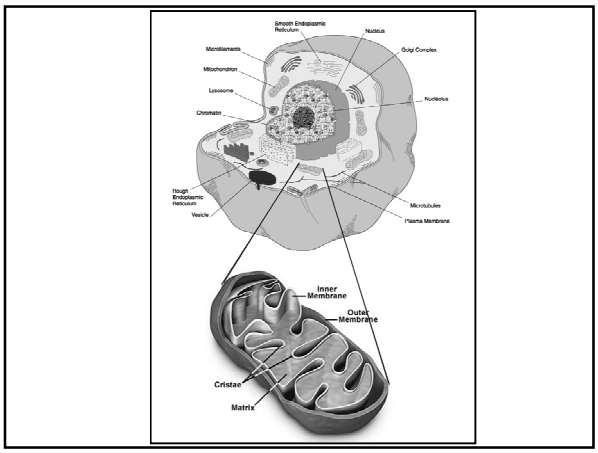
- ? Descendent of an ancestral purple, non-sulfur, photosynthetic bacteria
- Found in every cell, generate ATP (energy)
- Has its own DNA (genome)
- Many cells contain 500 to 2000 mitochondria
- Play a role in programmed cell death
- Mitochondria are the primary source of free radicals (reactive oxygen species, ROS) by electron leakage from the electron transport chain; 1-2% of oxygen normally produces free radicals

Mitochondria

- Build, break down, and recycle the cell's molecular building blocks
- Necessary for RNA and DNA synthesis
- Contain rate-limiting enzymes for synthesizing heme
- Detoxify ammonia in the liver
- Involved in cholesterol and neurotransmitter metabolism and estrogen and testosterone synthesis
- Oxidize fat, protein and carbohydrates

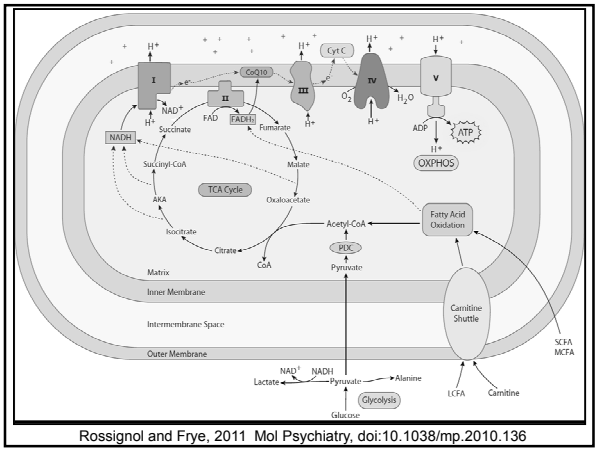
Mitochondria

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



Electron Transport Chain (ETC)

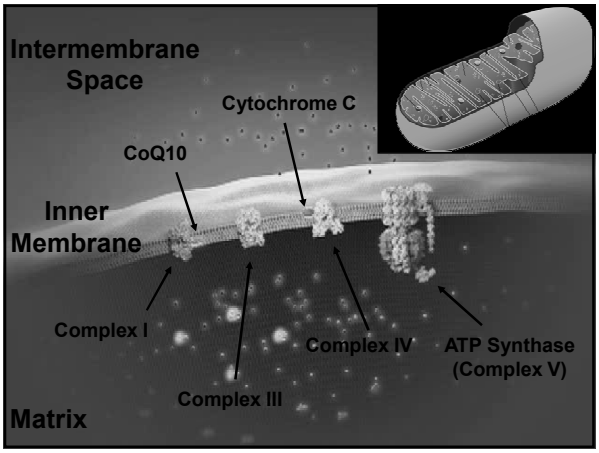
- Five complexes, located in inner mitochondrial membrane
- Mitochondrial oxidative phosphorylation occurs here; 2 main functions:
  - Generates ATP from electrons (from food) through ATP synthase (complex V)
  - Generates heat
- Electrons that leak out of the ETC produce free radicals



Rossignol and Frye, 2011 Mol Psychiatry, doi:10.1038/mp.2010.136

ETC

- If the ETC is damaged or inhibited, then free radical production may be increased
- ETC can be blocked or impaired ("leaky"):
  - Genetic defects in nDNA and mtDNA
  - Toxins (e.g., cyanide)
  - Medications



Mitochondrial Disease (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
- Some children have mitochondrial dysfunction (MtD) that does not reach the level of MD

Diseases/Disorders with MtD

- Parkinson's disease
- Alzheimer's disease
- Huntington's disease
- Multiple Sclerosis
- Amyotrophic lateral sclerosis (ALS)
- Friedreich ataxia
- Rett syndrome / ASD
- Aging
- Production of ROS correlates well with disease progression

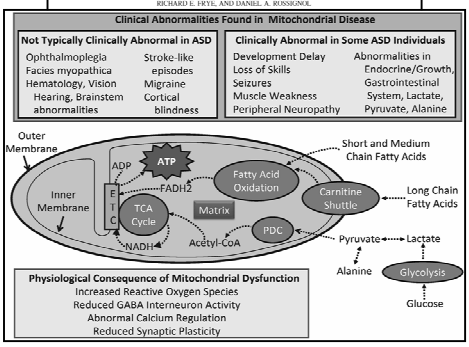
Symptoms / Signs of MtD

- "Any symptom in any organ at any age"
- Developmental or growth delay
- Motor delay
- Clumsiness
- Developmental regression
- Seizures
- Hypotonia (low muscle tone)
- Migraines
- GI Abnormalities (diarrhea, constipation)
- Slow cognitive processing speed
- Fatigue / lethargy
- Ataxia
- Cardiomyopathy
- Myopathy
- Oxidative stress

REVIEW ARTICLE

Mitochondrial Dysfunction Can Connect the Diverse Medical Symptoms Associated With Autism Spectrum Disorders

RICHARD E. FRYE, AND DANIEL A. ROSSIGNOL



Frye and Rossignol, 2011 Ped Res, Doi:10.1203/PDR.0b013e318212f16b

Brain region–specific deficit in mitochondrial electron transport chain complexes in children with autism

Abha Chauhan[1], Feng Gu, Musthafa M. Essa, Jerzy Wegiel, Kulbir Kaur, W. Ted Brown and Ved Chauhan

We observed significantly lower levels of complexes III and V in the cerebellum ($p < 0.05$), of complex I in the frontal cortex ($p < 0.05$), and of complexes II ($p < 0.01$), III ($p < 0.01$), and V ($p < 0.05$) in the temporal cortex of children with autism as compared to age-matched control subjects, while none of the five ETC complexes was affected in the parietal and occipital cortices in subjects with autism. A significant increase in the levels of lipid hydroperoxides, an oxidative stress marker, was also observed in the cerebellum and temporal cortex in the children with autism.

Chauhan et al., 2011 J Neurochem, in press

ORIGINAL ARTICLE

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

DA Rossignol[1] and RE Frye[2]

The prevalence of MD in the general population of ASD was 5.0% (95% confidence interval 3.2, 6.9%), much higher than found in the general population (approximately 0.01%). The prevalence of abnormal biomarker values of mitochondrial dysfunction was high in ASD, much higher than the prevalence of MD. Taken together, these findings suggest children with ASD have a spectrum of mitochondrial dysfunction of differing severity. Eighteen publications representing a total of 112 children with ASD and MD (ASD/MD) were identified. The prevalence of developmental regression (52%), seizures (41%), motor delay (51%), gastrointestinal abnormalities (74%), female gender (39%), and elevated lactate (78%) and pyruvate (45%) was significantly higher in ASD/MD compared with the general ASD population. 53% of children with ASD/MD had a complex I deficiency.

Rossignol and Frye, 2011 Mol Psychiatry, doi:10.1038/mp.2010.136

Objectives

I. Identify features of mitochondrial dysfunction in the general population of children with autism spectrum disorder (ASD)

II. Compare characteristics of mitochondrial dysfunction in children with ASD and concomitant mitochondrial disease (ASD/MD; 112 children) with published literature of two general populations: ASD children without MD, and non-ASD children with MD

Objectives

III. Examine studies correlating biomarkers of mitochondrial dysfunction with ASD symptoms
IV. Examine prevalence of ASD in MD
V. Examine animal models of ASD and mitochondrial dysfunction
VI. Examine treatments for mitochondrial dysfunction in ASD

I. Features of mitochondrial dysfunction in the general population of ASD

Prevalence: General ASD population (Table 1)

	Studies	*Total N*	*Overall prevalence*
General ASD population			
Mitochondrial disease in ASD	3	536	5.0% (3.2%, 6.9%)
Elevated lactate	6	479	31.1% (27.0%, 35.3%)
Elevated pyruvate	2	110	13.6% (7.2%, 20.1%)
Elevated lactate/pyruvate ratio	1	192	27.6% (21.2%, 33.9%)
Elevated alanine	1	36	8.3% (0.0%, 20.1%)
Low total carnitine	1	30	90.0% (81.0%, 99.0%)
Elevated creatine kinase	1	47	46.8% (32.4%, 61.2%)
Elevated ammonia	1	80	35.0% (24.5%, 45.5%)
Elevated AST	1	147	45.6% (37.5%, 53.7%)[a]
Elevated ALT	1	87	7.0% (0.5%, 13.5%)

Rossignol and Frye, 2011 Mol Psychiatry, doi:10.1038/mp.2010.136

Prevalence of MD in ASD: 5%

- 19% to 43% of children with ASD who had an elevated lactate had MD when fully assessed
- Underestimation of MD in ASD?
  - 2 of 3 studies used lactate alone to screen for MD, and excluded children with normal lactate from assessment for MD
  - Only 56% (Correia, 2006) to 79% (Oliveira, 2005) of children with ASD who had an elevated lactate were tested for MD

Biomarker values: General ASD population vs. controls (Table 2)

Biomarker	*Number of studies*	*ASD*		*Control*		*F-value*	*Hedge's g (CI)*
		Total N	*Mean (95% CI)*	*Total N*	*Mean (95% CI)*		
Lactate (mM^{-1})	5	114	1.73 (1.61, 1.88)	114	0.91 (0.87, 0.96)	8.72†	1.42 (0.92, 1.92)†
Pyruvate (nM^{-1})	1	24	0.12 (0.11, 0.14)	24	0.06 (0.06, 0.06)	20.25†	1.96 (0.85, 3.08)†
Carnitine ($mg\,mL^{-1}$)	1	30	3.83 (3.44, 4.31)	30	6.40 (6.22, 6.62)	4.61†	2.51 (1.61, 3.42)†
Ubiquinone	1	15	91.4 (81.9, 103.0)	15	144.2 (130.4,161.1)	2.13	1.90 (0.79, 3.01)†
Creatine kinase	2	55	178.8 (139.6, 226.9)	59	92.2 (89.9, 121.9)	6.93†	0.57 (−0.15, 1.30)
AST	1	147	36.3 (34.4, 38.6)	98	29.7 (28.1, 31.7)[a]	2.34†	0.49 (−0.22, 1.32)
ALT	1	87	24.6 (19.77, 30.52)	70	20.6 (18.7, 23.1)	7.37†	0.18 (−0.61, 0.97)

Rossignol and Frye, 2011 Mol Psychiatry, doi:10.1038/mp.2010.136

Significant Differences

- Lactate: 1.9 fold higher in ASD compared to controls, $p < 0.0001$
- Pyruvate: 2.0 fold higher in ASD compared to controls, $p < 0.0001$
- Carnitine: 1.7 fold lower in ASD compared to controls, $p < 0.0001$
- Ubiquinone: 1.6 fold lower in ASD compared to controls, $p < 0.0001$
- Not significantly different: creatine kinase, AST, ALT

In vitro studies: General population of ASD vs. controls

- Three controlled studies of lymphoblasts:
  - Lower mitochondrial function in ASD
  - Lower mitochondrial GSH reserve in ASD
  - Increased oxidative stress related to mitochondrial dysfunction
  - Larger reduction in mitochondrial function with both NO and glutamate in ASD
  - Greater generation of free radicals with thimerosal in ASD

II. ASD/MD compared to: (a) ASD children without MD, and (b) non-ASD children with MD

Literature search

<u>Three separate literature searches</u>:

- ASD/MD: 112 children from 18 studies (Supplemental Table S1)
  - 11/18 studies did not specify criteria for MD
  - 92% of children diagnosed with ASD before MD
  - 2% diagnosed with MD before ASD
- General childhood ASD population
- General childhood MD population

	ASD/MD		*General ASD*			*General MD*		
	%	N	%	χ^2	P	%	χ^2	P
Male	61	72	81	18.7	< 0.0001	58	0.26	0.61
Developmental regression	52	83	25	32.3	< 0.0001	60	2.2	0.14
Seizures	41	86	11	79.1	< 0.0001	33	2.48	0.11
Hypotonia	62	55	51	2.6	0.10	67	0.62	0.43
Fatigue/lethargy	54	61				19	48.6	< 0.0001
Ataxia	58	19				13	34.0	< 0.0001
Growth delay	21	73						
Motor delay	51	79	9	170.1	< 0.0001			
GI abnormalities	74	35	20	63.8	< 0.0001	39	18.0	< 0.0001
Cardiomyopathy	24	38				26	0.1	0.79
Myopathy	0	12				11	1.5	0.22
Elevated lactate	78	50	31	51.6	< 0.0001	54	12.4	< 0.001
Elevated pyruvate	45	22	14	17.6	< 0.0001			
Elevated lactate/pyruvate ratio	43	23	28	2.6	0.11			
Abnormal organic acids	36	36						
Elevated creatine kinase	34	29	47	1.96	0.16			
Elevated alanine	32	28						
Abnormal brain imaging	23	69				70	72.6	< 0.0001
Normal ETC activity	16	69				3	40.1	< 0.0001
Abnormal complex I	53	96				45	2.48	0.12
Abnormal complex II	9	65				8	0.09	0.76
Abnormal complex III	30	96				31	0.04	0.83
Abnormal complex IV	20	97				34	8.47	0.004
Abnormal complex V	23	44				12	5.0	0.03
Multiple complex deficiency	36	59				27	2.43	0.12
Elevated citrate synthase	24	17				44	2.76	0.10
Abnormal light microscopy	18	49				81	126.4	< 0.0001
mtDNA abnormality	23	87				16	3.17	0.08

Rossignol and Frye, 2011 Mol Psychiatry, doi:10.1038/mp.2010.136

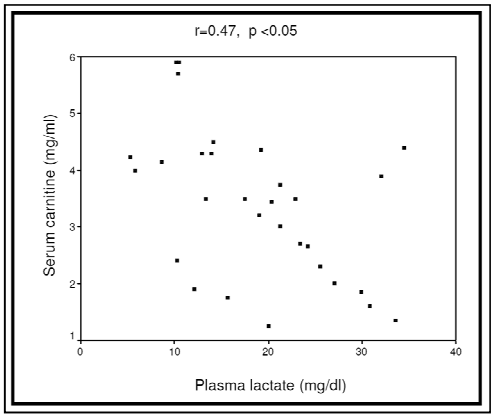
Genetic findings: ASD/MD

- 24 of 112 (21%) children with ASD/MD had an mtDNA, nDNA or chromosomal abnormality
  - mtDNA depletion: 3 children
  - mtDNA deletion: 5 children
  - mtDNA mutations (pathogenic): 12 children
  - nDNA mutation (SCO2): 1 child
  - Chromosomal abnormality: 3 children

III. Studies correlating biomarkers of mitochondrial dysfunction with ASD symptoms

III. Biomarkers and symptoms

- **Abnormal brain energy biomarkers significantly correlated with the severity of language impairments and neuro-psychological deficits in the ASD group but not in controls [Minshew, 1993]**
- **Children with severe ASD (measured by CARS) had both significantly lower carnitine and higher lactate than those with mild or moderate ASD [Mostafa, 2005]**



Mostafa et al., 2005 Int J Child Neuropsychiatry 2(2):179-188

IV. Prevalence of ASD in MD

IV. Prevalence of ASD in MD

- **Based on two studies, the overall prevalence of ASD or autistic features in children with MD was 4.6% (95% CI 0.3%, 8.9%)**
- **These two studies were not population-based and therefore probably do not reflect true prevalence**

V. Animal models of ASD and mitochondrial dysfunction

V. Animal models

- Propionic acid injection
- Mutations in SLC25A12: codes for mitochondrial AGC; myelination defects reversed with pyruvate
- Maternal ubiquitin protein ligase E3A deficiency: mouse model of Angelman's syndrome with complex III defect in hippocampal region

V. Animal models

- MECP2-null mouse (model of Rett syndrome): exhibits decoupling of respiratory complexes
- Mutation in SLC6A8 (creatine transporter): seizure and autistic-like behavior
- Neuronal glucose transporter isoform 3 deficiency: autistic features and ATP depletion

VI. Treatments for mitochondrial dysfunction in ASD

VI. Treatments

- Carnitine (6 studies)
- CoEnzyme Q10 (3 studies)
- B vitamins: thiamine and riboflavin (3 studies)
- Milk free diet in Cerebral Folate Deficiency and MD

Limitations

- Referral bias (only one study was population-based)
- Small sample sizes (< 100 patients)
- Some studies uncontrolled
- Many studies retrospective
- Variability in protocols selecting children for MD workup, collecting biomarkers, and defining MD

Limitations continued

- Certain characteristics in ASD/MD may have been due to features of criteria used to diagnose MD
- Limited evaluation of interrelationships between biomarkers
- MD population may have included undiagnosed ASD; ASD population may have included undiagnosed MD
- Uncertainty of a well-definable ASD/MD subgroup or if a continuum exists

Mitochondrial Dysfunction: ASD

- Prevalence of MD in ASD ~5% (probably an underestimation)
- Biomarker data suggest that mitochondrial dysfunction is present in ~1/3 children with ASD
- The significant variability in biomarkers suggests that a spectrum of mitochondrial dysfunction in ASD exists
- Mitochondrial dysfunction in many cases may be secondary in nature

Secondary Mitochondrial Dysfunction in ASD

Secondary mitochondrial dysfunction in autism

- Lowered glutathione concentrations
- Increased free radicals (ROS)
- Increased tumor necrosis factor (TNF)-α
- Increased nitric oxide (NO)
- Glutamate
- Environmental toxicants
- Abnormal calcium signaling
- Propionic acid / *Clostridia*
- Cerebral Folate Deficiency
- Medications: e.g., Risperidone: inhibits Complex I

Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1913–1917, March 1991
Biochemistry

Glutathione deficiency leads to mitochondrial damage in brain

(buthionine sulfoximine/glutathione ester/turnover/hydrogen peroxide/animal model)

AJEY JAIN*, JOHANNES MÅRTENSSON†, EINAR STOLE†, PETER A. M. AULD*, AND ALTON MEISTER†
Departments of *Pediatrics and †Biochemistry, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

Contributed by Alton Meister, December 5, 1990

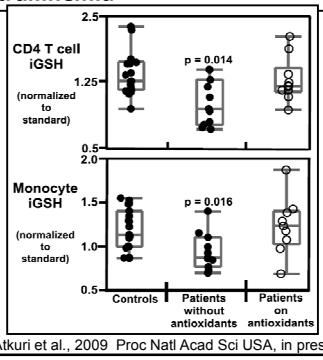
Jain et al., 1990 Proc Natl Acad Sci USA 88:1913-17

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

Secondary MtD: 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

Secondary MtD

- Medications: valproic acid (depletes carnitine), salicylates, antiretroviral HIV meds
- Estrogen increases mitochondrial efficiency
- Decreased metabolic reserve
  - Oxidative stress
  - Lowered glutathione
- Hypoxia

Selected medications causing MtD

- Disulfiram
- Aspirin
- Acetaminophen
- Diclofenac
- Indomethacin
- Naproxen
- Lidocaine
- Amiodarone
- Tetracycline
- Statins
- Metformin
- Amitriptyline
- Citalopram
- Fluoxetine
- Haloperidol
- Risperidone
- Alprazolam
- Diazepam
- Phenobarbital
- Propofol
- HIV medications
- Valproic acid

Neustadt and Pieczenik, 2008 Mol Nutr Food Res 52(7):780-8

Regression in ASD/MD

- 12 studies
- Regression in language, motor skills, eye contact, play skills, social interaction, and receptive skills
- Multiple regressions, regression with catabolic stress, regression after age 3, regression with fever, regression after vaccination

Mitochondrial Disease (MD) and Regression

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

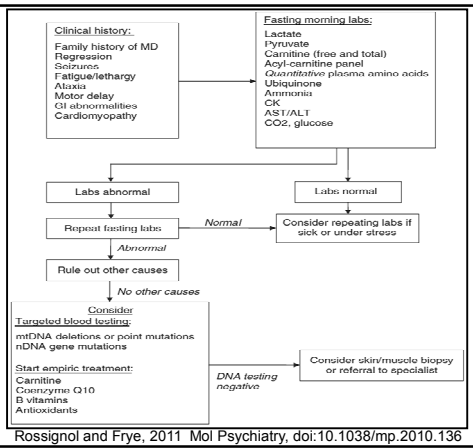
Testing: MtD

Labs: MtD (blood)

- Lactic acid and pyruvate
- Ammonia
- Carnitine, free and total
- Creatine kinase
- Fasting plasma amino acids: alanine (compared to lysine), glycine, proline, sacrosine, tyrosine
- Fasting plasma acylcarnitine analysis
- Basic chemistry (CO_2, anion gap)
- Liver enzymes (AST, ALT)

Labs: MtD (urine)

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



Rossignol and Frye, 2011 Mol Psychiatry, doi:10.1038/mp.2010.136

Treatments: MtD

Treatments: MD

- 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: MD

- Vitamin C: 25 mg/kg/day
- Alpha-lipoic acid: 15 mg/kg/day
- Vitamin K3: 5-80 mg/day
- Folinic acid: 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 the ETC
- Supplementation in high doses can lead to GI side effects and hyperactivity
- Compared to placebo, improves attention in ADHD and speech in autism

HBOT and mitochondrial dysfunction

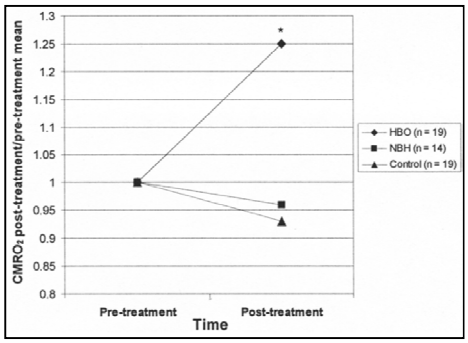
- HBOT decreases brain lactate and improves mitochondrial metabolism in traumatic brain injury (TBI)
- HBOT may lead to mitochondrial biogenesis
- Start with lower pressures (1.3 to 1.5 atm)
- Work with a knowledgeable physician

Oxygen treatment restores energy status following experimental neonatal hypoxia-ischemia

Hyperbaric oxygen and normobaric oxygen both attenuated brain injury, restored the levels of adenosine triphosphate (ATP) 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.

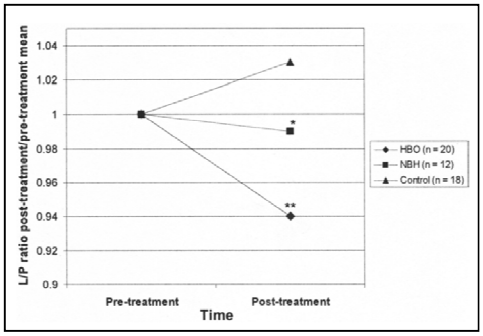
Calvert and Zhang 2007 Pediatr Cri Care Med 8(2):165-73

HBOT at 1.5 atm / 100% oxygen



Rockswold et al., 2010 J Neurosurg 112(5):1080-94

HBOT at 1.5 atm / 100% oxygen



Rockswold et al., 2010 J Neurosurg 112(5):1080-94

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 mitochondria cannot maintain ATP production, then cell undergoes apoptosis
- Cells normally remove old mitochondria (autophagy) and synthesize new mitochondria (biogenesis) from old ones

Neuroscience 137 (2006) 493–504

OXYGEN-INDUCED MITOCHONDRIAL BIOGENESIS IN THE RAT HIPPOCAMPUS

D. R. GUTSAEVA,[a,b] H. B. SULIMAN,[a] M. S. CARRAWAY,[a] I. T. DEMCHENKO[a,b] AND C. A. PIANTADOSI[a]*

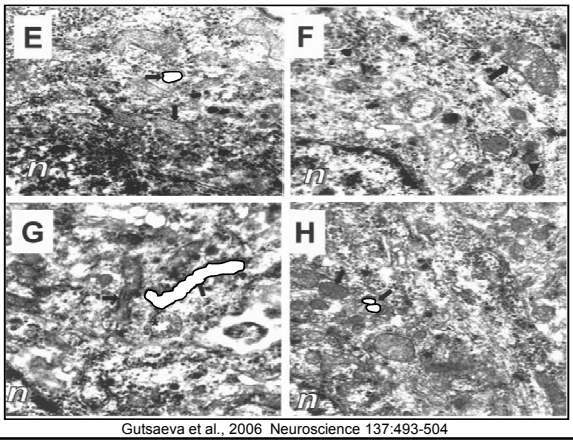
[a]Departments of Medicine and Anesthesiology and Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical Center, Box 3315, Durham, NC 27710, USA

[b]Institute of Evolutionary Physiology and Biochemistry Russian Academy of Science, St. Petersburg, Russia

1972; Balentine, 1982). The mechanisms of CI icity, although not fully understood, involve the of reactive oxygen and nitrogen species (ROS that disrupt the brain's oxidant/antioxidant bala chenko et al., 2002). This imbalance promotes ecule oxidation, including lipids, enzymes, and ids, which in theory produces the neurocher ations and manifestations of toxicity (Jamies Fridovich, 1998).

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.

Gutsaeva et al., 2006 Neuroscience 137:493-504



Gutsaeva et al., 2006 Neuroscience 137:493-504

Research article

Open Access

Hyperbaric treatment for children with autism: a multicenter, randomized, double-blind, controlled trial

Daniel A Rossignol*[1], Lanier W Rossignol[1], Scott Smith[1], Cindy Schneider[2], Sally Logerquist[2], Anju Usman[3], Jim Neubrander[4], Eric M Madren[5], Gregg Hintz[6], Barry Grushkin[7] and Elizabeth A Mumper[8]

CONCLUSION: Children with autism who received hyperbaric treatment at 1.3 atm and 24% oxygen for 40 hourly sessions had significant improvements in overall functioning, receptive language, social interaction, eye contact, and sensory/cognitive awareness compared to children who received slightly pressurized room air.

Rossignol et al., 2009 BMC Pediatr 9:21

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**

Handout for Calculating Probability of MD

Morava Criteria: MD

- **Clinical signs and symptoms (max 4 points)**
  - **Muscle weakness (1 point)**
  - **Developmental delay (1 point)**
  - **Loss of skills (1 point)**
  - **Seizures (1 point)**
  - **Multisystem involvement (1 point): GI, endocrine**
- **Metabolic/imaging studies (max 4 points)**
  - **Elevated lactate (2 points)**
  - **Elevated alanine (2 points)**
- **Mitochondrial morphology (max 4 points)**
- **Score: 2-4 possible MD; 5-7 probable MD; 8-12 definite MD**

Morava et al., 2006 Neurology 67(10):1823-6