May 2011 Research Update

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http://autism.asu.edu

Our research group is dedicated to finding the causes of autism, how to prevent autism, and how to best help people with autism.

Nutrition: vitamins, minerals, fatty acids, amino acids
Metabolism: glutathione, methylation, sulfation, oxidative stress
Mitochondria – ATP, muscle strength, carnitine
Toxic Metals and Chelation
Gastrointestinal Problems
Sleep
Inflammation
Seizures

Bringing Research to Families

Summary of Biomedical Treatments
28-page summary of 14 major treatments, including a summary of the research for each treatment and how to implement it
Free copy available at http://autism.asu.edu

Topics
GI Studies
Muscle Strength
Ribose and NADH Study
Carnitine Study
Vitamin/Mineral Study
Seizure Survey (reported on in fall, paper out in 1-2 months)
Future Research
Every study funded by Autism Research Institute (and Zoowalk for Autism)
Legacy Foundation also funded vitamin/mineral study

Gastrointestinal Flora and Gastrointestinal Status
Published April 2011 in BMC Pediatrics
Involved 58 children with autism and 39 typical children

Gut symptoms associated with much more severe autism

| Symptom                  | Low-GI Problem (n=22) | High-GI Problem (n=34) | % difference from test
|-------------------------|-----------------------|------------------------|------------------------
| GSI score               | 1.4 ± 0.8             | 5.4 ± 1.7              | 399%                   
| ATEC total              | 49.0 ± 21             | 81.5 ± 27.6            | 66%                    
| Gut Problems associated with worse symptoms (all four areas)

Short Chain Fatty Acids
Short chain fatty acids are produced in the gut when anaerobic bacteria consume fiber. One of them, propionic acid, is also a common food preservative.
Five studies (MacFabe et al 2007, 2008, 2009, 2010, 2011) have demonstrated that injection of propionic acid into blood of rats immediately produces ‘autistic-like’ symptoms, including social avoidance, hyperactivity, backwards walking, cognitive impairments, and seizures. Effects are temporary, and normal function returns when exposure to SCFA’s is stopped.

SCFA’s are lower in stool of children with autism, especially if taking probiotics
Possible interpretations of low SCFA’s in autism

Hypothesis 1) Possibly less fiber in diet, or less anaerobic bacteria that make SCFA’s
Hypothesis 2) More SCFA’s leak from gut into body, due to increased gut permeability and longer transit time

Need research to measure SCFA’s in blood to determine which hypothesis is correct
Probiotics may be helpful in reducing bacteria that make SCFA’s

Correlation of Low Muscle Tone and Autism Severity

Children with more severe autism have much lower muscle strength; suggests low ATP, since ATP is needed for muscle and brain

Importance of ATP

ATP is the primary energy for the body (muscles, brain, many biochemical reactions). Each person has only 250 g of ATP, but they recycle it hundreds of times a day.

ATP is recycled by mitochondria, “factories” within every cell whose primary role is to make ATP.

Many children with autism have low muscle tone (probably one indication of a mitochondrial disorder)

Recent study (C.A. Al-Mosalem, Clin. Biochem 2009) found that Saudi children with autism had impairments in ATP production compared to typical children.

ASU 2011 vitamin/mineral study found children with autism have 25% lower ATP in plasma than typical children.

New Study on NADH Therapy and Ribose Therapy

Two weeks of therapy with NADH (5-10 mg) or Ribose (5 g)

Many improvements in glutathione, methylation, NADH, NADPH, ribose, and ATP.

Ribose is a special sugar available in tiny amounts in food; mostly made in the body from glucose (requires NADP to make it, which is low in autism).

Ribose is a building block for DNA, RNA, ATP, GTP, NADH, NADPH, FADH, riboflavin, co-enzyme A and other nucleotides.

Ribose made from glucose; requires NADP at two steps

Very similar results, although NADH better for improving GSH, ribose better for improving ATP

Both therapies very effective in increasing level of Ribose.
Summary

Ribose and NADH have similar benefits, and can quickly improve methylation, glutathione, and ATP problems in autism. Larger, longer-term trials needed to determine effect on symptoms, but likely to be beneficial with minimal risk of side-effects.

Carnitine Treatment Study

Rationale – carnitine is needed to transport long-chain fatty acids (fuel) across membrane into mitochondria; One study found decreased carnitine in children with autism (Filipek et al) So, carnitine supplementation may be helpful. Study design: randomized, double-blind, placebo-controlled; 90 days treatment (Geier et al 2011, Med. Sci. Monitor)

Carnitine Study (cont.)

Dosage: 50 mg L-carnitine/kg bodyweight/day (similar to that for prescription carnitine)
Generally well-tolerated with minimal side effects (a few cases of irritability and/or stomach discomfort); 30 starting participants (19 treatment, 11 placebo); 7 withdrawals (4 in treatment group, 3 in placebo)

Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI</td>
<td>50% improved</td>
<td>0%</td>
</tr>
<tr>
<td>CARS</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>ATEC -speech</td>
<td>-21%</td>
<td>-4%</td>
</tr>
<tr>
<td>ATEC -social</td>
<td>-31%</td>
<td>-9%</td>
</tr>
<tr>
<td>ATEC -cognitive</td>
<td>-28%</td>
<td>-4%</td>
</tr>
<tr>
<td>ATEC -physical/behavioral</td>
<td>-28%</td>
<td>-25%</td>
</tr>
</tbody>
</table>

Muscle +5% 0% n.s.
Strength

Overall, modest but significant improvements in 50% of participants in only 90 days.

Summary of Carnitine Study

Modest benefits in 50% of participants, with statistically significant changes in CGI, CARS, ATEC -cognitive
Small study; larger study may yield greater significance
Longer study may yield greater benefits
Combination with other mitochondrial supplements (co-Q10, NADH, ribose, vitamins/minerals) may increase effectiveness

Nutrition Basics

Humans need to consume:
protein
vitamins/minerals
essential fatty acids
water
In US, most consume enough protein, but 80% low in omega 3 fatty acids (insufficient seafood), and many low in some vitamins/minerals (insufficient vegetables/fruits)
Worldwide, low consumption of protein, omega 3 fatty acids, iodine, and vegetables/fruits are a major concern

Vitamin/Mineral Supplements

Rationale: The definition of an essential vitamin or mineral is that lack of it results in disease or even death. Most people in the US consume too little of some vitamins and minerals. For example, many women lack enough calcium and iron, leading to osteoporosis and anemia, respectively.

Explanation of Treatment:
Vitamins and minerals are available in vegetables, fruits, meat, and other sources. However, the typical US diet is lacking in key vitamins and minerals, so many people need to take a supplement.

Research Study of Multivitamin/mineral supplement

- 3 month study of Spectrum Support
- Randomized, double-blind, placebo-controlled
- small study - 20 children only
- Many improvements, including statistically-significant improvements in sleep and GI

Adams et al., J Altern Complement Med. 2004

Pilot Studies

In 2006/2007 we conducted two pilot studies (each n=8), to determine the optimal dosage of a vitamin/mineral supplement.

Philosophy: broad-spectrum vitamin/mineral supplement, with high (but not mega-dose) levels of Vitamin B6 and Vitamin C.
Conclusion of Pilot Study

Appears that vitamin/mineral supplement is well-absorbed, and improves nutritional status, porphyrins, possibly, neurotransmitters, and possibly autism symptoms.

However, some need to adjust formulation slightly, and to improve anti-oxidants and glutathione support.

Study Design

1) Measure nutritional status of children with autism spectrum disorders vs. controls.
   No vitamin/mineral supplement in the 2 months prior to the study.
3) Randomized, double-blind, placebo-controlled treatment for 12 weeks. Dosage slowly raised over first 3 weeks.
4) Remeasure nutritional status and autism severity.
5) Break code and analyze data.

Study included 55 children with ASD, 44 typical children, ages 5-16 yr, 80% male.
Also national study with 88 children and adults with ASD (behavioral assessments only, no blood tests).

Reformulation

Based on the results of the 2 pilot studies, we made some changes to the formulation

1) small adjustments to some of the vitamins/minerals (increasing or decreasing dosage)
2) Primarily folic acid instead of folic acid
3) Addition of NAC (to boost glutathione)
4) Addition of CoQ10 (to assist mitochondria)

Nutritional Status: Pre and Post

Most vitamin levels initially similar in autism vs. controls.
(A, B1, B2, B3, folic acid, B12, B6, B, H)

Effect of supplement: VI B1 (+27%***), B3 (+9%)*, B12 (+20%)**.

Initially slightly lower:
Biotin: -20%***
B5: -10%* +6% good improvement
Carotenoids: -15%* -7% some improvement

Slightly higher:
VI, C: +18%* +54%** good increase

Overall, many good improvements

Biomarkers for need for vitamins

Pre:
Final
Figlu (need for folic acid): +23%* -18%**
MMA (need for vit B12): +14%* -33%*

The autism group had an increased need for folic acid and vitamin B12, despite normal levels of them.
Supplement resulted in substantial improvements for Figlu and MMA, suggesting that the supplemental folic acid and vit B12 was helpful.

Essential Minerals

Most minerals similar in children with autism and controls.

Lower level of lithium (consistent with our previous study of autistic children and their moms – Adams et al Biol. Tr. El. Res 2006 – supplement increased it greatly.
Low lithium associated with many psychiatric disorders – probably contributes to risk of autism

Iodine levels normal, but 30% have levels below 10th percentile of controls – iodine important for normal thyroid function and IQ. 99% of general population has thyroid problem, may be higher in autism – should test for thyroid function

Iron: Serum ferritin and serum iron normal. However, high RBC iron, with 42% of the children with autistic having levels above the 90th percentile for the typical children. May relate to problem with iron metabolism. Supplement normalized it

Effect of supplement on vitamins

Revised formulation

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000 IU</td>
<td>Calcium 100 mg (magnesium)</td>
</tr>
<tr>
<td>B1</td>
<td>25 mg</td>
<td>Chromium 70 mcg</td>
</tr>
<tr>
<td>B2</td>
<td>25 mg</td>
<td>Copper 6 mg (magnesium, cobalt)</td>
</tr>
<tr>
<td>B3</td>
<td>25 mg</td>
<td>Magendol 1 mg</td>
</tr>
<tr>
<td>B5</td>
<td>15 mg</td>
<td>Magnesium 100 mg</td>
</tr>
<tr>
<td>B6</td>
<td>40 mg</td>
<td>Magnesium 3 mg</td>
</tr>
<tr>
<td>B12</td>
<td>500 mcg</td>
<td>3 mg</td>
</tr>
<tr>
<td>Biotin</td>
<td>100 mcg</td>
<td>100 mg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>500 mcg</td>
<td>100 mcg</td>
</tr>
<tr>
<td>Folinic Acid</td>
<td>100 mcg</td>
<td>150 mcg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>150 mcg</td>
<td>Vitamin D 1000 IU</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>3 mg</td>
<td>50 mg (for mitochondria)</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>500 mcg</td>
<td>50 mg (for mitochondria)</td>
</tr>
<tr>
<td>Vitamin B5</td>
<td>200 mcg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Vitamin B3</td>
<td>250 mcg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>120 mcg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>100 mcg</td>
<td>50 mg (for mitochondria)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>500 mcg</td>
<td>100 mg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>400 mcg</td>
<td>500 mcg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>250 mcg</td>
<td>150 mcg</td>
</tr>
<tr>
<td>Methionine</td>
<td>100 mg</td>
<td>250 mg</td>
</tr>
</tbody>
</table>

Composition

• Mixed tocopherols 100 mg
• Mixed tocopherols 100 mg
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Effect of supplement: 

- Vitamin B1 (+27%***), B3 (+9%)*, B12 (+20%)**.
- Slightly higher: VI, C: +18%* +54%** good increase
- Overall, many good improvements

Essential minerals (continued)

- Slightly higher levels of RBC potassium, RBC phosphorus, copper (WB and RBC), serum magnesium, and RBC boron.
- Supplement also increased iodine, manganese, molybdenum, and selenium, and normalized magnesium, phosphorus, and potassium.
Summary of Neurotransmitters

Most major neurotransmitters (serotonin, norepinephrine, epinephrine, acetylcholine, GABA) low in children with autism.

Vitamin B6 and other vitamin/mineral co-factors needed to produce these neurotransmitters.

Supplement dramatically improves and normalizes neurotransmitter levels.

This study clearly shows that nutritional supplements are an alternative to standard psychiatric medications for altering neurotransmitter levels.

Biochemical Summary

Many abnormalities in autism in vitamins, minerals, glutathione, methylation, oxidative stress, sulfation, and neurotransmitters.

These abnormalities are associated with variation in autism severity.

Supplement seems to improve or normalize levels of almost all of them.

Need more anti-oxidants, carotenoids, and sulfation.

Behavioral Effects

Includes averages of national and Arizona studies, total of 141 children and adults with autism.

Significant improvement on PGI-R, little change in others.

PGI-R more sensitive, since: 1) it uses a 7-point scale (vs. 3-4 point scale for others) and 2) it measures changes, rather than differences between two large numbers.

Summary of Regression Analysis

Many biomarkers significantly associated with variations in autism severity.

Biomarker adj. R2 most significant terms

Toxic Metals 0.38-0.47 mercury, cadmium, lead, tin

Vitamins 0.25-0.57 Vit B6, Vit C, NMNA

Minerals 0.22-0.38 Ca, Zn, Fe, Se

Amino Acids 0.18-0.39 serine, proline, ethanolamine, beta-amino-isobutyrate

Sulfate/SAM/Glut 0.15-0.24 Sulfate, SAM, GSSG

ATP/NADH 0.0-0.13 NADH, ATP

Summary of Neurotransmitters

Some important neurotransmitters:

- Norepinephrine, epinephrine, acetylcholine, dopamine
- GABA, serotonin
- Glutamate, aspartate

ATP, NADH, NADPH

NADH/NADPH - important for amino acid metabolism and energy metabolism.

Many improvements in sulfate, methylation, glutathione, oxidative stress.

Comparison of treatment and placebo groups:

- Control: Pre-treatment, Post-treatment
- Supplement: Pre-treatment, Post-treatment

Overall, improvements in calcium, iodine, iron, lithium, manganese, and possible improvements in magnesium and selenium.

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Supplement seemed to most help children up to age 18, possibly less benefit for adults (need to try higher dosage).

Who improved the most?
Improvement on the PGI-R was strongly correlated with level of vitamin K (-0.71) and biotin (-0.67), p<0.01
ie, children with lower levels of vitamin K and biotin tended to improve the most

Regression analysis also found that variation in degree of improvement strongly associated with initial nutrients (adjusted R2 = 0.61, p<0.0005), with vitamin K and biotin being most significant

Summary
Supplement greatly improved nutrition and metabolism, including:
- Vitamins
- Minerals
- ATP/NADH/NADPH
- Methylation
- Glutathione
- Oxidative stress
- Sulfate
- Neurotransmitters

Current and Future Research
Supplement currently available as Syndion (www.syndion.com)
Revised version (based on data from this study) available June 2011 – will provide improved antioxidants, vit D, and methylation.
Suggest Epsom salt baths for extra sulfation
Suggest extra vitamin D for most children (1000-5000 IU)

Future Research (continued)
Nutrition and Diet Intervention Study
12-month, single-blind design
vitamin/mineral supplement (revised) essential fatty acids (fish oil)
carnitine (for mitochondrial function, seizures)
digestive enzymes
healthy, GFCF diet

Thanks
Many thanks to the families who participated in our study!
Many thanks to ARI, Arizona Zoowalk, and Legacy Foundation for their support!
Thanks to Health Diagnostics and Doctor’s Data for laboratory measurements.
For Summary of Biomedical Treatments, go to http://autism.asu.edu

Supplementation: Our Recovery Story
- Development slowed by 1 year, then began regressing
- Autism diagnosis at 26 months
- Immediately began GFCF low-allergenic diet
- Habilitation, therapies, and preschool

Supplement Study Results: Age 4 ½
- Day 11: Increased energy level, more echolalia
- Day 18: First formed stool, willing to eat a greater variety of foods
- Day 25: Uncomfortable in wet diaper
- Day 40: Uncomfortable in wet diaper
- Day 48: Spontaneously learning new words, initiating conversations, 100% dry all day, playing with toys, willing to shift attention from TV/video games to family members
- Day 76: 100% dry day and night, bowels solid and fully formed, great appetite and energy level, has conversations with stuffed animals, prefers interaction with real people to TV & video games, used “I” when answering a question, fine motor improvement
Supplementation: Our Recovery Story