Pilot Study of a Moderate Dose Multivitamin/Mineral Supplement for Children with Autistic Spectrum Disorder

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ABSTRACT

Objective: Determine the effect of a moderate dose multivitamin/mineral supplement on children with autistic spectrum disorder.

Design: Randomized, double-blind, placebo-controlled 3-month study.

Subjects: Twenty (20) children with autistic spectrum disorder, ages 3–8 years.

Results: A Global Impressions parental questionnaire found that the supplement group reported statistically significant improvements in sleep and gastrointestinal problems compared to the placebo group. An evaluation of vitamin B6 levels prior to the study found that the autistic children had substantially elevated levels of B6 compared to a control group of typical children (75% higher, p < 0.0000001). Vitamin C levels were measured at the end of the study, and the placebo group had levels that were significantly below average for typical children, whereas the supplement group had near-average levels.

Discussion: The finding of high vitamin B6 levels is consistent with recent reports of low levels of pyridoxal-5-phosphate and low activity of pyridoxal kinase (i.e., pyridoxal is only poorly converted to pyridoxal-5-phosphate, the enzymatically active form). This may explain the functional need for high-dose vitamin B6 supplementation in many children and adults with autism.

INTRODUCTION

Nutritional deficiencies are widespread in the United States. For example, ~30% of the general population has a marginal vitamin C status, and ~15% of Americans are vitamin C deficient (Hampl et al., 2000; Johnston and Thompson, 1998). Low levels of other vitamins and minerals, including calcium, iron, folic acid, magnesium, and chromium continue to be a significant problem in the United States and other countries (Anderson, 1992; Murray, 1996). Based on these findings, it is not surprising that a number of studies and reviews have demonstrated the benefits of targeted multivitamin/mineral nutritional supplementation in the improvement of IQ, scholastic test scores, early neurological development, and behavioral, cognitive, and academic gains in children with learning disabilities (Carlton et al., 2000; Fernstrom, 2000; Shoenthaler, 2000).

Many studies find or suggest that people with autism have functionally low level of essential vitamins and minerals.

Measurements of vitamin/mineral levels

Audhya and colleagues recently reported measurements of vitamin and mineral levels in the blood of over 150 children with autism compared to 50–100 controls of the same age (Adams et al., 2003). They found that the children with autism on average had much lower levels of most vitamins (vitamins A, C, D, and E, and all B vitamins except choline) and some minerals (zinc, magnesium, selenium).

High copper:zinc ratio

Analysis of data from the Pfeiffer laboratory found that over 99% of 500 children with autism had an elevated serum copper:plasma zinc ratio, higher than that for any other
group they had tested (Walsh, 2001). It has been hypothesized that this imbalance is related to a metallothionein defect, and suggests that people with autism should avoid copper and take extra zinc.

**B12 deficiency**

A functional B12 deficiency has been suggested by a study that found elevated urinary methylmalonic acid in autistics (Wakefield et al., 1998).

**Calcium deficiency**

Landgreme found that 22% of autistic children measured low 24-hour urinary calcium excretion (Landgreme and Landgrebe, 1976).

**Sulfate deficiency**

Two studies have found that children with autism often have abnormal sulfate metabolism, leading to excess urinary excretion of sulfate and very low plasma levels of sulfate (Alberti et al., 1999; O’Reilly and Waring, 1993).

**Vitamin A**

Megson (2000) has hypothesized that children with autism may have a Vitamin A deficiency.

**Reasons for low levels of nutrients in children with autism**

**Chronic diarrhea/constipation.** Malabsorption problems in autism were first documented over 30 years ago (Goodwin et al., 1971). A recent study by the Southwest Autism Research Center of over 400 people with autism has found that 48% reported chronic diarrhea or chronic constipation (Schneider and Melmed, 2000).

**Gastrointestinal inflammation.** There is substantial evidence of chronic gastrointestinal (GI) inflammation and structural compromise in the digestive tract in many people with autism, especially in the terminal ileum, which is the major site for nutrient absorption (Horvath et al., 1999; Wakefield et al., 2000). This intestinal inflammation likely reduces nutrient absorption.

**Dietary restrictions.** One of the classic symptoms of autism is restricted interests and behaviors and, in our clinical experience, this often includes choices of food. Restricted, self-limited diets are more likely to be deficient in one or more essential nutrients.

**Benefits of nutritional supplements**

Rimland (2001) conducted an open survey of thousands of parents of children with autism to determine which treatments they found to be helpful or harmful. The study found that parents reported supplements of calcium, vitamin C, folic acid, and vitamin B6 with magnesium, zinc, niacin, niacinamide, and dimethylglycine resulted in improvement in 41–58% of cases versus 1–8% responding that the supplements worsened symptoms.

**Benefits of B6/magnesium.** Eighteen (18) studies on the efficacy and safety of very high doses of vitamin B6 with magnesium, including 11 double-blind placebo-controlled studies, have been conducted over the past 35 years and the results have repeatedly shown this combination to be effective in reducing many of the deficits and symptoms of autism (Barthulemey et al., 1981, 1983; Bonish, 1968; Coleman et al., 1979; Ellman, 1981; Garner et al., 1986; Gaulterir et al., 1981; Jonas et al., 1984; LeLord et al., 1981, 1988; Martineau et al., 1982, 1985, 1986; Rimland, 1973, 1974, 1987; Rimland et al., 1978; Rossel et al., 1990).

**Vitamin C.** Vitamin C has also been shown in a clinical trial to facilitate a reduction in symptom severity in children with autism (Dolske et al., 1993).

**MATERIALS AND METHODS**

The objectives of this study were to determine the levels of vitamin B6, vitamin C, and alpha lipoic acid in children with autistic spectrum disorder; and to determine if a moderate dose multivitamin/mineral supplement is effective in partially reducing some of the symptoms of autism.

The study methodology was approved by the Human Subjects Institutional Review Board at Arizona State University. The parents of all participants signed informed consent forms.

The study was advertised by a mass mailing sent to approximately 1000 families of people with autism in the state of Arizona, using the combined mailing lists of the Greater Phoenix Chapter of the Autism Society of America and the Southwest Autism Research Center.

The selection criteria for the study included age 3–8 years; diagnosis of an autism spectrum disorder (autism, pervasive developmental disorder—not otherwise specified [PDD/NOS], or Asperger’s syndrome) by a psychiatrist or developmental pediatrician; no changes in any treatment therapies during the previous 2 months, including psychiatric medications, nutritional supplements, diet, or behavioral therapy program, as well as an agreement to make no such changes during the 3 months of the study; and no prior use of a multivitamin/mineral supplement other than a standard children’s multivitamin/mineral. Any standard supplements were discontinued prior to the study.

Twenty-five (25) applicants who applied met the criteria, and they were enrolled in the study. The children were matched in pairs by age and sex, and then a random selec-
tion was made to determine which child received the supplement and which the placebo.

The study began with an initial physical evaluation conducted by a physician to determine that the children were in reasonable physical health. Then a blood and first morning urine sample were collected. The children took the supplements for 3 months, and were then re-examined by the physician, and additional blood specimens were collected. The parents, physicians, and children were blinded regarding who received the supplement and who received the placebo.

The levels of vitamin B6 in plasma were measured at the beginning and end of the study by Vitamin Diagnostics Laboratory. The measurements were carried out using the ciliate protozoan Tetrahymena pyriformis (Baker et al., 1966). The standards were prepared by mixing pyridoxal hydrochloride, pyridoxamine dihydrochloride, and pyridoxal-5-phosphate in equal ratio. The assay measures the total amount of vitamin B6, including pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated forms. This is an older method, still used by commercial labs due to its reliability.

At the beginning of the study, prior to supplementation, the level of alpha lipoic acid was also measured by Vitamin Diagnostics. The measurements were carried out using a protozoan assay with T. pyriformis (Baker et al., 1998).

Vitamin C was measured on the last day of the study (due to logistical problems it could not be measured at the beginning). Blood was collected in EDTA-treated vacutainers, immediately processed, and an aliquot of plasma was mixed with an equal volume of 10% TCA. The supernatant was frozen (−45°C) for later vitamin C analysis using the colorimetric 2,4-dinitrophenylhydrazine method (Omaye et al., 1979).

Statistical analysis was conducted with a simple t-test comparison, assuming a normal distribution and setting a level of p = 0.05 for statistical significance.

Choice of supplement

We wanted to investigate a commercial supplement that was in use by children with autism. We chose Spectrum Support (BrainChild Nutritional, Santa Cruz, CA) because it contains a broad range of most vitamins and minerals, does not contain copper, and has moderate amounts of vitamin B6 (Table 1). Also, the liquid formulation and taste helped with compliance (only one child had a significant compliance problem).

Dosage

The dosage was gradually increased during the study. The dosage began with the Spectrum Support II (SSII) formulation, starting at 1/8 of the dose, and increased linearly over 24 days to the maximum dose of SSII, which was held constant until day 34. During days 35–50, there was a gradual transition to Spectrum Support III (SSIII), which was continued until day 90 at the same volume (SSIII is similar to SSII, with the major difference being a slightly higher concentration of nutrients). The full dosage was 1 mL/5 pounds bodyweight, t.i.d. with food, for a total daily intake of 3 mL/5 pounds bodyweight.

Both SSII and SSIII are liquid suspensions in a Kosher vegetable glycerine base, flavored with lemon, lime, and grapefruit essential oils and preserved with grapefruit seed extract, pau d’arco, and potassium sorbate. Trace amounts of burdock root, chinese astragalus root, chinese licorice root, ginkgo, gotu kola, siberian ginseng, and slippery elm are added.

Placebo

The placebo was the same Kosher vegetable glycerine base, with hibiscus added to match the coloring of the supplement. The coloring faded over time, so it was not an exact match, but the consistency remained very similar to the supplement. Because the parents and children did not know what the true SSII or SSIII looked like, this did not appear to be a significant issue, and at the end of the study the parents were still unsure whether or not they had received the supplement.

Elimination of participants

Five (5) children who began the study were not included in the final evaluation. In the placebo group, 3 children withdrew. One child, with a history of diarrhea, developed diarrhea 2 weeks into the study; the parent stopped the “supplement,” but diarrhea continued for another week. The second child began risperidal therapy 2 weeks into the study, and was removed from the study. In the third case, the child dropped out prior to beginning supplement, to enter another study, on secretin. In the supplement group, 1 child withdrew after two months, when the family went on a one-month vacation and stopped giving the supplement, and another child did not take the supplement consistently, due to several factors including unwillingness, travel, ear surgery, and flu.

Final participant list

The 9 participants in the placebo group who completed the study were 8 boys and 1 girl; mean age 5 years 5 months; 6 with autism and 3 with PDD/NOS. The 11 participants in the supplement group who completed the study were 10 boys and 1 girl; mean age 5 years 2 months; 9 with autism and 2 with PDD/NOS.

Adverse side-effects

There were no adverse events reported in children who followed the dosage instructions. However, 2 of the children (twins) took the supplement with meals for breakfast and
lunch, but at night they took it at 9 PM on an empty stomach (4 hours after dinner). There were no problems until week 9 of the study, when they began to have nausea and vomiting in the late evening after taking the supplement (no problems during the day). This lasted for several weeks. When the investigators learned of the problem, they corrected the dosage time. The children completed the study, and their results are included in Table 2, but their outcome

### Table 1. Ingredients of Spectrum Support II and III (per 30 mL)

<table>
<thead>
<tr>
<th></th>
<th>SSII</th>
<th>SSIII</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro-vitamin A mixed carotenoids from Dunaliela Salina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha and beta-carotene and cryptoxanthin</td>
<td>7500 IU</td>
<td>10,500 IU</td>
</tr>
<tr>
<td>Lutien and zeaxanthan</td>
<td>60 IU</td>
<td>84 IU</td>
</tr>
<tr>
<td>B1 (thiamin hydrochloride)</td>
<td>20 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>B2 (riboflavin-5-phosphate-coenzyme B2)</td>
<td>25 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>B3 (inositol hexaniacinate)</td>
<td>25 mg</td>
<td>35 mg</td>
</tr>
<tr>
<td>B5 (calcium pantothenate)</td>
<td>45 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>B6 (pyridoxyl-5-phosphate-coenzyme B6)</td>
<td>30 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>B12 (cyanocobalamin)</td>
<td>1200 mcg</td>
<td>1600 mcg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>800 mcg</td>
<td>800 mcg</td>
</tr>
<tr>
<td>Biotin</td>
<td>100 mcg</td>
<td>150 mcg</td>
</tr>
<tr>
<td>Choline (citrate)</td>
<td>50 mg</td>
<td>60 mg</td>
</tr>
<tr>
<td>Inositol (monophosphate)</td>
<td>50 mg</td>
<td>60 mg</td>
</tr>
<tr>
<td>Vitamin C ascorbates (magnesium, potassium, and zinc ascorbates)</td>
<td>650 mg</td>
<td>800 mg</td>
</tr>
<tr>
<td>Mixed bioflavonoids</td>
<td>200 mg</td>
<td>400 mg</td>
</tr>
<tr>
<td>Vitamin D-3 (cholecalciferol)</td>
<td>150 IU</td>
<td>150 IU</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>175 IU</td>
<td>250 IU</td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (Kreb’s chelates)</td>
<td>175 mg</td>
<td>200 mg</td>
</tr>
<tr>
<td>Calcium D-glucarate</td>
<td>75 mcg</td>
<td>100 mcg</td>
</tr>
<tr>
<td>Chromium (picolinate)</td>
<td>75 mcg</td>
<td>100 mcg</td>
</tr>
<tr>
<td>Magnesium (Kreb’s chelates and magnesium ascorbate)</td>
<td>175 mg</td>
<td>200 mg</td>
</tr>
<tr>
<td>Manganese (succinate)</td>
<td>3 mg</td>
<td>3 mg</td>
</tr>
<tr>
<td>Molybdenum (Kreb’s chelate)</td>
<td>0</td>
<td>75 mcg</td>
</tr>
<tr>
<td>Potassium (alpha ketoglutarate and potassium ascorbate)</td>
<td>75 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td>Selenium (selenomethionine)</td>
<td>70 mcg</td>
<td>85 mcg</td>
</tr>
<tr>
<td>Silicon (sodium metasilicate)</td>
<td>3 mg</td>
<td></td>
</tr>
<tr>
<td>Sulfur (methylsulfonylmethane, or MSM)</td>
<td>175 mg</td>
<td>300 mg</td>
</tr>
<tr>
<td>Zinc (picolinate, Kreb’s chelates, and zinc ascorbate)</td>
<td>15 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td><strong>Other ingredients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betaine (trimethylglycine)</td>
<td>300 mg</td>
<td>400 mg</td>
</tr>
<tr>
<td>DMAE (bitartrate)</td>
<td>100 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>N-acetyl cysteine</td>
<td>25 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Alpha lipoic acid (thiocitic acid)</td>
<td>0</td>
<td>25 mg</td>
</tr>
</tbody>
</table>

*The minerals are reported by the mass of the total molecule, not by the mass of the active mineral. Thus the dose of the active mineral is actually lower.

### Table 2. Impressions by Mothers of 20 Children at the End of the Study (9 on Placebo, 11 on Supplement), Based on Changes They Observed, Ranked on a 7-Point Scale*a

<table>
<thead>
<tr>
<th>Category</th>
<th>Placebo score</th>
<th>Supplement score</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep</td>
<td>3.9</td>
<td>5.0</td>
<td>+1.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>3.9</td>
<td>4.9</td>
<td>+1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Receptive language</td>
<td>4.9</td>
<td>5.5</td>
<td>+0.6</td>
<td>NS</td>
</tr>
<tr>
<td>General behavior</td>
<td>4.3</td>
<td>4.8</td>
<td>+0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Eye contact</td>
<td>4.9</td>
<td>5.3</td>
<td>+0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Expressive language</td>
<td>5.6</td>
<td>5.5</td>
<td>-0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sociability</td>
<td>5.1</td>
<td>5.0</td>
<td>-0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Overall</td>
<td>5.1</td>
<td>5.1</td>
<td>0.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a1 = much worse; 2 = worse; 3 = slightly better; 4 = same; 5 = slightly better; 6 = better; 7 = much better
NS, nonsignificant
RESULTS AND DISCUSSION

Global impressions

On the last day of the study, the mothers of the children filled out a simple Global Impressions survey, to evaluate changes during the study. The forms were filled out privately by the mothers, with no input from the investigators. As can be seen in Table 2, the mothers reported some improvement for the children on the placebo (presumably due to a placebo effect), but there was generally more improvement for the children on the supplement than for those on the placebo. The improvements in sleep and GI symptoms were statistically significant (based on a simple t-test, assuming a normal distribution), with \( p < 0.05 \).

If the data are analyzed without including the two twins who mistakenly took the supplements on an empty stomach and suffered from vomiting/nausea, the mean improvements in GI and sleep symptoms are slightly higher (average 5.2 and 5.2) and more statistically significant (\( p = 0.005 \) and \( p = 0.01 \), respectively).

Vitamin C

According to a t-test, there was a statistically significant difference (\( p < 0.05 \)) between the placebo and supplement group, which is to be expected as the supplement group received a high dose of vitamin C. The children in our study had a mean age of 5.5 years. The average vitamin C level for that age group is 1.45 mg/100 mL (National Center for Health Statistics, 1982). The placebo group had a low level of vitamin C, 1.0 mg/100 mL, but even the supplemented group receiving a high dose of vitamin C still had a below average level of vitamin C of 1.33 mg/mL (Table 3). It is important to point out that a level of 0.5 mg/mL is considered a marginal deficiency, and a level of 0.2 mg/mL is considered a clinical deficiency. So, although the children with autism were not clinically deficient, they generally had low levels of vitamin C, and high-dose supplementation appeared to only partially help. Our results are consistent with a previous study (Dolske et al., 1993), which found that high-dose vitamin C supplementation led to clinical improvements in children with autism.

Vitamin B6

The results are shown in Table 4. Prior to the start of supplementation, the children in the placebo group and the supplement group had similar vitamin B6 levels (54 and 56 \( \mu \)g/mL, respectively). Those levels are well above the Vitamin Diagnostic laboratory’s reference range of 22–47 for typical children ages 3–16; their reference range is defined as the lowest and highest values of the typical children. We also sent a set of plasma samples from typical children not taking vitamin supplements (age 6–9 years, \( n = 11 \)) to the laboratory in a blinded fashion, and found that their average level was 32 \( \mu \)g/mL, consistent with the reference range. The difference between the total autism group and the typical children was highly statistically significant (\( p = 0.00000006 \)). It is important to note that this measurement assesses total vitamin B6, including pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated forms: pyridoxine-5-phosphate (PNP), pyridoxal-5-phosphate (PLP), and pyridoxamine-5-phosphate (PMP).

A previous study by Adams et al. (2003) found low levels of PLP in children with autism versus controls. PLP is a co-factor for many enzymatic reactions in the body, and is the active form of vitamin B6. A study by Audhya (2002)

<table>
<thead>
<tr>
<th>Placebo group</th>
<th>Placebo group</th>
<th>Supplement group</th>
<th>Supplement group</th>
<th>All children beginning study</th>
<th>Typical children (ages 6–9)</th>
<th>Typical children (ages 8–11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>End</td>
<td>Beginning</td>
<td>End</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n = 8 )</td>
<td>( n = 7 )</td>
<td>( n = 11 )</td>
<td>( n = 10 )</td>
<td>( n = 24 )</td>
<td>( n = 11 )</td>
<td>( n = 11 )</td>
</tr>
<tr>
<td>54</td>
<td>65</td>
<td>56</td>
<td>92</td>
<td>55.5</td>
<td>32</td>
<td>0.00000006</td>
</tr>
</tbody>
</table>

\*Placebo, supplement, and drop-outs.
found a very low activity of pyridoxal kinase (high $K_m$), the enzyme which converts pyridoxal and pyridoxamine into their active forms, PLP and PMP. Thus, a very slow conversion of pyridoxal and pyridoxamine would lead to elevated pyridoxal and pyridoxamine levels and low PLP and PMP levels, which is consistent with this study and the one by Audhya. This would explain why many studies of vitamin B6 have demonstrated a functional need for high doses of vitamin B6 (so that a sufficient amount is converted into the active forms prior to being excreted). Note that consumption of PLP is not necessarily any better than pyridoxal or pyridoxamine, as the phosphate group is likely removed during digestion.

Alpha lipoic acid

Alpha lipoic acid is currently being used in some heavy-metal detoxification treatments for autism, and we investigated whether its level was lower in the children with autism. The mean level was $3.7 \pm 0.6 \mu g/mL$. The reference group (typical adults, no data for children) has a range of 2.3–5.0 $\mu g/mL$. Thus, assuming that the extended time in frozen storage (12 months) did not significantly lower the value, it seems that the alpha lipoic acid levels are not abnormal, but a control group of children rather than adults is needed to properly interpret these results.

Limitations of this study

This pilot study had several limitations. First, the number of participants was small. Although some results were statistically significant according to a simple t-test, a larger study is needed to increase the statistical power.

Second, the behavioral assessment was limited to a very simple parental assessment, based on their observations over 3 months. More rigorous assessment tools are needed, including evaluations by professionals.

Third, the vitamin C measurements would be more valid if pretesting had been done in addition to the post-assessment. Also, the use of controls for vitamin C levels, rather than relying on national norms, would also increase the validity.

CONCLUSION

Based on a simple parental assessment, it appears that a 3-month treatment with a moderate-dose vitamin/mineral supplement (Spectrum Support) did result in a statistically significant improvement in sleep and GI problems in this group. However, a larger study is needed to increase the statistical power to determine if those results are generally valid.

Vitamin C levels were below average in children with autism not receiving the supplement, whereas those taking the supplement had near-normal levels.

Vitamin $B_6$ is significantly elevated in children with autism, probably due to defective pyridoxal kinase, implying a functional need for more. This may explain why very high doses of vitamin B6 have been shown in numerous studies to benefit children with autism. A future study could measure pyridoxal, pyridoxamine, PMP, and PLP simultaneously to validate this interpretation.

Alpha lipoic acid levels were found to be similar to those found in typical adults, but data for children are needed to fully interpret these results.

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