Abnormally High Plasma Levels of Vitamin B₆ in Children with Autism Not Taking Supplements Compared to Controls Not Taking Supplements

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ABSTRACT

Background: There have been many studies of the effect of high-dose supplementation of vitamin B₆ on children and adults with autism, with all but one reporting benefits.

Objective: The aim of this study was to investigate the biochemical basis for vitamin B₆ therapy by measuring the level of total vitamin B₆ in the plasma of unsupplemented children with autism spectrum disorder compared to unsupplemented control subjects.

Participants: Children with autism spectrum disorders (n = 35, age 3–9 years) and unrelated typical children (n = 11, age 6–9 years), all from Arizona, were studied. (This includes the data from 24 children with autism from our previous study.)

Methodology: A microbiologic assay was used to measure the level of total vitamin B₆ (including phosphorylated and unphosphorylated forms), in a blinded fashion.

Results: Children with autism had a 75% higher level of total vitamin B₆ than the controls (medians of 56 versus 32 ng/mL, respectively, p = 0.00002). Most of the autistic children (77%) had levels that were more than 2 standard deviations above the median value of the controls. The autistic girls (n = 5) also had elevated levels (mean of 54.6 ng/mL, median of 60 ng/mL).

Discussion: These results are consistent with previous studies that found that: (1) pyridoxal kinase had a very low activity in children with autism and (2) pyridoxal 5 phosphate (PLP) levels are unusually low in children with autism. Thus, it appears that the low conversion of pyridoxal and pyridoxine to PLP results in low levels of PLP, which is the active cofactor for 113 known enzymatic reactions, including the formation of many key neurotransmitters.

Conclusions: Total vitamin B₆ is abnormally high in autism, consistent with previous reports of an impaired pyridoxal kinase for the conversion of pyridoxine and pyridoxal to PLP. This may explain the many published studies of benefits of high-dose vitamin B₆ supplementation in some children and adults with autism.

INTRODUCTION

There have been many studies of the effect of high-dose vitamin B₆ supplementation on children and adults with autism, and all but one of those studies have reported positive benefits, usually in about half of the participants.¹⁻²⁷ (A summary of these studies is available also.²⁷) Most studies of vitamin B₆ have included magnesium (Mg) to prevent Mg deficiency and hyperactivity. These studies have been somewhat controversial because of some limitations in their methodology, primarily caused by limitations in the diagnostic and assessment tools that were available when the studies were con-
ducted. Eleven (11) of the 12 double-blind, placebo-controlled studies reported a favorable response in terms of various behavioral assessments. Only one study of high-dose vitamin B₆ reported negative results, and that study had two limitations. First, it involved only 10 participants, so it was unlikely that any results would be statistically significant. Second, it was a double-blind, placebo-controlled, cross-over study with each phase being only 4 weeks long and with no washout between the phases; the lack of a washout invalidated the “placebo” arm, because B₆ benefits can last for several weeks. (For example, a study by Coleman et al. of “hyperkinetic” children found that B₆ greatly raised serotonin levels with no drop even 3 weeks after stopping B₆.) Although all of the studies had some methodologic limitations, all but one found positive benefits from high-dose vitamin B₆ with Mg (in roughly one half of the children and adults with autism).

There was also one study by Tolbert et al. that used a much lower dose (2.86 mg/kg body weight, versus typically 30 mg/kg in most of the other studies). That study did not find a positive benefit, which suggests that the higher dose used in the other studies was necessary.

There have only been two previous studies of the level of vitamin B₆ in children with autism. One study was done by Sankar, who found high levels of vitamin B₆ in approximately 19 autistic children compared to a reference range in the literature, but the reference range was determined with a different method and is questionable. (Other disability groups in their study also had high levels of vitamin B₆). The second study was done by the current authors’ investigative group. In that study the level of total vitamin B₆ (including both phosphorylated and unphosphorylated forms) was measured in the plasma of 24 unsupplemented children with autism, which was found to be unusually high compared to the testing laboratory’s reference range for typical children who were not taking the supplement. Table 1 shows the results of that study. It should be noted that the children with autism were somewhat younger than most of the typical children; and because vitamin B₆ levels increase with age, the difference between children with autism and age-matched typical children is probably even slightly greater. That study was limited because: it depended on the reference range of the testing laboratory (Vitamin Diagnostics, Clifford Beach, NJ); the samples were not tested in a blinded manner; and the samples were not simultaneously measured. Therefore an additional study was needed, which is the focus of the present paper.

This paper reports the results of testing of the plasma levels of vitamin B₆ in 11 more children with autism, who were compared to an additional 11 control subjects. The testing method is identical to that used in a previous study by the current authors’ investigative group, so the data for meta-analysis can be pooled. A simple test was used to compare the groups, assuming a normal distribution, with a value of \( p < 0.05 \) being considered statistically significant.

## METHODS

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

### Participant selection

The participants were recruited in Arizona. The selection criteria for the study included:

1. Age 3–9
2. No use of vitamin B₆ supplements, or supplements that contained vitamin B₆, in the 2 months before testing
3. Autism Spectrum Disorders (ASD) group: diagnosis of an autism spectrum disorder (autism, Pervasive Development & Disorders Not Otherwise Specified [PDD/NOS], or Asperger’s syndrome) by a psychiatrist or developmental pediatrician
4. Control group: unrelated to a child with ASD (not a sibling or cousin), and in good mental and physical health.

### Table 1. Participant Information and Total Vitamin B₆ Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Age range</th>
<th>Age: Mean ± standard deviation</th>
<th>Male/female</th>
<th>Total vitamin B₆ in plasma (ng/mL): Mean, median, and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism, previous study</td>
<td>3–8</td>
<td>4.9 ± 1.4 years</td>
<td>22 male, 2 female</td>
<td>55.5, 55, ± 7.5²⁄₃²⁄₃³²⁄₃³²⁄₃³²⁄₃³</td>
</tr>
<tr>
<td>Autism, present study</td>
<td>5–9</td>
<td>7.2 ± 1.4 years</td>
<td>8 male, 3 female</td>
<td>56.0, 56, ± 21²⁄₃²⁄₃³²⁄₃³²⁄₃³</td>
</tr>
<tr>
<td>Controls, present study</td>
<td>6–9</td>
<td>7.8 ± 1.2 years</td>
<td>10 male, 1 female</td>
<td>36.0, 32, ± 8.8</td>
</tr>
</tbody>
</table>

³⁻Test of B₆ levels of previous autism versus controls: \( p = 0.0000001 \).
²⁻Test of B₆ levels of present autism versus controls: \( p = 0.001 \).
¹⁻Test of B₆ levels of previous and present autism versus controls: \( p = 0.00002 \).
VITAMIN B₆ LEVELS IN CHILDREN WITH AUTISM

The present results confirm the previous finding by this investigative group of much higher levels of vitamin B₆ in children with autism not taking the supplement compared to control subjects. In fact, only two of 35 children with autism had values at or below the median of the typical children. Most of the children with autism (77%) had levels that were 2 standard deviations around the median of the control children.

In a previous study, levels of pyridoxal 5 phosphate had been measured and found to be generally much lower than levels in control subjects. In another study, it was found that in autistic children the enzyme pyridoxal kinase (which phosphorylates vitamin B₆ to pyridoxal-5-phosphate—the biochemically active form of vitamin B₆) has decreased binding affinity (increased Michaelis constant or $K_m$) for vitamin B₆, possibly because of the polymorphic nature of the enzyme. Therefore, it is possible that high doses of vitamin B₆ increased intracellular substrate concentrations and thus activated the defective enzyme. An extensive review by Ames et al. on the molecular basis of disease arising from the mutations in the genes of many enzymes has recently been published. So, a low activity of pyridoxal kinase would ultimately result in low levels of PLP and high levels of pyridoxal. Those results are consistent with the present study, which finds a high amount of total vitamin B₆ (including phosphorylated and unphosphorylated forms), as typically the amount of the unphosphorylated forms is much higher than the phosphorylated forms in the blood.

PLP is an enzymatic cofactor for 113 of the 3870 enzymes cataloged in the ENZYME database (www.expasy.org/enzyme), including the formation of major neurotransmitters such as serotonin, GABA, and the catecholamines. Thus low levels of PLP could have wide-ranging effects on human metabolism, including those on mental function. Normalization of PLP would be expected to improve mental and physical function in some cases. This may explain the many reports of improvement in autistic symptoms upon treatment with high-dose vitamin B₆.

One might wonder whether similar improvements would occur by simply giving PLP. However, it appears that the phosphate group is removed during digestion, so that PLP would likely have no additional benefit over pyridoxal. Also, the previous study by this investigative group compared 6 months of treatment with PLP or pyridoxine HCl in 184 children with autism, and found adverse effects (worsening of behaviors) in 10% of those children receiving PLP (half the group) versus none in those receiving pyridoxine HCl. Therefore it appears that vitamin B₆ should be given as pyridoxal HCl or pyridoxine HCl, not as PLP.

CONCLUSIONS

Children with autism have abnormally high plasma levels of vitamin B₆ compared to controls. This is consistent with previous reports of an impaired pyridoxal kinase for...
the conversion of pyridoxine to pyridoxal to PLP. This may explain the many published reports of high-dose vitamin B₆ supplementation in some children and adults with autism.

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REFERENCES


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