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Abnormally High Plasma Levels of Vitamin B_6 in Children with Autism Not Taking Supplements Compared to Controls Not Taking Supplements

JAMES B. ADAMS, Ph.D.,[1] FRANK GEORGE, D.O.,[2] and T. AUDHYA, Ph.D.[3]

ABSTRACT

Background: There have been many studies of the effect of high-dose supplementation of vitamin B_6 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 B_6 therapy by measuring the level of total vitamin B_6 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 B_6 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 enymatic reactions, including the formation of many key neurotransmitters.

Conclusions: Total vitamin B_6 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 B_6 supplementation in some children and adults with autism.

INTRODUCTION

There have been many studies of the effect of high-dose vitamin B_6 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.[1–26,*] (A summary of these studies is available also.[27]) Most studies of vitamin B_6 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-

[1]Arizona State University, Tempe, AZ.
[2]Integrative Medical Health Care, Scottsdale, AZ.
[3]Vitamin Diagnostics, Clifford Beach, NJ.

*Hopkins JN. The effects of vitamin B_6 supplements on the behavior and brain activity of subjects with autism. Unpublished master's thesis, Swinburne University of Technology, Victoria, Australia, 1999.

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 B_6 reported negative results, and that study[7] 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 B_6 benefits can last for several weeks. (For example, a study by Coleman et al.[28] of "hyperkinetic" children found that B_6 greatly raised serotonin levels with no drop even 3 weeks after stopping B_6.) Although all of the studies had some methodologic limitations, all but one found positive benefits from high-dose vitamin B_6 with Mg (in roughly one half of the children and adults with autism).

There was also one study by Tolbert et al.[29] 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 B_6 in children with autism. One study was done by Sankar,[30] who found high levels of vitamin B_6 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 B_6). The second study was done by the current authors' investigative group.[31] In that study the level of total vitamin B_6 (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 B_6 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 B_6 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 B_6 supplements, or supplements that contained vitamin B_6, 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

Table 1. Participant Information and Total Vitamin B_6 Results

Group	*Age range*	*Age: Mean ± standard deviation*	*Male/female*	*Total vitamin B_6 in plasma (ng/mL): Mean, median,and standard deviation*
Autism, previous study	3–8	4.9 ± 1.4 years	22 male, 2 female	55.5, 55, ± 7.5[a,c]
Autism, present study	5–9	7.2 ± 1.4 years	8 male, 3 female	56.0, 56, ± 21[b,c]
Controls, present study	6–9	7.8 ± 1.2 years	10 male, 1 female	36.0, 32, ± 8.8

[a] *t*-Test of B_6 levels of previous autism versus controls: $p = 0.0000001$.
[b] *t*-Test of B_6 levels of present autism versus controls: $p = 0.001$.
[c] *t*-Test of B_6 levels of previous and present autism versus controls: $p = 0.00002$.

health (no attention-deficit disorder/attention-deficit/hyperactivity, etc.).

There were 22 applicants (11 children with autism and 11 control subjects) who applied and met the criteria, and they were enrolled in the study. Their demographic characteristics are listed in Table 1. (This control group was referenced in a previous paper.[31])

Sample mesurement

The samples were measured in a blinded fashion by Vitamin Diagnostics Laboratory using the same methods as in the previous study.[31]

RESULTS

The results of the current and previous measurements are shown in Table 1. The vitamin B_6 levels of the children with autism in the present study were very close to those of the previous study, with averages of 56 and 55.5 ng/mL, respectively. Also, the vitamin B_6 levels for the controls in the present study (median of 32 ± 9 ng/mL) are similar to those of the laboratory reference range used in the previous study (22–47 ng/mL). (Note that because of a typographic error the units in the previous study were printed incorrectly, and were actually ng/mL).

A comparison of the two groups in the present study shows that the children with ASD had much higher levels of vitamin B_6 than the control subjects (averages of 56 and 36 ng/mL, respectively, medians of 56 and 32 ng/mL, respectively). Based on a two-sided *t*-test, the difference was statistically significant ($p = 0.001$), although the small number of participants meant that the statistical power was weak.

As the data in the present study were derived using the same equipment and methods as in the previous study, and as the autism groups in both studies had nearly identical means and medians, it was appropriate to pool the data for the children with autism from both studies for a meta-analysis. In comparing both autism groups ($n = 36$) and the present control group (the previous study used this control group as a reference), the difference was highly statistically significant ($p = 0.00002$).

It is interesting to note that the five autistic girls in the combined past/present study had a mean level of 54.6 ng/mL and a median of 60 ng/mL, extremely similar to that of the autistic boys. Larger numbers are needed to be certain, but these limited data suggest that autistic girls also tend to have elevated total vitamin B_6 plasma levels.

DISCUSSION

The present results confirm the previous finding by this investigative group of much higher levels of vitamin B_6 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,[31] levels of pyridoxal 5 phosphate had been measured and found to be generally much lower than levels in control subjects. In another study,[2] it was found that in autistic children the enzyme pyridoxal kinase (which phosphorylates vitamin B_6 to pyridoxal-5-phosphate—the biochemically active form of vitamin B_6) has decreased binding affinity (increased Michaelis constant or K_m) for vitamin B_6, possibly because of the polymorphic nature of the enzyme. Therefore, it is possible that high doses of vitamin B_6 increased intracellular substrate concentrations and thus activated the defective enzyme. An extensive review by Ames et al.[32] 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 B_6 (including phosphorylated and unphosphorylated forms), as typically the amount of the unphosphorylated forms is much higher than the phsophorylated forms in the blood.

PLP is an enzymatic cofactor for 113 of the 3870 enzymes catalogued 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 B_6.

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 B_6 should be given as pyridoxal HCl or pyridoxine HCl, not as PLP.

CONCLUSIONS

Children with autism have abnormally high plasma levels of vitamin B_6 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 B_6 supplementation in some children and adults with autism.

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REFERENCES

1. Adams JB, Audhya T, Vogelaar E. Nutritional Abnormalities in Autism, and the Effect of Nutritional Supplementation. 2003 conference proceedings of the National Autism Society of America, Pittsburgh, PA.
2. Audhya T. Laboratory Indices of Vitamin and Mineral Deficiency in Autism. Conference Proceedings of the Fall DAN! 2002 Conference, San Diego, CA, 239–244.
3. Baker H, Frank O, Ning M, et al. A protozoological method for detecting clinical vitamin B_6 deficiency. Am J Clin Nutr 1966;18:123–133.
4. Barthelmey C, Garreau B, Leddet I, et al. Behavioral and biological effect of oral magnesium, vitamin B6, and combined magnesium–B6 administration in autistic children. Magnes Bull 1981;3;150–153.
5. Barthelmey C, Garreau B, Leddet I, et al. Behavioral and biological effects of oral magnesium, vitamin B_6, and combined magnesium–B_6 administration in autistic children [in French]. Neuropsychiaurie de l'Enfance 1983;31;289–301.
6. Bonisch VE. Erfahrungen with pryithosin bei hirngeschadigten kinder mit autischem syndrom [in German]. Proxis der Kinderpsychologie 1968;8:308–310.
7. Ellman G. Pyridoxine effectiveness on autistic patients at Sonoma State Hospital. Paper presented at Research Conference on Autism, San Diego, CA. November 1, 1981.
8. Findling RL, Maxwell K, Scotese-Wojtila L, et al. High-dose pyridoxine and magnesium administration in children with autistic disorder: An absence of salutary effects in a double-blind, placebo-controlled study. J Autism Dev Disorders 1997;27:467–478.
9. Garner C, Conroy E, Barthelemy Cl, et al. Dopamin-beta hyrozalase(DBH) and homovanillic acid (HVA) in autistic children. J Autism Develop Disorders 1986;16:23–29.
10. Gualtieri CT, Von Bourgonndien ME, Hartx C. Pilot study of pyridozine treatment in autistic children. Paper presented at American Psychiatric Association meeting, New Orleans, LA, May 1981.
11. Heeley AF, Roberts GE. A study of tryptophan metabolism in psychotic children. Develop Med Child Neurol 1966;8:708–718.
12. Jonas C, Etienne T, Barthelemy C, et al. Clinical and biochemical value of magnesium + vitamin B_6 conbination in the treatment of residual autism in adults [in French]. Therapie 1984;39:661–669.
13. LeLord G, Muh JP, Barthelemy C, et al. Effects of pryidozine and magnesium on autistic symptoms: Initial observations. J Autism Develop Disorders 1981;11:219–230.
14. LeLord G, Muh JP, Barthelemy C, et al. Clinical and biological effects of bitamin B6 + magnesium in autistic subjects. In: Leklem J, Reynolds R, eds. Vitamin B_6 Responsive Disorders in Humans. New York: Alan R. Liss, 1988.
15. Martineau J, Garreau B, Barthelemy C, Alelord O. Comparitive effects of oral B6, B6-Mg, and Mg administration on evoked potentials conditioning in autistic children. In: Rothenberger A, ed. Proceedings: Symposium on Event-Related Potentials in children, Essen, FRG 11–13 June 1982. Amsterdam: Elsevier Biomedical, 1982:411–416.
16. Martineau J, Barthelemy C, Garreau B, Lelord G. Vitamin B6, magnesium and combined B6-Mg: Therapeutic effects in childhood autism. Biol Psychiatry 1985;20:467–468.
17. Martineau J, Barthelemy C, Lelord G. Long-term effects of combined vitamin B6-magnesium administration in an autistic child. Biol Psychiatry 1986;21:511–518.
18. Martineau J, Barthelemy C, Cheliakine C, Lelord G. Brief report: An open middle-term study of combined vitamin B6-magnesium in a subgroup of autistic children selected on their sensitivity to this treatment. J Autism Dev Disord 1988;18: 435–447.
19. Martineau J, Barthelemy C, Roux S, et al. Electrophysiological effects of fenfluramine or combined vitamin B6 and magnesium on children with autistic behaviour. Dev Med Child Neurol 1989;31:721–727.
20. Menage P, Thibault G, Barthelemy C, et al. CD4+ CD45RA+ T lymphocyte deficiency in autistic children: Effect of a pyridoxine-magnesium treatment. Brain Dysfunct 1999;5:326–333.
21. Moreno H, Borjas L, Arrieta A, et al. Clinical heterogeneity of the autistic syndrome: A study of 60 families. Invest Clin 1992;33:13–31.
22. Rimland B. High dosage levels of certain vitamins in the treatment of children with severe mental disorders. In: Orthomolecular Psychiatry. Hawkins D, Pauling L, eds. New York; WH Freeman, 1973:513–538.
23. Rimland B. An orthomolecular study of psychotic children. Orthomol Psychiatry 1974;3:371–377.
24. Rimland B, Callaway E, Dreyfus P. The effects of high doses of vitamin B6 on autistic children: A double-blind crossover study. Am J Psychiatry 1978;135:472–475.
25. Rimland B. Controversies in the treatment of autistic children: Vitamin and drug therapy. J Child Neurol 1988;3(Suppl): S68–S72.
26. Rimland B, Edelson SM. Parent Ratings of Behavior Effects of Biomedical Interventions. Autism Research Institute San Diego, CA: 2003.
27. Rossi P, Visconti P, Bergossi A, Balestra V. Effects of vitamin B6 and magnesium therapy in autism. Conference on the Neurobiology of Infantile Autism, Tokyo, Japan, Nov. 10–11, 1990. Online document at: www.autismwebsite.com/ari/treatment/b6studies.htm
28. Coleman M, Steinberg G, Tippett J, et al. A preliminary study of the effect of pyridoxine administration in a subgroup of hy-

perkinetic children: A double blind crossover comparison with methylphenidate. Biol Psychiatry 1979;14:741–751.
29. Tolbert L, Haigler T, Waits MM, Dennis T. Brief report: Lack of response in an autistic population to a low dose clinical trial of pyridoxine plus magnesium. J Autism Dev Disord 1993; 23:193–199.
30. Sankar DV. Plasma levels of folates, riboflavin, vitamin B_6, and ascorbate in severely disturbed children. J Autism Dev Disorders 1979;9:73–82.
31. Adams JB, Holloway C. Pilot study of a moderate dose multivitamin/mineral supplement for children with autistic spectrum disorder. J Altern Complement Med 2004;10:1033–1039.
32. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K_m): Relevance to genetic disease and polymorphisms. Am J Clin Nutr 2002;75:616–658.

Address reprint requests to:
James B. Adams, Ph.D.
Arizona State University
PO Box 876006
Tempe, AZ 85287-6006

E-mail: jim.adams@asu.edu