Decreased Serum Cu/Zn SOD in Children with Autism

A.J. Russo
Research Director, Health Research Institute/Pfeiffer Treatment Center, 4575 Weaver Parkway, Warrenville, Illinois 60555, USA. Email: ajrusso@hriptc.org

Abstract
Aim: To assess serum Cu/Zn SOD (Superoxide Dismutase) concentration in autistic children and evaluate its possible relationship to GI Symptoms.

Subjects and Methods: Serum from 50 autistic children (31 with chronic digestive disease (most with ileo-colonic lymphoid nodular hyperplasia (LNH) and inflammation of the colorectum, small bowel and/or stomach) and 19 autistic children without GI disease), and 29 non autistic controls (20 age matched non autistic children with no GI disease and 9 age matched non autistic children with GI disease) were tested for Cu/Zn SOD using ELISAs.

Results: Serum Cu/Zn SOD levels of autistic children were significantly lower than all non autistic controls (p < 0.0001). Serum Cu/Zn SOD of autistic children with severe GI disease was significantly lower than autistic children with no GI disease (p < 0.0001), non autistic children without GI disease (<0.0001) and non autistic children with GI disease (p = 0.0003).

Discussion: These results suggest an association between Cu/Zn SOD serum levels and autism, particularly autistic children with GI disease, and that the concentration of serum Cu/Zn SOD may be a useful biomarker for autistic children with severe GI disease.

Keywords: autism, Cu/Zn SOD, super oxide dismutase, GI disease
Introduction

Autism is a complex, behaviorally defined neurodevelopmental disorder characterized by social deficits, language impairments, and repetitive behaviors with restricted interests.

There has been a dramatic increase in the diagnosis of autism over the past decade.\(^1\)

While genes play a major role in the etiology, the cause of autism remains elusive, and is considered multifactorial, influenced by genetic, environmental, and immunological factors, as well as increased vulnerability to oxidative stress. No single gene has been found to be associated with autism, and involvement of multiple genes has been postulated.\(^2-5\)

Environmental factors, such as mercury, lead, measles, rubella virus, retinoic acid, maternal thalidomide, valproic acid and alcohol use during pregnancy have been suggested to be involved in the etiology the disease,\(^6-10\) and behavioral impairments, gastrointestinal disturbances,\(^11-15\) epilepsy,\(^16\) immune,\(^3,17-19\) autoimmune,\(^20-22\) and infectious factors\(^8,9,23-27\) have also been suggested to play role in autism pathophysiology.

In vivo, oxygen radicals are produced as byproducts of normal oxidative metabolism.\(^28\) Hence, activated cells with increased metabolism produce more oxygen radicals. In addition, macrophages, which are phagocytic cells, produce and release reactive oxygen species (ROS)\(^29\) in response to phagocytosis or stimulation with various agents. It has long been known that control of the intracellular redox environment is vital for proper cellular function. To protect themselves from the constant oxidative challenge, cells have developed defense mechanisms that ensure a proper balance between pro- and antioxidant molecules.\(^30\) Cu/Zn superoxide dismutase (SOD-1) is a key enzyme in the dismutation of superoxide radicals resulting from cellular oxidative metabolism into hydrogen peroxide.\(^29\)

Increasing evidence suggests a role for oxidative stress in the manifestation of autism.\(^31,32\) In fact, oxidative stress has also been implicated in the pathogenesis of other neuropsychiatric diseases, including schizophrenia,\(^33-35\) major depressive disorder,\(^35\) anxiety disorders such as panic disorder,\(^37\) and obsessive-compulsive disorder.\(^38\) It is probable that autism may result from an interaction between genetic, environmental, and immunological factors, with oxidative stress as a mechanism linking these risk factors.

Several studies have suggested that modifications in anti-oxidant enzymes may play a role in the etiology of autism. For instance, compared to controls, patients with autism showed decreased activity of glutathione peroxidase in plasma\(^39\) and in erythrocytes,\(^39,40\) reduced levels of total glutathione, lower redox ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) in autistic children with GI disease and controls.

Figure 1. The mean ± SD Cu/Zn SOD concentration (ng/ml) of 50 autistic children (31 with chronic digestive disease (most with ileo-colonic lymphoid nodular hyperplasia (LNH) and inflammation of the colorectum, small bowel and/or stomach) and 19 autistic children without GI disease), and 29 non autistic controls (20 age matched non autistic children with no GI disease and 9 age matched non autistic children with GI disease).
Serum Cu/Zn SOD in autistic children

Table 1. Significant difference between Cu/Zn SOD concentration (pg/ml) of 50 autistic children (with and without GI disease) and 29 age matched non autistic controls (with and without GI disease).

| Group | Autistic | Non autistic |
|-------|---------|-------------|
| Mean  | 0.854   | 1.751       |
| SD    | 0.651   | 0.817       |
| SEM   | 0.092   | 0.152       |
| N     | 50      | 29          |

$p < 0.0001$.

(GSSG) in plasma, and decreased catalase and SOD activity in erythrocytes.

Zinc to copper ratio is abnormally low in individuals with autism and zinc is also antagonistic to copper absorption, and therefore zinc deficiency often occurs simultaneously with an excess of copper. The loss of copper and zinc homeostasis is purportedly a likely indicator of a metallothionein deficiency.

Low zinc levels have been directly related to low Cu/Zn SOD concentration. Because of this, we hypothesized that autistic children, particularly those with GI disease, would have abnormal levels of Cu/Zn SOD.

Materials and Methods

ELISA to measure serum Cu/Zn SOD (Bender Systems)

All reagents and specimens were equilibrated to room temperature before the assay was performed. A 1:51 dilution of the patient samples was prepared by mixing 10 µl of the patient’s sera with 0.5 ml of Serum Diluent. One hundred microliters of calibrators (0.08–2.5 ng/ml Cu/Zn SOD), serum diluent alone, and diluted patient samples were added to the appropriate microwells of a microculture plate (each well contained affinity purified polyclonal IgG to Cu/Zn SOD). Wells were incubated for 60 minutes (±5 min) at room temperature, then washed 4x with wash buffer. One hundred microliters of pre-diluter antihuman Cu/Zn SOD IgG conjugated with HRP was added to all microwells, incubated for 30 minutes (±5 min) at room temperature, then wash 4x with wash buffer. One hundred microliters of enzyme substrate was added to each microwell. After approximately 30 minutes at room temperature, the reaction was stopped by adding 50 µl of 1 M sulfuric acid, then the wells were read at 405 nm with an ELISA reader (BioRad Laboratories, Inc., Hercules, CA, USA).

Subjects

The diagnosis of autism for all subjects in this study was made using the standard Autism Diagnostic Interview-Revised (ADI-R) algorithm, and ASSQ assessment was used to exclude autism in controls.

GI pathology was determined through medical history data and, in the case of those with GI disease, through endoscopic diagnosis.

Experimental

Serum from autistic individuals with GI disease (n = 31) was obtained from the Thoughtful House, Austin, Texas and chosen randomly. All of these children (median age 6 years; range 2–16; 8 male) had chronic digestive disease, most characterized with...
Table 3. Serum Cu/Zn SOD associated with severity of GI disease and auto antibodies.

| Patient | OD_1  | OD_2  | Mean  | STD DEV | Cu/Zn SOD (ng/ml) | Mean O.D. anti-PR3** | Mean O.D. anti-MPO*** | Mean O.D. ASCA**** |
|---------|-------|-------|-------|---------|-------------------|----------------------|-----------------------|---------------------|
| 1       | 0.247 | 0.222 | 0.235 | 0.018   | 0.408             | 0.227                | 0.306                 | 0.169               |
| 2       | 0.150 | 0.140 | 0.145 | 0.007   | 0.118             | 0.289                | 0.327                 | 0.282               |
| 3       | 0.170 | 0.154 | 0.162 | 0.011   | 0.173             | 0.173                | 0.394                 | 0.195               |
| 4       | 0.170 | 0.135 | 0.153 | 0.025   | 0.142             | 0.166                | 0.313                 | 0.169               |
| 5       | 0.160 | 0.159 | 0.160 | 0.001   | 0.165             | 0.141                | 0.414                 | 0.169               |
| 6       | 0.120 | 0.124 | 0.122 | 0.003   | 0.044             | 0.482                | 0.566                 | 0.78                |
| 7       | 0.142 | 0.143 | 0.143 | 0.001   | 0.110             | 0.235                | 0.435                 | 0.29                |
| 8       | 0.157 | 0.156 | 0.157 | 0.001   | 0.155             | 0.274                | 0.506                 | 0.343               |
| 9       | 0.173 | 0.160 | 0.167 | 0.009   | 0.188             | 0.221                | 0.537                 | 0.249               |
| 10      | 0.119 | 0.134 | 0.127 | 0.011   | 0.058             | 0.140                | 0.356                 | 0.151               |
| 11      | 0.111 | 0.121 | 0.116 | 0.007   | 0.024             | 0.533                | 0.784                 | 0.696               |
| 12      | 0.916 | 0.788 | 0.852 | 0.091   | 0.872             | 0.196                | 0.360                 | 0.299               |
| 13      | 0.884 | 0.884 | 0.884 | 0.000   | 0.916             | 0.259                | 0.463                 | 0.248               |
| 14      | 0.592 | 0.636 | 0.614 | 0.031   | 0.546             | 0.174                | 0.457                 | 0.174               |
| 15      | 0.674 | 0.671 | 0.672 | 0.002   | 0.626             | 0.199                | 0.503                 | 0.196               |
| 16      | 0.569 | 0.569 | 0.569 | 0.000   | 0.484             | 0.443                | 0.539                 | 0.426               |
| 17      | 0.721 | 0.736 | 0.728 | 0.011   | 0.703             | 0.278                | 0.529                 | 0.228               |
| 18      | 0.594 | 0.585 | 0.589 | 0.006   | 0.513             | 0.147                | 0.302                 | 0.176               |
| 19      | 1.415 | 1.364 | 1.389 | 0.036   | 0.610             | 0.251                | 0.351                 | 0.31                |
| 20      | 0.527 | 0.486 | 0.506 | 0.029   | 0.399             | 0.129                | 0.322                 | 0.162               |
| 21      | 0.626 | 0.638 | 0.632 | 0.008   | 0.571             | 0.121                | 0.286                 | 0.161               |
| 22      | 0.688 | 0.643 | 0.665 | 0.032   | 0.617             | 0.197                | 0.361                 | 0.253               |
| 23      | 0.717 | 0.676 | 0.696 | 0.029   | 0.659             | 0.140                | 0.356                 | 0.151               |
| 24      | 0.709 | 0.665 | 0.687 | 0.031   | 0.646             | 0.099                | 0.225                 | 0.128               |
| 25      | 0.67 | 0.713 | 0.691 | 0.030   | 0.652             | 0.175                | 0.329                 | 0.169               |
| 26      | 0.841 | 0.795 | 0.818 | 0.033   | 0.826             | 0.576                | 0.629                 | 0.793               |
| 27      | 0.553 | 0.531 | 0.542 | 0.016   | 0.447             | 0.245                | 0.416                 | 0.219               |
| 28      | 1.039 | 1.058 | 1.048 | 0.013   | 1.142             | 0.189                | 0.341                 | 0.169               |
| 29      | 0.489 | 0.515 | 0.502 | 0.018   | 0.393             | 0.366                | 0.450                 | 0.556               |
| 30      | 1.05 | 1.049 | 1.049 | 0.001   | 1.143             | 0.134                | 0.274                 | 0.157               |
| 31      | 0.811 | 0.943 | 0.877 | 0.093   | 0.907             | 0.544                | 0.504                 | 0.277               |
Table 3. (Continued)

| Diagnosis | LNH | Eryth | Total GI | AutoAb | No AutoAb | LNH | No LNH | High total GI | Low total GI |
|-----------|-----|-------|----------|--------|-----------|-----|--------|---------------|-------------|
| RA        | 3   | 0     | 5        | 0.408  | 0.408     |     |        | 0.408         | 0.408       |
| RA        | 6   | 0     | 10       | 0.118  | 0.118     | 0.118|        |               |             |
| A         | 4   | 0     | 7        | 0.173  | 0.173     | 0.173|        |               |             |
| RA        | 3   | 2     | 7        | 0.142  | 0.142     | 0.142|        |               |             |
| RA        | 2   | 0     | 3        | 0.165  | 0.165     | 0.165|        |               |             |
| RA        | 3   | 2     | 6        | 0.044  | 0.044     | 0.044|        |               |             |
| RA        | 3   | 0     | 6        | 0.110  | 0.110     | 0.110|        |               |             |
| R-PDD/NOS | 2   | 1     | 4        | 0.155  | 0.155     | 0.155|        |               |             |
| RA        | 2   | 2     | 7        | 0.188  | 0.188     | 0.188|        |               |             |
| RA        | 2   | 0     | 4        | 0.058  | 0.058     | 0.058|        |               |             |
| A         | NA  | NA    | NA       | 0.024  |           |      |        |               |             |
| RA        | 0   | 2     | 5        | 0.872  | 0.872     | 0.872|        |               |             |
| RA        | 3   | 0     | 4        | 0.916  | 0.916     | 0.916|        |               |             |
| A         | 3   | 4     | 6        | 0.546  | 0.546     | 0.546|        |               |             |
| RA        | 3   | 2     | 5        | 0.626  | 0.626     | 0.626|        |               |             |
| R-PDD     | 5   | 0     | NA       | 0.484  | 0.484     | 0.484|        |               |             |
| R-PDD     | 3   | 1     | 8        | 0.703  | 0.703     | 0.703|        |               |             |
| RA        | 3   | 1     | 7        | 0.513  | 0.513     | 0.513|        |               |             |
| R-UD      | 4   | 2     | 8        | 1.610  | 1.610     | 1.610|        |               |             |
| A         | 3   | 1     | 6        | 0.399  | 0.399     | 0.399|        |               |             |
| RA        | 1   | 0     | 7        | 0.571  | 0.571     | 0.571|        |               |             |
| PDD       | 2   | 1     | 4        | 0.617  | 0.617     | 0.617|        |               |             |
| RA        | 2   | 0     | 4        | 0.659  | 0.659     | 0.659|        |               |             |
| R-ASP     | 2   | 1     | 6        | 0.646  | 0.646     | 0.646|        |               |             |
| RA        | 3   | 1     | NA       | 0.652  | 0.652     | 0.652|        |               |             |
| A         | 4   | 4     | 11       | 0.826  | 0.826     | 0.826|        |               |             |


\[ \text{p} = 0.2829 \quad \text{p} = 0.5164 \quad \text{p} = 0.3477 \]
ileo-colonic lymphoid nodular hyperplasia (LNH) and inflammation of the colorectum, small bowel and/or stomach (identified by endoscopy).

**Controls**
Three control groups (n = 48) were studied, 19 autistic children without GI disease, and 29 non autistic controls (20 age matched non autistic children with no GI disease and 9 age matched non autistic children with GI disease). Serum and medical history of controls were obtained from the Autism Genetic Resource Exchange (AGRE*).

**Serums**
Experimental (Thoughtful House**) and control (AGRE) serums were all morning draws and then treated in an identical fashion—frozen at −70°C immediately after collection and cell/serum separation, then stored at −70°C until thawed for use in ELISAs.

**Statistics**
Inferential statistics were derived from t-test and odds ratios with 95% confidence intervals. ANOVA analysis was used to do an analysis of variance and multiple comparisons.

**Approval**
This project has been approved by the Institutional Review Board of the Pfeiffer Treatment Center, Warrenville, Illinois.

**Results**
Serum from 50 autistic children; including 31 with chronic digestive disease (most with ileo-colonic lymphoid nodular hyperplasia and inflammation of the colorectum, small bowel and/or stomach) and 20 autistic children without GI disease, and 29 non autistic controls; including 20 age matched non autistic children with no GI disease and 9 age matched non autistic children with GI disease, was tested for Cu/Zn SOD using ELISAs designed to quantitate Cu/Zn SOD levels (described above). Each assay was repeated two or more times, with multiple wells for each serum in each assay. The results of a typical assay are represented on Figure 1. (Fig. 1: Controls).

Serum Cu/Zn SOD levels of autistic children were significantly lower than non autistic children (p < 0.0001) (Table 1), and serum Cu/Zn SOD levels in autistic children with GI disease were significantly lower than each of the other groups (autistic children with no GI disease (p < 0.0001), non autistic children without GI disease (<0.0001) and non autistic children with GI disease (p = 0.0003)) (Table 2). A one-way ANOVA analysis was also performed on the four groups (F = 24.83; p < 0.0001).

Cu/Zn SOD concentration of autistic children with GI disease was compared to GI disease severity (including LNH and erythema). There was no significant association between Cu/Zn SOD levels and severity of the GI disease, including severity of LNH and erythema.

We previously reported that some of these same autistic children with GI disease had serum autoantibodies, measured by ELISA. We found borderline association between the presence of three autoantibodies (anti-PR3, anti-MPO and ASCA) and low Cu/Zn SOD (Table 3).

**Discussion**
Evidence suggests that increased oxidative stress is associated with autism, with likely contributions from environmental, genetic and immunological factors. This may be due to (a) increased production of endogenous pro-oxidants (such as NO, xanthine oxidase, homocysteine) or environmental pro-oxidants, or deficiencies of antioxidants (ceruloplasmin, transferrin, superoxide dismutase, glutathione peroxidase, catalase, reduced glutathione), or both. Reduced levels of serum ceruloplasmin (a copper-transport protein) and transferrin (an iron-transport protein) in autism suggest that metabolism of iron and copper (pro-oxidant components of oxidative stress) may be abnormal.

Increased oxidative stress, in turn, may lead to membrane lipid abnormalities, mitochondrial dysfunction, excitotoxicity, inflammation and immune...
dys-regulation\textsuperscript{77,80–82} in autistic children, and might contribute to behavioral aberrations, sleep disorder, and gastrointestinal disturbances.\textsuperscript{83,84} Preliminary results of clinical trials have suggested improved behavior in individuals who receive antioxidant therapy.\textsuperscript{85–87}

A major cause of damage to cells results from reactive oxygen species (ROS)-induced alteration of proteins and DNA by reactive electrophilic oxidation products from polyunsaturated fatty acyls in membrane lipids. Oxidative stress and ROS have been implicated in disease states such as Alzheimer’s disease, Parkinson’s disease, cancer, atherosclerosis, age-related macular degeneration (AMD), and aging. Under normal conditions, ROS are cleared from the cell by the action of superoxide dismutase (SOD),\textsuperscript{88} and abnormal concentrations may lead to an atypical brain phenotype in autistic children.\textsuperscript{89}

Our results show that a significant number of autistic children, particularly those with severe GI disease, have a lower concentration of serum Cu/Zn SOD when compared to controls. Because low zinc concentrations have been associated with autism and related to lower Cu/Zn SOD levels, it may be associated with low Zn levels in autistic children. Also, low Cu/Zn SOD suggests a relationship between this anti-oxidant, thus oxidative stress, and autism—particularly in autistic children with GI disease.

Disclosure
This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author reports no conflicts of interest.

References
1. Muhle R, Trentacoste SV, Rapin I. The Genetics of Autism. Pediatrics. 2004;113:472–86.
2. Lamb JA, Moore J, Bailey A, Monaco AP. Autism: recent molecular genetic advances. \textit{Hum Mol Genet.} 2000;9:861–8.
3. Korvatska E, Van de Water J, Anders TF, Gershwin ME. Genetic and immunologic considerations in autism. \textit{Neurobiol Dis.} 2002;9:107–25.
4. Keller F, Persico AM. The neurobiological context of autism. \textit{Mol Neurobiol.} 2003;28:1–22.
5. Sung YJ, Dawson G, Munson J, Schellenberg GD, Wijsman EM. Genetic investigation of quantitative traits related to autism: use of multivariate polygenic models with ascertainment adjustment. \textit{Am J Hum Genet.} 2005;76:68–81.
6. London EA. The environment as an etiologic factor in autism: a new direction for research. \textit{Environ Health Perspect.} 2000;108:401–4.
7. Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? \textit{Neuro Endocrinol Lett.} 2005;26:439–46.
8. Wakefield AJ, Montgomery SM. Autism, viral infection and measles-mumps-rubella-vaccination. \textit{Isr Med Assoc J.} 1999;1:183–7.
9. Fombonne E. Are measles infections or measles immunizations linked to autism? \textit{J Autism Dev Disord.} 1999;29:349–50.
10. Edelson SB, Cantor DS. Autism: xenobiotic influences. \textit{Toxicol Ind Health.} 1998;14:799–811.
11. Horvath K, Pernan JA. Autism and gastrointestinal symptoms. \textit{Curr Gastroenterol Rep.} 2002;4:251–8.
12. White JE. Intestinal pathology in autism. \textit{Exp Biol Med.} (Maywood). 2003;228:639–49.
13. Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon JT. Gastrointestinal abnormalities in children with autism. \textit{J Pediatr.} 1999;135:559–63.
14. Wakefield AJ, Anthony A, Murch SH, et al. Walker-Smith, Entero-colitis in children with developmental disorders. \textit{Am J Gastroenterol.} 2000;95:2285–95.
15. Taylor B, Miller E, Lingam R, Andrews N, Simmons A, Stone J. Measles, mumps, and rubella vaccination and bowel prob-[26] Stubbs EG, Ash E, Williams CP. Autism and congenital cytomegavirus. \textit{J Autism Dev Disord.} 1984;14:183–9.
16. Tuchman R, Rapin I. Epilepsy in autism. \textit{Lancet Neurol.} 2002;1:152–8.
17. Krause I, He XS, Gerswin ME, Shoenfeld Y. Brief report: immune factors in autism: a critical review. \textit{J Autism Dev Disorder.} 2002;32:337–45.
18. Hornig M, Lipkin WI. Infectious and immune factors in the pathogenesis of neurodevelopmental disorders: epidemiology, hypotheses, and animal models. \textit{Ment Retard Dev Disabil Res Rev.} 2001;7:200–10.
19. Pardo CD, Vargas DL, Zimmerman AW. Immunity, neuroglia and neuroinflammation in autism. \textit{Int Rev Psychiatry.} 2005;17:485–95.
20. Ashwood P, Van de Water J. Is autism an autoimmune disease? \textit{Autoimmunity Rev.} 2004;3:557–62.
21. Coni AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. \textit{J Child Neurol.} 1999;14:388–94.
22. Sweeten TL, Bowyer SL, Posse DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. \textit{Pediatrics.} 2003;112:420–4.
23. Chess S. Follow-up report on autism in congenital rubella. \textit{J Autism Child Schizophr.} 1977;7:69–81.
24. Chess S, Fernandez P, Korn S. Behavioral consequences of congenital rubella. \textit{J Pediatrics.} 1978;93:699–703.
25. Yamashita Y, Fujimoto C, Nakajima E, Isagi T, Matsuishi T. Possible association between congenital cytomegavirus infection and autistic disorder. \textit{J Autism Dev Disord.} 2003;33:455–9.
26. Stubbs EG, Ash E, Williams CP. Autism and congenital cytomegavirus. \textit{J Autism Dev Disorder.} 1984;14:183–9.
27. Delong GR, Bean SC, Brown FR. Acquired reversible autistic syndrome in acute encephalopathic illness in children. \textit{Arch Neurol.} 1981;38:191–4.
28. Malmstrom BG. Enzymology of oxygen. \textit{Toxicol Ind Health.} 1999;15:287–310.
29. Prabakaran S, Swatton JE, Ryan MM, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. \textit{Mol Psychiatry.} 2004;9:684–97.
30. Abdalla DSP, Monteiro HP, Oliveira JAC, Bechara EJ. Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. \textit{Clin Chem.} 1986;32:805–7.
36. Biliçi M, Efe H, Koroglu MA, Udu HA, Bekaroglu M, Deger O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J Affect Disord*. 2001;64:43–51.

37. Kuloglu M, Atmaca M, Tezcan E, Ustundag B, Bulut S. Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. *Neuropsychobiology*. 2002;46:186–9.

38. Kuloglu M, Atmaca M, Tezcan E, Gecci O, Tuncelik H, Ustundag B. Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. *Neuropsychobiology*. 2002;46:27–32.

39. Yorbik O, Sayal A, Akay C, Akbiyik DJ, Solhnen T. Investigation of anti- oxidant enzymes in children with autistic disorder. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67:341–3.

40. Pasca SP, Nemes B, Vlase L, et al. High levels of homocysteine and low serum paraoxonase 1 alysesterase activity in children with autism. *Life Sci*. 2006;78:2244–8.

41. James SJ, Cutler P, Melnyk S, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr*. 2004;80:1611–7.

42. Faber S, Zinn GM, Kern JC 2nd, Kingston HM. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. *Biomarkers*. 2009;14:171–80.

43. Walsh WJ, Usman A, Tarpey J. Disordered Metal Metabolism in a Large Autism Population, Proceedings of the *Amer Psych Assn*; New Research: Abstract NR109. New Orleans, May, 2001.

44. Fischer WP, Giroux A, L’abbe M. Effects of Zinc on Mucosa! Copper Binding and on the Kinetics of Copper Absorption. *The Journal of Nutrition*. 1981;25:462–9.

45. Nalini Pandey, Girish Chandra Pathak, Amit Kumar Singh, Chandra Prakash Sharma. Enzyme changes in response to zinc nutrition. *Journal of Plant Physiology*. 2002;159:1151–3.

46. Sajdel-Sulkowska EM, et al. Oxidative Stress in Autism: Elevated Cerebellar 3-nitrotyrosine Levels. *American Journal of Biochemistry and Biotechnology*. 2008;4(2):73–84.

47. Edelson SB, Cantor DS. The neurotoxic etiology of the autistic spectrum disorder: a replicative study. *Toxicol Ind Health*. 2000;16:239–47.

48. Hovatta I, Tennant RS, Helton R, et al. Glyoxalase I and glutathione reductase 1 regulate anxiety in mice. *Neuroscience*. 2005;134:662–6.

49. Junaid MA, Kowal D, Barua M, Pullarakt PS, Sklover Brooks S, Pullarakt RK. Proteomic studies identified a single nucleotide polymorphism in glyoxalase I as autism susceptibility factor. *Am J Med Genet*. 2004;131:11–7.

50. Cohen IL, Liu X, Schutz C, et al. Association of autism severity with a basic protein in children with autistic behavior. *J Neuroimmunol*. 2006;173:126–34.

51. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic disorder: a replicative study. *Neurology*. 2000;55:2539–42.

52. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamine alterations by antidepressant treatments. *J Neurochem*. 2005;94:271–80.

53. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biol Psychiatry*. 2002;52:805–10.

54. Aldred S, Moore KM, Fitzgerald M, Waring RH. Plasma amino acid levels in children with autism and their families. *J Autism Dev Disord*. 2003;33:93–7.

55. Uchida K, Shiraiishi M, Naito Y, Torii N, Nakamura Y, Otsawa T. Activation of stress signalling pathways by the end product of lipid peroxidation. *J Biol Chem*. 1999;274:2234–42.

56. Parola M, Bellomo G, Robino G, Barrera G, Dianzani MU. 4-hydroxyxynonale as a biological signal: molecular basis and pathophysiological implications. *Antioxidant Redox Signal*. 1999;1:255–84.

57. de la Fuente M, Victor VM, Guayerbas N. The amount of thiolic antioxidant ingestion needed to improve several immune functions of stress signalling pathways by the end product of lipid peroxidation. *J Biol Chem*. 1992;267:190–7.

58. de la Fuente M, Victor VM. Ascorbic acid and acetylcysteine improve the function of lymphocytes from mice with endotoxin-induced oxidative stress. *Free Radic Res*. 2001;35:73–84.

59. de la Fuente M, Miguel J, Catalan MP, Victor VM, Guayerbas N. The amount of thiolic antioxidant ingestion needed to improve several immune functions is higher in aged than in adult mice. *Free Radic Res*. 2002;36:119–26.
Nutrition and Metabolic Insights 2009:2

82. Viora M, Quarante MG, Straface E, Vari R, Masella R, Maloni W. Redox imbalance and immune functions: opposite effects of oxidized low-density lipoproteins and N-acetylcysteine. *Immunology*. 2001;104:431–8.

83. McGinnis WR. Oxidative stress in autism. *Altern Ther Health Med*. 2004;10:22–36.

84. Ashwood P, Wakefield AJ. Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. *J Neuroimmunol*. 2006;173:126–34.

85. Chez MG, Buchanan CP, Aimonovitch MC, Becker M, Black C, Komen J. Double-blind, placebo-controlled study of 1-carnosine supplementation in children with autism spectrum disorders. *J Child Neurol*. 2002;17:833–7.

86. Rimland B, Callaway E, Dreyfus P. The effect of high doses of Vitamin B6 on autistic children: a double-blind crossover study. *Am J Psychiatry*. 1978;135:472–5.

87. Kleijnen J, Knipschild P. Niacin and Vitamin B6 in mental functioning: a review of controlled trials in humans. *Biol Psychiatry*. 1991;29:931–41.

88. Zoroglu SS, Armutcu F, Ozen S, et al. Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. *Eur Arch Psychiatry Clin Neurosci*. 2004;254:143–7.

89. Evans TA, et al. The Autistic Phenotype Exhibits a Remarkably Localized Modification of Brain Protein by Products of Free Radical-Induced Lipid Oxidation. *American Journal of Biotechnology and Biochemistry*. 2008;4(2):61–72.