

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

Nutrition and Metabolic Insights 2009:2 27–35

This article is available from <http://www.la-press.com>.

© the author(s), publisher and licensee Libertas Academica Ltd.

This is an open access article. Unrestricted non-commercial use is permitted provided the original work is properly cited.

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 plasma³⁹ and in erythrocytes,^{39,40} reduced levels of total glutathione, lower redox ratio of reduced glutathione (GSH) to oxidized glutathione

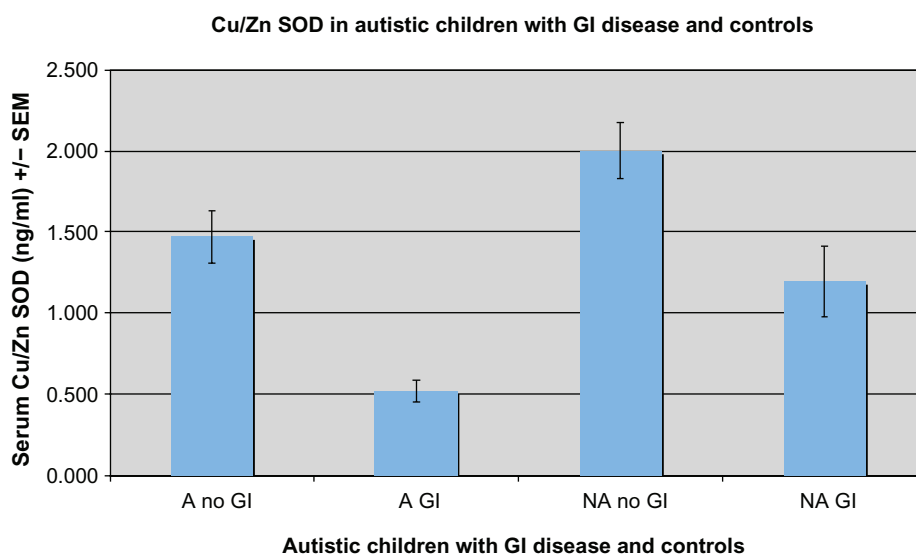


Figure 1. The mean \pm SD Cu/Zn SOD concentration (ng/ml) of 50 autistic children (31 with chronic digestive disease (most with ileo-colonic lymphoid nodular hyperplasia (LNH) and inflammation of the colorectum, small bowel and/or stomach) and 19 autistic children without GI disease), and 29 non autistic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

**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,⁴¹ and decreased catalase and SOD³⁹ activity in erythrocytes.

Zinc to copper ratio is abnormally low in individuals with autism^{42,43} and zinc is also antagonistic to copper absorption, and therefore zinc deficiency often occurs simultaneously with an excess of copper.⁴⁴ The loss

Table 2. Significant difference between Cu/Zn SOD concentration (ng/ml) of 31 autistic children with chronic digestive disease (most with ileo-colonic lymphoid nodular hyperplasia (LNH) and inflammation of the colorectum, small bowel and/or stomach) and 20 autistic children without GI disease, and 20 age matched non autistic children with no GI disease and 9 age matched non autistic children with GI disease.

Group	A GI	A No GI
Mean	0.524	1.469
SD	0.378	0.725
SEM	0.068	0.162
N	31	20

$p < 0.0001$.

Group	A GI	NA No GI
Mean	0.524	2.000
SD	0.378	0.773
SEM	0.068	0.173
N	31	20

$p < 0.0001$.

Group	A GI	NA GI
Mean	0.524	1.197
SD	0.378	0.646
SEM	0.068	0.215
N	31	9

$p = 0.0003$.

of copper and zinc homeostasis is purportedly a likely indicator of a metallothionein deficiency.⁴³

Low zinc levels have been directly related to low Cu/Zn SOD concentration.⁴⁵ 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 μ l 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 anti-human 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 μ l 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	0.6725	0.002	0.626	0.199	0.503	0.196
16	0.569		0.569		0.484	0.443	0.539	0.426
17	0.721	0.736	0.7285	0.011	0.703	0.278	0.529	0.228
18	0.594	0.585	0.5895	0.006	0.513	0.147	0.302	0.176
19	1.415	1.364	1.3895	0.036	0.610	0.251	0.351	0.31
20	0.527	0.486	0.5065	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	0.6655	0.032	0.617	0.197	0.361	0.253
23	0.717	0.676	0.6965	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	0.6915	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	1.0485	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	1.0495	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 A/RA/ASP/PDD	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
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	
R-PDD	1	0	3		0.447		0.447		0.447
A	1	2	6		1.142		1.142		1.142
RA	2	2	6		0.393		0.393		0.393
RA	2	0	8		1.143		1.143	1.143	
RA	3	0	7		0.907		0.907	0.907	
			Mean	0.298	0.549	0.642	0.521	0.627	0.492
			SD	0.457	0.370	0.610	0.322	0.476	0.302
				p = 0.2829		p = 0.5164		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.^{57,58} 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,^{46,47} genetic^{48–51} and immunological^{52–58} factors. This may be due to (a) increased production of endogenous pro-oxidants (such as NO, xanthine oxidase, homocysteine)^{59–64} or environmental pro-oxidants,^{65,66} or deficiencies of antioxidants (ceruloplasmin, transferrin, superoxide dismutase, glutathione peroxidase, catalase, reduced glutathione), or both.^{67–70} 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,^{71,72} mitochondrial dysfunction,^{73–75} excitotoxicity,^{76,77} inflammation,^{78,79} and immune

*The Autism Genetic Resource Exchange (AGRE) is the first collaborative gene bank for the study of autism spectrum disorders and one of the world's largest shared resources for the study of autism and related disorders, with a collection of over 900 well-characterized multiplex and simplex families made available to the greater scientific community. Founded by Cure Autism Now (CAN) in 1997, AGRE is currently funded by the National Institute of Mental Health (NIMH) and Autism Speaks (AS), which merged with CAN in 2006.

**The Thoughtful House is a comprehensive treatment and research center, specializing in the care of children with neurological disorders, including autism.

dys-regulation^{77,80–82} in autistic children, and might contribute to behavioral aberrations, sleep disorder, and gastrointestinal disturbances.^{83,84} Preliminary results of clinical trials have suggested improved behavior in individuals with autism who receive anti-oxidant therapy.^{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),⁸⁸ and abnormal concentrations may lead to an atypical brain phenotype in autistic children.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 levels 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:e472–86.
2. Lamb JA, Moore J, Bailey A, Monaco AP. Autism: recent molecular genetic advances. *Hum Mol Genet*. 2000;9:861–8.
3. Korvatska E, Van de Water J, Anders TF, Gershwin ME. Genetic and immunologic considerations in autism. *Neurobiol Dis*. 2002;9:107–25.
4. Keller F, Persico AM. The neurobiological context of autism. *Mol Neurobiol*. 2003;28:1–22.
5. Sung YJ, Dawson G, Munson J, Estes A, Schellenberg GD, Wijsman EM. Genetic investigation of quantitative traits related to autism: use of multivariate polygenic models with ascertainment adjustment. *Am J Hum Genet*. 2005;76:68–81.
6. London EA. The environment as an etiologic factor in autism: a new direction for research. *Environ Health Perspect*. 2000;108:401–4.
7. Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? *Neuro Endocrinol Lett*. 2005;26:439–46.
8. Wakefield AJ, Montgomery SM. Autism, viral infection and measles-mumps-rubella-vaccination. *Isr Med Assoc J*. 1999;1:183–7.
9. Fombonne E. Are measles infections or measles immunizations linked to autism? *J Autism Dev Disord*. 1999;29:349–50.
10. Edelson SB, Cantor DS. Autism: xenobiotic influences. *Toxicol Ind Health*. 1998;14:799–811.
11. Horvath K, Perman JA. Autism and gastrointestinal symptoms. *Curr Gastroenterol Rep*. 2002;4:251–8.
12. White JF. Intestinal pathology in autism. *Exp Biol Med*. (Maywood). 2003;228:639–49.
13. Horvath K, Papadimitriou JC, Rabsztyrn A, Drachenberg C, Tildon JT. Gastrointestinal abnormalities in children with autism. *J Pediatr*. 1999;135:559–63.
14. Wakefield AJ, Anthony A, Murch SH, et al. Walker-Smith, Enterocolitis in children with developmental disorders. *Am J Gastroenterol*. 2000;95:2285–95.
15. Taylor B, Miller E, Lingam R, Andrews N, Simmons A, Stowe J. Measles, mumps, and rubella vaccination and bowel prob-[26] Stubbs EG, Ash E, Williams CP. Autism and congenital cytomegalovirus. *J Autism Dev Disord*. 1984;14:183–9.
16. Tuchman R, Rapin I. Epilepsy in autism. *Lancet Neurol*. 2002;1:352–8.
17. Krause I, He XS, Gershwin ME, Shoenfeld Y. Brief report: immune factors in autism: a critical review. *J Autism Dev Disord*. 2002;32:337–45.
18. Hornig M, Lipkin WI. Infectious and immune factors in the pathogenesis of neurodevelopmental disorders: epidemiology, hypotheses, and animal models. *Ment Retard Dev Disabil Res Rev*. 2001;7:200–10.
19. Pardo CD, Vargas DL, Zimmerman AW. Immunity, neuroglia and neuroinflammation in autism. *Int Rev Psych*. 2005;17:485–95.
20. Ashwood P, Van de Water J. Is autism an autoimmune disease? *Autoimmunity Rev*. 2004;3:557–62.
21. Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *J Child Neurol*. 1999;14:388–94.
22. Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Pediatrics*. 2003;112:420–4.
23. Chess S. Follow-up report on autism in congenital rubella. *J Autism Child Schizophr*. 1977;7:69–81.
24. Chess S, Fernandez P, Korn S. Behavioral consequences of congenital rubella. *J Pediatrics*. 1978;93:699–703.
25. Yamashita Y, Fujimoto C, Nakajima E, Isagai T, Matsuishi T. Possible association between congenital cytomegalovirus infection and autistic disorder. *J Autism Dev Disord*. 2003;33:455–9.
26. Stubbs EG, Ash E, Williams CP. Autism and congenital cytomegalovirus. *J Autism Dev Disord*. 1984;14:183–9.
27. DeLong GR, Bean SC, Brown FR. Acquired reversible autistic syndrome in acute encephalopathic illness in children. *Arch Neurol*. 1981;38:191–4.
28. Malmstrom BG. Enzymology of oxygen. *Annu Rev Biochem*. 1982;51:21.
29. Fridovich I. The biology of oxygen radicals. *Science*. 1978;201:875.
30. Forman HJ, Torres M. Redox signaling in macrophages. *Mol Aspects Med*. 2001;22:189.
31. Chauhan A, Chauhan V, Brown WT, Cohen IL. Oxidative stress in autism: Increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferring—the antioxidant proteins. *Life Sci*. 2004;75:2539–49.
32. McGinnis WR. Oxidative stress in autism. *Altern Ther Health Med*. 2004;10:22–36.
33. Yao JK, Reddy RD, van Kammen DP. Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. *CNS Drugs*. 2001;15:287–310.
34. Prabakaran S, Swatton JE, Ryan MM, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry*. 2004;9:684–97.
35. Abdalla DSP, Monteiro HP, Oliveira JAC, Bechara EJ. Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. *Clin Chem*. 1986;32:805–7.



36. Bilici M, Efe H, Koroglu MA, Uydu 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, Gecici O, Tunckol 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 DI, Sohmen T. Investigation of antioxidant 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 arylesterase 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 PW, Giroux A, L'abbe3a M. Effects of Zinc on Mucosal 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. Enzymic 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 1 and glutathione reductase 1 regulate anxiety in mice. *Nature.* 2005;438:662–6.
49. Junaid MA, Kowal D, Barua M, Pullarkat PS, Sklower Brooks S, Pullarkat 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 monoamine oxidase A functional polymorphism. *Clin Genet.* 2003;64:190–7.
51. Thornalley PJ. Glyoxalase I-structure, function and a critical role in the enzymatic defense against glycation. *Biochem Soc Trans.* 2003;31:1343–8.
52. Gupta S, Agarwal S, Heads C. Brief report: dysregulated immune system in children with autism: beneficial effects of intravenous immune globulin on autistic characteristics. *J Autism Dev Disord.* 1996;26:439–52.
53. Ferrari P, Marescot MR, Moulins R, et al. Immune status in infantile autism. Correlation between the immune system, autistic symptoms and levels of serotonin. *Encephale.* 1988;5:339–44.
54. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol.* 2005;57:67–81.
55. 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.
56. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immunol.* 1993;7:97–103.
57. Russo AJ, Krigsman A, Jepson B, Wakefield A. Anti-PR3 and Anti-MPO IgG ANCA in autistic children with chronic GI disease. *Immunology and Immunogenetics Insights.* 2009;2:21–8.
58. Russo AJ, Krigsman A, Jepson B, Wakefield A, Low Serum Alpha-1 Antitrypsin associated with Anti-PR-3 ANCA in autistic children with GI disease, accepted for publication. *Genomics Insights.* 2009;2:1–9.
59. 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.
60. Upchurch GR, Welch GN, Fabian AJ, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem.* 1997;272:17012–7.
61. Hindley S, Juurlink BHJ, Gysbers JW, Middlemiss PJ, Herman MAR, Rathbone MP. Nitric oxide donors enhance neurotrophin-induced neurite outgrowth through a cGMP-dependent mechanism. *J Neurosci Res.* 1997;47:427–39.
62. Truman JW, De Vente J, Ball EE. Nitric oxide-sensitive guanylate cyclase activity is associated with the maturational phase of neuronal development in insects. *Development.* 1996;122:3949–58.
63. Wong JM, Billiar TR. Regulation and function of inducible nitric oxide synthase during sepsis and acute inflammation. *Adv Pharmacol.* 1995;34:155–70.
64. Nussler AK, Di Silvio M, Billiar TR, et al. Stimulation of the nitric oxide synthase pathway in human hepatocytes by cytokines and endotoxin. *J Exp Med.* 1992;176:261–4.
65. Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? *Neuro Endocrinol Lett.* 2005;26:439–46.
66. James SJ, Slikker W, Melnyk S, New E, Pogribna M, Jernigan S. Thimerosal neurotoxicity is associated with glutathione depletion: protection with glutathione precursors. *Neurotoxicology.* 2005;26:1–8.
67. Gutteridge JMC, Richmond R, Halliwell B. Oxygen free-radicals and lipid peroxidation. Inhibition by the protein ceruloplasmin. *FEBS Lett.* 1980;112:269–72.
68. Loeffler DA, Connor JR, Juneau PL, et al. Transferrin and iron in normal, Alzheimer's disease, and Parkinson's disease brain regions. *J Neurochem.* 1995;65:710–24.
69. Chauhan A, Chauhan V, Brown WT, Cohen IL. Oxidative stress in autism: Increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin—the antioxidant proteins. *Life Sci.* 2004;75:2539–49.
70. McGinnis WR. Oxidative stress in autism. *Altern Ther Health Med.* 2004;10:22–36.
71. Horrobin DF. The phospholipid concept of psychiatric disorders to the neurodevelopmental concept of schizophrenia. In: Peet M, Glen I, Horrobin DF, editors. *Phospholipid Spectrum Disorder in Psychiatry.* Lancashire, UK: Maurius Press; 1999. pp. 3–20.
72. Puri BK, Richardson A. Brain phospholipid metabolism in dyslexia assessed by magnetic resonance spectroscopy. In: Peet M, Glen I, Horrobin DF, editors. *Phospholipid Spectrum Disorder in Psychiatry.* Lancashire, UK: Maurius Press; 1999. pp. 243–50.
73. Lenaz G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life.* 2001;52:159–64.
74. Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med.* 1999;26:463–71.
75. Kroemer G, Dallaporta B, Resch-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. *Annu Rev Physiol.* 1998;60:619–42.
76. Fatemi SH, Halt AR, Sary 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.
77. 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.
78. Uchida K, Shiraishi M, Naito Y, Torii N, Nakamura Y, Osawa T. Activation of stress signalling pathways by the end product of lipid peroxidation. *J Biol Chem.* 1999;274:2234–42.
79. Parola M, Bellomo G, Robino G, Barrera G, Dianzani MU. 4-hydroxynonenal as a biological signal: molecular basis and pathophysiological implications. *Antioxidant Redox Signal.* 1999;1:255–84.
80. de la Fuente M, Victor VM. Ascorbic acid and N-acetylcysteine improve in vitro the function of lymphocytes from mice with endotoxin-induced oxidative stress. *Free Radic Res.* 2001;35:73–84.
81. de la Fuente M, Miguel J, Catalan MP, Victor VM, Guayerbas N. The amount of thiol antioxidant ingestion needed to improve several immune functions is higher in aged than in adult mice. *Free Radic Res.* 2002;36:119–26.



82. Viora M, Quarante MG, Straface E, Vari R, Masella R, Malomi 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. 33.
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.

Publish with Libertas Academica and every scientist working in your field can read your article

“I would like to say that this is the most author-friendly editing process I have experienced in over 150 publications. Thank you most sincerely.”

“The communication between your staff and me has been terrific. Whenever progress is made with the manuscript, I receive notice. Quite honestly, I’ve never had such complete communication with a journal.”

“LA is different, and hopefully represents a kind of scientific publication machinery that removes the hurdles from free flow of scientific thought.”

Your paper will be:

- Available to your entire community free of charge
- Fairly and quickly peer reviewed
- Yours! You retain copyright

<http://www.la-press.com>