

Altered Vascular Phenotype in Autism

Correlation With Oxidative Stress

Yuemang Yao, BSc; William J. Walsh, PhD; Woody R. McGinnis, MD; Domenico Praticò, MD

Background: Autism is a neurologic disorder characterized by impaired communication and social interaction. Results of previous studies showed biochemical evidence for abnormal platelet reactivity and altered blood flow in children with autism.

Objective: To evaluate the vascular phenotype in children with autism.

Design and Main Outcome Measures: Urinary levels of isoprostane $F_{2\alpha}$ -VI, a marker of lipid peroxidation; 2,3-dinor-thromboxane B_2 , which reflects platelet activation; and 6-keto-prostaglandin $F_{1\alpha}$, a marker of endothelium activation, were measured by means of gas chromatography-mass spectrometry in subjects with autism and healthy control subjects.

Setting and Subjects: Children with a clinical diagnosis of autism attending the Pfeiffer Treatment Center.

Results: Compared with controls, children with autism had significantly higher urinary levels of isoprostane $F_{2\alpha}$ -VI, 2,3-dinor-thromboxane B_2 , and 6-keto-prostaglandin $F_{1\alpha}$. Lipid peroxidation levels directly correlated with both vascular biomarker ratios.

Conclusion: Besides enhanced oxidative stress, platelet and vascular endothelium activation also could contribute to the development and clinical manifestations of autism.

Arch Neurol. 2006;63:1161-1164

AUTISM IS A NEUROLOGIC DISORDER with typical onset before the age of 3 years and is characterized by impairments in social, behavioral, and communicative functions.¹ The condition is highly prevalent, and in recent years there has been an apparent increase in the diagnosis of this disorder.² Although the biological basis for autism remains unknown, there is increasing evidence that genetic, environmental, and immunologic factors could contribute to its development.³⁻⁵

Several lines of emerging evidence suggest a potential role for enhanced oxidative stress in autism. Thus, increased levels of nitric oxide have been reported in individuals with autism in association with markers of lipid peroxidation, which would suggest excessive reactive oxygen species formation.⁶ Moreover, there are reports of alteration in energy metabolism, possibly linked to mitochondrial dysfunction, and a significant reduction in endogenous antioxidants in autism

compared with that in control subjects.⁷⁻⁹ Recent research results indicate that the F_2 -isoprostane 8-iso-prostaglandin $F_{2\alpha}$ is enhanced in children with autism.¹⁰ This isoprostane is a nonenzymatic oxidation product of arachidonic acid and is widely recognized as a reliable marker of lipid peroxidation. Moreover, it induces in vivo vasoconstriction and amplifies agonist-induced platelet adhesion and aggregation.¹¹ Thus, the enhanced formation of biologically active F_2 -isoprostane in this syndrome also could result in platelet and vascular activation. Previous study results indicated indirect biochemical evidence for altered platelet reactivity in autism, and most of the work has been focused on serotonin and its receptor in platelets.¹² However, no data are available regarding systemic platelet activation as reflected by levels of 2,3-dinor-thromboxane (Tx) B_2 , the stable metabolite of the platelet cyclooxygenase-derived TxA_2 .¹³ The same consideration is valid for markers of vascular activation as reflected by 6-keto-prostaglandin (PG) $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$), the

Author Affiliations:

Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia (Ms Yao and Dr Praticò); and Pfeiffer Treatment Center, Warrenton, Ill (Dr Walsh). Dr McGinnis is in private practice.

Table. Demographics of the Study Population*

Population	Age, y	Sex, M/F	Regression, Yes/No	Isoprostane F _{2α} -VI, ng/mg Creatinine	Creatinine (mg/dL)
Autistic (n = 26)	4.6 ± 0.2	22/4	14/12	5.2 ± 0.5†	15.3 ± 1.7
Control (n = 12)	6.7 ± 0.4‡	10/2	NA	3.1 ± 0.3	14.1 ± 2.6

Abbreviation: NA, not applicable.

SI conversion factor: To convert creatinine to micromoles per liter, multiply by 88.4.

*Data are expressed as mean ± SEM and number of subjects.

†*P* < .01.

‡*P* < .05.

stable metabolite of the endothelium cyclooxygenase-derived prostacyclin.¹³ Moreover, the relative contribution of oxidative stress in inducing persistent abnormalities in platelet and endothelial function remains to be determined.

Here, we report the first observation that oxidative stress is increased and directly correlated with the rate of systemic TxA₂ and prostacyclin biosynthesis in a group of subjects with autism compared with findings in controls.

METHODS

STUDY PARTICIPANTS

The participants in the study were 26 children with autism who had not undergone any treatment and 12 healthy control children. The test subjects were outpatients at the Pfeiffer Treatment Center who had a diagnosis of autism on the basis of the criteria for autistic disorder as defined in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* and a diagnostic interview conducted by a developmental pediatrician.^{14,15} Children with autism were excluded from the study if they had ever been treated with antioxidants, chelators, nutritional supplements, or medications with known antioxidant effect. They were also excluded if they were sick within 2 weeks of the sample collection or if they had a chronic inflammatory disorder (such as asthma or arthritis), seizures, depression, psychosis, head injury, schizophrenia, or other mental disorders or were taking psychiatric or anti-inflammatory medications. All subjects were carefully screened for signs of infection or other illnesses on the day of specimen acquisition, and their parents attested to the absence of these potential confounders in the prior month.

The same criteria applied to all control subjects, each of whom (1) was a healthy, well-adjusted child who was not receiving any kind of therapy; (2) had no relatives with a diagnosis of autism spectrum disorder and had good behavior and academic standing; (3) had no mental disorder, including autism spectrum disorder; and (4) received no medication for at least 1 month and stopped receiving any multivitamin 2 weeks before sample acquisition. The study was approved by the institutional review board of the Pfeiffer Treatment Center, and each family provided a signed consent form authorizing their participation in the study.

BIOCHEMICAL ANALYSES

Data were always analyzed in a blinded fashion. Urinary isoprostone F_{2α}-VI (iPF_{2α}-VI), 2,3-dinor-TxB₂, and 6-keto-PGF_{1α} were measured in urine spot samples by using standardized gas chromatography–mass spectrometry assays, as previously de-

scribed. Briefly, each sample was first spiked with the corresponding deuterated internal standard, extracted by means of a solid phase extraction column, purified by means of a thin-layer chromatography step, and finally assayed by means of negative ion chemical ionization gas chromatography–mass spectrometry.^{16,17} A urine aliquot (0.1 mL) was used to measure creatinine concentration with a commercially available, standardized colorimetric assay (Sigma-Aldrich Co, St Louis, Mo). Results were always normalized for urinary creatinine concentration, as previously described.¹⁷

STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Statistical analysis was performed by using nonparametric 1-way analysis of variance (Kruskal-Wallis test) and Dunn posttest comparison. Correlations between variables were examined by using linear regression analysis. Only *P* values lower than .05 were regarded as significant.

RESULTS

As shown in the **Table**, we recruited 38 individuals: 26 subjects with autism and 12 control subjects. Although they were well matched for sex (autistic, 22 [85%] boys; controls, 10 [80%] boys), subjects with autism were younger than the controls (mean, 4.6 vs 6.7 years) (Table).

First, we compared the iPF_{2α}-VI contents in the urine of children with autism with that of the controls. The urinary levels of iPF_{2α}-VI were significantly higher in children with autism (5.2 ± 0.5 ng/mg creatinine) than in the controls (3.1 ± 0.3 ng/mg creatinine, *P* < .01) (Table). The data were also significant when children with autism were compared with the controls of the same sex (boys, 4.9 ± 0.5 vs 2.9 ± 0.4 ng/mg creatinine; girls, 6.6 ± 0.8 vs 3.5 ± 0.8 ng/mg creatinine; *P* < .05 for both). No significant difference in urinary creatinine levels was observed between the 2 groups of subjects (Table). No effect of age was observed on iPF_{2α}-VI levels either in the subjects with autism or control subjects (*r*² = 0.05, *P* = .18) (**Figure 1**).

Figure 2 (upper panel) illustrates the urinary levels of 2,3-dinor TxB₂ in the 2 groups investigated. Similar to the findings for iPF_{2α}-VI, 2,3-dinor TxB₂ content in the urine of subjects with autism was significantly higher than that in control subjects (2.9 ± 0.2 vs 1.8 ± 0.1 ng/mg creatinine, *P* < .01).

Next, we investigated the endogenous biosynthesis of prostacyclin by assaying the urinary levels of its stable metabolite 6-keto-PGF_{1α}. As shown in **Figure 2** (lower panel), subjects with autism had a much higher urinary

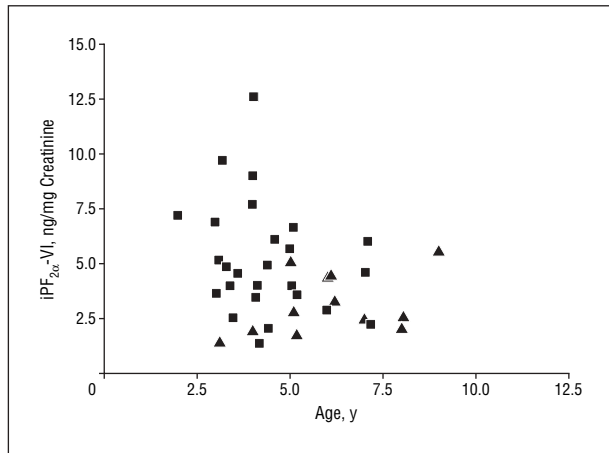


Figure 1. Correlation between urinary levels of isoprostane $F_{2\alpha}$ -VI ($iPF_{2\alpha}$ -VI) in the subjects investigated and their age ($r^2=0.05$, $P=.18$).

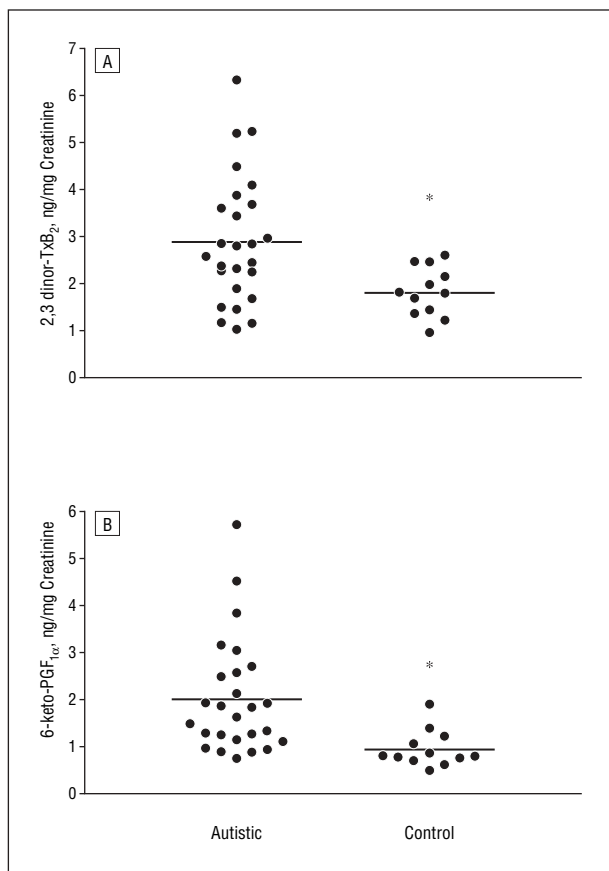


Figure 2. A, Urinary levels of 2,3-dinor-thromboxane B_2 (2,3-dinor- TxB_2) in subjects with autism and control subjects. B, Urinary levels of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto- $PGF_{1\alpha}$) in subjects with autism and control subjects. Asterisk indicates $P<.01$; horizontal lines, means.

excretion of 6-keto- $PGF_{1\alpha}$ than did controls (2.01 ± 0.2 vs 1.07 ± 0.3 ng/mg creatinine, $P=.005$).

Levels of 2,3-dinor- TxB_2 directly correlated with 6-keto- $PGF_{1\alpha}$ in subjects with autism ($r^2=0.22$, $P=.01$), suggesting a common mechanism of formation. Finally, we observed a linear relationship between those 2 metabolites and urinary $iPF_{2\alpha}$ -VI levels (2,3-dinor- TxB_2 : $r^2=0.30$, $P=.003$; and 6-keto- $PGF_{1\alpha}$: $r^2=0.33$, $P=.002$) (**Figure 3**).

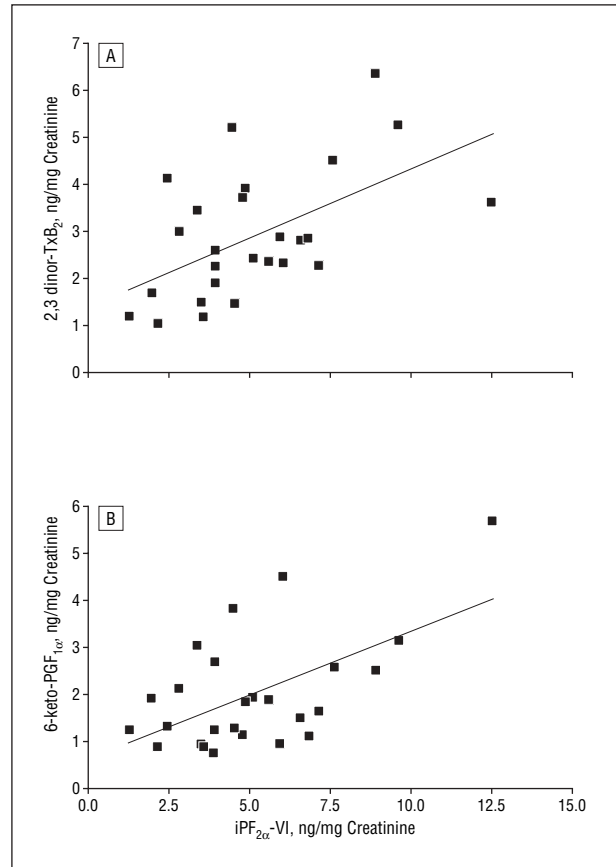


Figure 3. Correlation between urinary levels of isoprostane $F_{2\alpha}$ -VI ($iPF_{2\alpha}$ -VI) with 2,3-dinor-thromboxane B_2 (2,3-dinor- TxB_2) ($P=.003$) (A) $iPF_{2\alpha}$ -VI with 6-keto-prostaglandin $F_{1\alpha}$ (6-keto- $PGF_{1\alpha}$) ($P=.002$) in subjects with autism (B).

COMMENT

The major finding of this study is that autism is characterized by increased in vivo oxidative stress, which associates and directly correlates with biochemical signatures of platelet and vascular endothelial activation.

Autism is a complex neurologic disorder; its pathogenesis involves an interaction among multiple genetic, epigenetic, and environmental factors.¹⁻⁵ Oxidative imbalance is a feature of the autistic syndrome, and several lines of evidence support the hypothesis that oxidative stress also may play a functional role in this disease. Thus, autism is characterized by a lower antioxidant defense system, higher free radical production, and improvement of behavioral symptoms after antioxidant administration.^{6-9,18-20}

Consistent with a previous report,¹⁰ our results indicated that children with autism showed higher rates of in vivo lipid peroxidation than did controls. More important, in our study, this increase was independent of the age of the subjects investigated. A subgroup of children with autism is characterized by regression, a potential confounding factor. However, we found no difference between regressed vs early-onset subgroups along the variables we measured. Moreover, we observed that girls in the autism group and in the control group did not show a significant difference in isoprostane levels when compared with levels in boys. However, this con-

clusion may be secondary to the relatively small size of the girls in our study. Finally, by contrast with a previous study, isoprostane levels in the subjects with autism in our study did not show a bimodal distribution.¹⁰ This difference could be due to different inclusion/exclusion criteria adopted in recruiting the subjects. Thus, by contrast with that study, the subjects with autism in our study were not taking any medication. Another possible reason is the fact that while Ming et al¹⁰ measured 8-isoprostaglandin F_{2α}, a class III F₂-isoprostane, we assayed iPF_{2α}-VI, a member of class VI.¹¹ Thus, previous reports have shown that in vivo, for mechanisms not completely understood, there could be a preferential formation of one isomer vs the other of this large family.¹¹

Measurement of iPF_{2α}-VI has been characterized as a reliable and specific method of investigating lipid peroxidation in vivo and reflects a status of enhanced oxidative stress, regardless of the underlying pathophysiologic triggers. Interestingly, iPF_{2α}-VI belongs to a class of lipids, the F₂-isoprostanes that are also characterized by biological activities. Thus, they modulate the vascular phenotype of the oxidative stress response by inducing, among other phenomena, platelet activation and vasoconstriction.¹¹ For this reason, we investigated the relationship between oxidative stress and the vascular phenotype in subjects with autism. This study represents the first observation, to our knowledge, that the rates of TxA₂ and prostacyclin biosynthesis, markers of platelet and endothelial activation, respectively, are not only significantly increased in autism but also are closely correlated with the rate of oxidative stress.

Because F₂-isoprostanes promote platelet aggregation and vasoconstriction, the correlation of these variables in our study allows some speculation about a direct effect of these bioactive lipids on the vascular phenotype in autism. However, our data do not establish a causal relationship among the variables we measured. Abnormal markers of platelet activation and vasoconstriction in autism may result from many factors, including other biologically active species resulting from free radicals.¹¹ In general, abnormalities of the vascular phenotype can be reflected clinically by an abnormal blood flow. In this regard, it is interesting that results of multiple neuroimaging studies demonstrate brain hypoperfusion in autism.^{21,22} Further elucidation of the relationship of oxidative stress and vascular homeostasis to the pathogenesis of autism, including the possible influence of F₂-isoprostane on tissue perfusion, could lead to improvements in therapy.

Accepted for Publication: February 21, 2006.

Correspondence: Domenico Praticò, MD, Department of Pharmacology, University of Pennsylvania, 3620 Hamilton Walk, John Morgan Building, Room 124, Philadelphia, PA 19104 (domenico@spirit.gerc.upenn.edu).

Author Contributions: *Study concept and design:* Walsh, McGinnis, and Praticò. *Acquisition of data:* Praticò. *Analysis and interpretation of data:* Yao and McGinnis. *Drafting of the manuscript:* Yao and Praticò. *Critical revision of the manuscript for important intellectual content:* Walsh,

McGinnis, and Praticò. *Statistical analysis:* Praticò. *Obtained funding:* Walsh and McGinnis. *Administrative, technical, and material support:* Yao, McGinnis, and Praticò. *Study supervision:* Walsh and Praticò.

Funding/Support: We acknowledge the financial support of Alexander and Bo MacInnis to the Pfeiffer Treatment Center.

Acknowledgment: We thank the subjects and their families, whose generosity made this research possible.

REFERENCES

- Eigsti IM, Shapiro T. A systems neuroscience approach to autism: biological, cognitive, and clinical perspectives. *Ment Retard Dev Disabil Res Rev.* 2003; 9:205-215.
- Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. *JAMA.* 2003;289:49-55.
- Bauman ML, Kemper TL. The neuropathology of the autism spectrum disorders: what have we learned? *Novartis Found Symp.* 2003;251:112-122.
- Korvatska E, Van de Water J, Anders TF, Gershwin ME. Genetic and immunologic considerations in autism. *Neurobiol Dis.* 2002;9:107-125.
- Newschaffer CJ, Fallin D, Lee NL. Heritable and nonheritable risk factors for autism spectrum disorders. *Epidemiol Rev.* 2002;24:137-153.
- Sogut S, Zoroglu SS, Ozyurt H, et al. Changes in nitric oxide levels and antioxidant activities may have a role in the pathophysiological mechanisms involved in autism. *Clin Chim Acta.* 2003;331:111-117.
- Filipek PA, Juranek J, Nguyen MT, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. *J Autism Dev Disord.* 2004;34:615-623.
- 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;80:1611-1617.
- Chauhan A, Chauhan V, Brown WT, Cohen I. Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin—the antioxidant proteins. *Life Sci.* 2004;75:2539-2549.
- Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC. Increased excretion of a lipid peroxidation biomarker in autism. *Prostaglandins Leukot Essent Fatty Acids.* 2005;73:379-384.
- Praticò D, Lawson JA, Rokach J, FitzGerald GA. The isoprostanes in biology and medicine. *Trends Endocr Metabol.* 2001;12:243-247.
- Safai-Kutti S, Kutti J, Gillberg C. Impaired in vivo platelet reactivity in infantile autism. *Acta Paediatr Scand.* 1985;74:799-800.
- Praticò D, Cyrus T, Li H, FitzGerald GA. Endogenous biosynthesis of thromboxane and prostacyclin in 2 distinct murine models of atherosclerosis. *Blood.* 2000;96:3823-3826.
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.* Washington, DC: American Psychiatric Association; 1994.
- Lord C, Pickles A, McLennan J, et al. Diagnosing autism: analyses of data from the Autism Diagnostic Interview. *J Autism Dev Disord.* 1997;27:501-517.
- Ding T, Yao Y, Praticò D. Increase in peripheral oxidative stress during hypercholesterolemia is not reflected in the central nervous system: evidence from two mouse models. *Neurochem Int.* 2005;46:435-439.
- Cyrus T, Sung S, Zhao L, Funk CD, Tang S, Praticò D. Effect of low-dose aspirin on vascular inflammation, plaque stability and atherogenesis in low-density lipoprotein receptor deficient mice. *Circulation.* 2002;106:1282-1287.
- Dolske MC, Spollen J, McKay S, Lancashire E, Talbert L. A preliminary trial of ascorbic acid as supplemental therapy for autism. *Prog Neuropsychopharmacol Biol Psychiatry.* 1993;17:765-774.
- 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;67:341-343.
- 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;254:143-147.
- Boddaert N, Zilbovicius M. Functional neuroimaging and childhood autism. *Pediatr Radiol.* 2002;32:1-7.
- Wilcox J, Tsuang MT, Ledger E, Algeo J, Schnurr T. Brain perfusion in autism varies with age. *Neuropsychobiology.* 2002;46:13-16.