Altered Vascular Phenotype in Autism
Correlation With Oxidative Stress

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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 F₂α-VI, a marker of lipid peroxidation; 2,3-dinor-thromboxane B₂, which reflects platelet activation; and 6-keto-prostaglandin F₃α, 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 F₂α-VI, 2,3-dinor-thromboxane B₂, and 6-keto-prostaglandin F₃α. 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.

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stable metabolite of the endothelium cyclooxygenase-
derived prostacyclin.13 Moreover, the relative contribu-
tion of oxidative stress in inducing persistent abnormali-
ties 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 sys-
temic TxA2 and prostacyclin biosynthesis in a group of sub-
jects 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 chil-
dren. The test subjects were outpatients at the Pfeiffer Treat-
ment Center who had a diagnosis of autism on the basis of the
criteria for autistic disorder as defined in the Diagnostic and Sta-
tistical Manual of Mental Disorders, Fourth Edition
and a diag-
nostic interview conducted by a developmental pediatri-
cian.15 Children with autism were excluded from the study
if they had ever been treated with antioxidants, chelators, nu-
tritional supplements, or medications with known antioxi-
dant effect. They were also excluded if they were sick within 2
weeks of the sample collection or if they had a chronic inflam-
matory disorder (such as asthma or arthritis), seizures, depres-
sion, psychosis, head injury, schizophrenia, or other mental dis-
orders 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 acquisi-
tion, and their parents attested to the absence of these poten-
tial 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 re-
ceiving any kind of therapy; (2) had no relatives with a diag-
nosis of autism spectrum disorder and had good behavior and
academic standing; (3) had no mental disorder, including au-
tism 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 in-
stitutional 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 iso-
prostane F2α-VI(iPF2α-VI), 2,3-dinor-TxB2, and 6-keto-PGF1α
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 corre-
sponding 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 nega-
tive ion chemical ionization gas chromatography–mass spec-
 trometry.16 A urine aliquot (0.1 mL) was used to measure creatinine concentration with a commercially available, stan-
dardized colorimetric assay (Sigma-Aldrich Co, St Louis, Mo).
Results were always normalized for urinary creatinine concen-
tration, as previously described.17

#### STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Statistical analysis was per-
formed by using nonparametric 1-way analysis of variance
(Kruskall-Wallis test) and Dunn posttest comparison. Corre-
lations between variables were examined by using linear re-
gression analysis. Only P values lower than .05 were regarded
as significant.

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; con-
trols, 10 [80%] boys), subjects with autism were younger than the controls (mean, 4.6 vs 6.7 years) (Table).

First, we compared the iPF2α-VI contents in the urine of children with autism with that of the controls. The ur-
inary levels of iPF2α-VI were significantly higher in chil-
dren 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 diffe-
rence in urinary creatinine levels was observed between
the 2 groups of subjects (Table). No effect of age was ob-
erved on iPF2α-VI levels either in the subjects with au-
tism or control subjects (r² = 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 iPF2α-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-PGF1α. As shown in Figure 2 (lower
panel), subjects with autism had a much higher urinary

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Table. Demographics of the Study Population

<table>
<thead>
<tr>
<th>Population</th>
<th>Age, y</th>
<th>Sex, M/F</th>
<th>Regression, Yes/No</th>
<th>Isoprostan F2α-VI, ng/mg Creatinine</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autistic (n = 26)</td>
<td>4.6 ± 0.2</td>
<td>22/4</td>
<td>14/12</td>
<td>5.2 ± 0.5†</td>
<td>15.3 ± 1.7</td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>6.7 ± 0.4‡</td>
<td>10/2</td>
<td>NA</td>
<td>3.1 ± 0.3</td>
<td>14.1 ± 2.6</td>
</tr>
</tbody>
</table>

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.
excretion of 6-keto-PGF$_{1\alpha}$ than did controls (2.01±0.2 vs 1.07±0.3 ng/mg creatinine, $P=0.005$).

Levels of 2,3-dinor-TxB$_2$ directly correlated with 6-keto-PGF$_{1\alpha}$ in subjects with autism ($r^2=0.22$, $P=0.01$), suggesting a common mechanism of formation. Finally, we observed a linear relationship between those 2 metabolites and urinary iP$\alpha$F$_{2\alpha}$-VI levels (2,3-dinor-TxB$_2$: $r^2=0.30$, $P=0.003$; and 6-keto-PGF$_{1\alpha}$: $r^2=0.33$, $P=0.002$) (Figure 3).

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-

Figure 1. Correlation between urinary levels of isoprostane F$_{2\alpha}$-VI (iPF$_{2\alpha}$-VI) in the subjects investigated and their age ($r^2=0.05$, $P=0.18$).

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 $P<0.01$; horizontal lines, means.

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$_2$) ($P=0.003$) (A) iPF$_{2\alpha}$-VI with 6-keto-prostaglandin F$_{1\alpha}$ (6-keto-PGF$_{1\alpha}$) ($P=0.002$) in subjects with autism (B).
...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. 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 al measured 8-isoprostaglandin F$_{2\alpha}$, a class III F$_2$-isoprostane, we assayed iPF$_{2\alpha}$-VI, a member of class VI. 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.

Measurement of iPF$_{2\alpha}$-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 pathophysiological triggers. Interestingly, iPF$_{2\alpha}$-VI belongs to a class of lipids, the F$_2$-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. 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 TxA$_2$ 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 F$_2$-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. 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. Further elucidation of the relationship of oxidative stress and vascular homeostasis to the pathogenesis of autism, including the possible influence of F$_2$-isoprostane on tissue perfusion, could lead to improvements in therapy.

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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.

REFERENCES


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