

# Autism: A Multi-System Oxidative and Inflammatory Disorder

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## Abstract

Autism is a complex, multifactorial disorder of uncertain etiology. Initially described as a psychological disorder involving communication failure, relationship dysfunction, and ritualistic, repetitive behavior, autism is associated with measurable defects in many organ systems.

Neurotransmitter, enzymatic, and structural abnormalities have been observed in the nervous system. Autoimmunity is common, as are loss of gut integrity and coagulation abnormalities. At a metabolic level, not only is mitochondrial function seriously impaired, but also numerous enzymes and substrates are deficient in quantity or activity. Various therapies addressing isolated metabolic impairments have shown partial benefit in limited subgroups.

The complex range of stigmata seen in autism is consistent with a pathological state of oxidative stress and inflammation. Various therapies addressing oxidative stress and inflammation have had some success and are reviewed here. We postulate that effective strategies dealing with the primary causative sources of oxidative stress can result in definitive resolution of many of the symptoms associated with the autism spectrum disorders. Suggestions are made for future therapeutic modalities addressing possible underlying causes of oxidative stress.

**Keywords:** autism, inflammation, oxidative stress, xenobiotics, heavy metals

Autism was first described in 1943 by Leo Kanner, a psychologist. The case histories he presented were defined by the absence or loss of language; the inability to understand social reciprocity; and a desire for sameness as exhibited in ritualistic or intensely habitual behavior.<sup>1</sup> Inevitably, given the postwar focus on psychoanalysis, attempts were made to discover causative factors in the family structure of autistic patients, usually focusing on the role of the mother.

These attempts proved unproductive, and in recent decades, the search has shifted toward empirical treatment and the search for biological causative factors. This search has become more pressing, as the incidence of autism in the US and UK has apparently increased.<sup>2-4</sup> The search for causative factors has, however, generated great controversy, in some cases pitting angry parents or concerned caregivers against perceived commercial and governmental interests. That the controversy has generated more heat than light emphasizes the need for a dispassionate review of autism spectrum disorders, tying together observed metabolic data into a unified hypothesis that could serve as a basis for therapeutic progress.

Oxidative stress and inflammation are key features of the pathophysiology of most chronic illness. Cardiac

disease, for example, is thought to result from the impact of oxidative stress and inflammation on the vascular endothelium.<sup>5</sup> Similarly, oxidative stress and inflammation are increasingly seen to play an important role in the pathophysiology of neurodegenerative disorders,<sup>6-9</sup> and in insulin resistance and its various manifestations.<sup>10,11</sup> Oxidative stress and resulting inflammation also contribute extensively to gastrointestinal<sup>12-14</sup> and rheumatological<sup>15</sup> disorders and, most likely, aging.<sup>16</sup>

Patients with autism spectrum disorders have measurable evidence of oxidative stress in virtually every organ system that has been assessed. For the sake of brevity, only an illustrative sampling of findings will be given. In the autistic brain, for example, the consistent autopsy finding of lipofuscin (a complex of oxidized lipid and protein) deposits localized to Wernicke's speech recognition area and Broca's speech generation area suggest a role for oxidative stress in the pathogenesis of autism.<sup>17</sup> This observation may correlate with functional studies showing decreased temporal blood flow in autism.<sup>18,19</sup>

Metabolic indices of oxidative stress such as serum lipid peroxides,<sup>20</sup> urine isoprostanes<sup>21</sup> and red cell thiobarbituric reactive substance<sup>22</sup> are consistently elevated in autistic patients. Stores of the key antioxidant



glutathione are markedly reduced in autism.<sup>23</sup> Glutathione deficiency may result from overuse, depletion, or dietary deficiency of amino acid precursors, or may be the product of a deficiency in sulfation capacity – an almost universal finding in autism.<sup>24</sup>

Since glutathione is so central to intracellular redox balance, glutathione deficiency in itself tends toward allowing oxidative stress to persist, thereby predisposing sensitive cells to apoptosis (a catastrophic process in post-mitotic tissues like the brain). But glutathione is not the only antioxidant deficiency. Red cell and plasma glutathione peroxidase, which catalyzes the reaction from hydrogen peroxide to water, is deficient in most autistic patients, as is red cell and platelet superoxide dismutase, which reduces superoxide to hydrogen peroxide.<sup>25</sup> These deficiencies further diminish the body's capacity for neutralizing reactive oxygen species. **In autism, these deficiencies are aggravated by relatively lower levels of various antioxidant cofactors such as vitamins A, C, and E, magnesium, selenium, and zinc,<sup>26</sup> and vitamin B6.<sup>27</sup>**

The presence of oxidative stress is further documented by increased concentrations of nitric oxide in autism (measured as the total of nitrite and nitrate)<sup>28</sup> and by increased concentrations of red cell xanthine oxidase.<sup>29</sup> Indeed, generalized mitochondrial dysfunction seems to be a consistent feature of autism.<sup>30</sup> Mitochondrial dysfunction would be expected to generate oxidative stress, which in turn will generate an inflammatory response and clinical symptoms in sensitive organs. As evidence of this process, circulating cytokines are increased in autism.<sup>31</sup> Cytokines are both a result of oxidative stress and producers of additional reactive oxygen species. The interaction between oxidative stress and inflammation produces a self-reinforcing cycle, and immune abnormalities appear to play an important role in the pathogenesis of autism.<sup>32,33</sup>

Oxidative stress has a serious impact on the brain, as would be predicted in an organ chiefly composed of lipids, with high energy requirements, considerable sensitivity to excitotoxins, and no meaningful ability to replace cells destroyed by apoptosis.<sup>34</sup> Nitric oxide has been shown to damage the blood brain barrier<sup>35</sup> and to cause or correlate with demyelination.<sup>36</sup> Autoantibodies to myelin basic protein are present in autism.<sup>37</sup> Nitric oxide also damages cholinergic receptors, and these are less active in the cerebral cortex of autistic patients,<sup>38</sup> as are gamma-aminobutyric acid (GABA) receptors. Similarly, the concentration of glutamic acid decarboxylase (GAD), which converts the excitotoxin glutamate to GABA, has been found to be decreased in numerous studies.<sup>39</sup> Diminished GABA activity will lessen resistance to apoptosis of neurons exposed to glutamate under oxidative stress conditions, and, indeed, the plasma concentration of glutamate is increased in autism.<sup>40</sup> Apoptosis may explain the diminished number of neurons found in the cortex, brainstem, and cerebellum of autistic patients at autopsy.<sup>41</sup>

**Oxidative stress also has a serious impact on the gut, which could easily be considered a second sentinel organ in autism.** Majorities of autistic patients in most series suffer from significant GI complaints, with a loose correlation between severity of autistic regression and severity of GI symptoms.<sup>42</sup> The gut is highly sensitive to oxidative injury.<sup>43</sup> Excessive nitric oxide degrades mucin,<sup>44</sup> increases intestinal permeability, relaxes the esophageal sphincter,<sup>45</sup> and mediates slow-transit constipation,<sup>46</sup> all of which are issues in autism. **Increased intestinal permeability is associated in autism with absorption of opioid-like polypeptides from the partial digestion of casein and gliadin; these are thought to have an adverse impact on alertness and general behavior.<sup>47</sup>**

Clinical therapies addressing individual biochemical abnormalities have had some success. Many

investigators have reported behavioral improvements with avoidance of dietary gluten and casein, presumably by lowering exposure to exorphins.<sup>48</sup> A cholinesterase antagonist has had a positive impact on behavior in some patients,<sup>49</sup> perhaps by partially compensating for the damage to cholinergic receptors described above. **High-dose combined vitamin B6 and magnesium (Mg) therapy has been found effective in a double-blind trial;<sup>50</sup> neither was effective individually.** This combination may improve cholinergic status via B6-dependent kinase, which has a positive influence on GABA and muscarinic receptors and requires both B6 and Mg for function. Both B6 and Mg levels are generally low in autistic children. Magnesium is also involved in mitochondrial energy production as a cofactor for NADPH and ATP production. Vitamin B6, in turn, is required for glutathione peroxidase and reductase activity and for the synthesis of mitochondrial Complexes I, II, and III, heme for Complex IV and Coenzyme Q10, with its vital role in electron transport.<sup>51,52</sup> The combination of Mg and B6 may therefore improve clinical status by augmenting mitochondrial function, thereby diminishing oxidative stress.

Vitamin C in a double-blind university trial at eight grams per seventy kilograms body weight was found to have a significantly positive impact on several measured autistic behaviors.<sup>53</sup> Whether this effect was due to vitamin C's antioxidant power, its ability to protect against the damage caused by nitric oxide and peroxynitrite, its ability to protect neurons against glutamate toxicity, or some combination thereof was not clear.<sup>54</sup>

Zinc picolinate in high doses (2-3 mg per kg body weight) has been found to improve behavior in autistic patients.<sup>55</sup> Whether this results from simple repletion of zinc deficiency – common in autism – or from zinc's ability to antagonize excessive copper – also common in autism – is not clear. Zinc is necessary



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► for the synthesis of metallothionein, which binds, transports, and regulates heavy metals. Zinc is a competitive inhibitor of glutamate, protects sulfhydryl groups against oxidative damage, competes with manganese (which is believed by some to play a role in neurodegeneration), and has numerous other antioxidant effects.

Selenium is low in autistic patients and is part of many autism protocols. It has the theoretical value of being a necessary cofactor for glutathione peroxidase. It has not been the subject of a controlled trial.

Various vitamins and amino acids have been reported to improve behavior in autistic patients, including B12, thiamine, and folic acid.<sup>56,57</sup> Each of these is involved in oxidative phosphorylation, presumably improving mitochondrial function and lessening oxidative stress. Similarly, amino acid precursors of glutathione<sup>58</sup> have had a positive effect, also probably because of glutathione's ability to lessen oxidative stress.

Polyunsaturated fatty acids have also been found to be beneficial in treating autism.<sup>59</sup> Since membrane levels of essential fatty acids are commonly depleted in autism, perhaps by oxidation, repletion may restore healthier membrane and receptor function.

Each of these modalities has produced some benefit, chiefly by ameliorating one or more of the results of oxidative stress. None has satisfactorily addressed causation. Oxidative stress is a symptom, not a cause. Search for etiology has suggested a substantial genetic component in the pathophysiology of autism. The monozygotic twin of an autistic patient has a 50% risk of also being autistic.<sup>60</sup> Non-monozygotic siblings of autistic children have a greatly increased risk of autism.<sup>61</sup> On first glance, this would seem to suggest that one or more chromosomal defects in genes relating to neural development might

be primary factors in the development of autism. However, since the risk for monozygotic twins is 50%, not 100%, and since none of the various genetic abnormalities thus far detected occurs in more than three percent of autistic patients,<sup>62</sup> it would seem more likely that the heritable component exists at the level of genes coding for factors affecting overall gene expression and not necessarily in genes specifically responsible for neurodevelopment.

We propose that heritable defects in detoxification pathways might be the controlling factors in development of autism. As we have observed above, deficiencies in sulfation ability or in glutathione concentration are common in autism. Sulfation and glutathione deficiencies adversely affect two major Phase Two detoxification pathways. These deficiencies would allow the accumulation of toxic environmental chemicals and heavy metals, which could, in sufficient concentration or if introduced early enough during critical neurodevelopmental periods, be capable of influencing the expression of genes controlling neurological development. We have coined the term "non-excretor" to describe this type of patient, who is apparently unable to excrete various toxic chemicals and heavy metals, presumably because of demonstrably impaired or compromised detoxification pathways.<sup>63</sup>

Similarly, persons who are homozygous for apolipoprotein E4, which has no sulfhydryl group, might have a lesser capacity for eliminating xenobiotics dependent on binding to sulfhydryl groups, such as heavy metals and some xenobiotic chemicals. This might explain the high incidence of ApoE4 genotypes in autism and Alzheimer's disease.<sup>64</sup>

Damage to the expression of genes controlling neurological development could occur either through direct toxicity of accumulated xenobiotics and metals, or through initiation by these substances of chronic oxidative stress, from which would ensue phenotypic damage and clinical symptoms. There is evidence for such a process occurring *in utero*,

leading to autism.<sup>65</sup> Analogous injury could occur during early childhood, while the brain is still developing. Substances routinely used during the early developmental years that may cause severe neurodevelopmental deficits including, but not limited to, autism spectrum disorder, have been described in the literature and detailed in the material safety data sheets of these substances<sup>66</sup> as well as referenced in reports by the Environmental Protection Agency<sup>67</sup> and the Environmental Working Group.<sup>68-70</sup>

What environmental factors might be causative? The two leading suspects would seem to be neurotoxic chemicals and heavy metals, probably in some synergistic combination. The Second National Report on Human Exposure to Environmental Chemicals (NHANES)<sup>71</sup> paints a grim picture of maternal and fetal exposures to multiple chemicals and metals. A small study by Edelson and Cantor found 100% of autistic probands had deficient liver detoxification ability, as assessed by the usual excretory tests, and 90% had high levels of many neurotoxic chemicals.<sup>72</sup> In Edelson's earlier study,<sup>73</sup> all the probands also had evidence of unusually high levels of heavy metals.

Lead and mercury are known neurotoxins and known initiators of oxidative stress<sup>74,75</sup> and other clinical disorders.<sup>76-80</sup> Cadmium, arsenic, and less common heavy metals may also play a role, perhaps through synergistic augmentation of the toxicity of lead and/or mercury.<sup>81,82</sup> Synergy between metals and neurotoxic chemicals is probably also present, but has not been systematically measured.

Mercury has been suspected for some time to play a role in autism, in part because of the inclusion of thimerosal in several vaccines, in part because the apparent increase in autism rates paralleled the increase in mercury-containing immunizations. While this association was not found persuasive by the Institute of Medicine (IOM) in 2001, the IOM nonetheless agreed to the 1999 request by the Public Health Service and American

Academy of Pediatrics to remove thimerosal from vaccines,<sup>83</sup> and with some exceptions, this appears to have been accomplished on a limited basis.

Bernard's extensive review<sup>84</sup> outlines in exquisite detail the immense overlap between described symptoms and findings in mercury poisoning and in autism. If one were to draw Venn diagrams of the two conditions, they would be almost perfectly superimposed, according to Bernard's data. Mercury is not only capable of producing clinical results closely approximating autism, it is also well-known as a powerful initiator of oxidative stress.<sup>85</sup>

Future therapy directed at eliminating causative factors of autism will have to address neurotoxic chemicals and heavy metals. Reduction of total body load of both groups of xenobiotics will most likely diminish oxidative stress and may create conditions under which healing and reversal of symptoms might occur.

We have an ongoing series of approximately 450 autistic patients, each of whom is being treated with the full range of nutritional and biomedical modalities referred to above – also with transdermal DMPS and, for the removal of mercury and other heavy metals, by chelation. Individuals with lead are also treated with EDTA. Thus far, out of approximately 450 probands, approximately 15% have had total remission of symptoms. The remainder show various levels of recovery. Some of these patients only underwent treatment for a limited time period and showed minimal response, compared to those who sustained the entire treatment course, which varies depending on biological individuality and level of toxicity.

In our series, patients were monitored for RBC, urine, fecal, and hair levels of toxic and essential minerals. On initial DMPS challenge, elevated levels of arsenic were observed in the urine of 100% of a subgroup studied in detail. Abnormal levels of cadmium, nickel, and tin were recovered from 86%

and mercury in only 57%, on initial challenge. However, on further treatment and subsequent challenges, excreted mercury levels tended to increase over time, while the other toxic metals, including aluminum, decreased. Clinical improvement continued over time and correlated with overall lowering of total body burden of heavy metals, primarily mercury.

The benefits of lowering total body burden of heavy metals are well-established in the literature.<sup>86,87</sup> Our experience with this series of well-documented autism spectrum patients so far includes approximately 15% who have completely resolved their symptoms and another more than 50% who have made rapid progress since the start of this treatment regimen.

We infer from this initial experience with treatment of autistic spectrum patients that reducing the body burden of one likely cause of oxidative stress – heavy metals – greatly adds to the benefits observed from merely treating the symptoms of oxidative stress. We will fully report our ongoing experience in the near future.

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