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OXIDATIVE STRESS IN AUTISM

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STATEMENT OF PURPOSE

Indirect markers are consistent with greater oxidative stress in autism. They include greater free-radical production, impaired energetics and cholinergics, and higher excitotoxic markers. Brain and gut, both abnormal in autism, are particularly sensitive to oxidative injury.

Higher red-cell lipid peroxides and urinary isoprostanes in autism signify greater oxidative damage to biomolecules. A pre-liminary study found accelerated lipofuscin deposition—consistent with oxidative injury to autistic brain in cortical areas serving language and communication.

Double-blind, placebo-controlled trials of potent antioxidants—vitamin C or carnosine—significantly improved autistic behavior. Benefits from these and other nutritional interventions may be due to reduction of oxidative stress. Understanding the role of oxidative stress may help illuminate the pathophysiology of autism, its environmental and genetic influences, new treatments, and prevention.

OBJECTIVES

Upon completion of this article, participants should be able to:

1. Be aware of laboratory and clinical evidence of greater oxidative stress in autism.
2. Understand how gut, brain, nutritional, and toxic status in autism are consistent with greater oxidative stress.
3. Describe how anti-oxidant nutrients are used in the contemporary treatment of autism.

W hen oxidants exceed the antioxidant defense, biological systems suffer oxidative stress, with damage to biomolecules and functional impairment. Autism is a behavioral disorder, with hallmark communication and social deficits. It has been suggested that oxidative stress may play a role in the pathophysiology underlying the behaviors that define autism.[1] Another serious behavioral disorder, schizophrenia, features high oxidative biomarkers[2] and documentation of clinical response to antioxidant.[3] Many neuroleptic medications used in the treatment of schizophrenia are, in fact, potent antioxidants.[4]

DIRECT EVIDENCE OF OXIDATIVE INJURY IN AUTISM

Bodily lipids, proteins, glycoproteins, and nucleic acids are subject to oxidative injury, and a number of analytical methods exist for measurement of oxidative by-products in urine, blood, breath, and organ tissue samples. Oxidized lipids and their protein adducts are commonly used as oxidative biomarkers. Lipids, which comprise biological membranes, are easily oxidized, particularly if highly unsaturated.

Direct markers for lipoxidation are higher in autism. In a published study which carefully eliminated dietary and medicinal confounders, red-cell thiobarbituric reactive substance (TBARS, a measure of lipoxidation) was twice higher in autistic children than in age-matched controls.[5] Other preliminary studies found serum lipid peroxides[6] and urinary isoprostanes[7] significantly higher in autistic children.

Indirect markers are consistent with greater lipoxidation in

autism. Low concentrations of highly unsaturated lipids in autistic red-cell membrane[8] suggest oxidative depletion. Higher phospholipase A2[8] and loss of membrane lipoprotein asymmetry[9] in autism comport with oxidative effects.

Lipofuscin is a non-degradable matrix of oxidized lipid and cross-linked protein which forms in tissue as a result of oxidative injury. Co-localization of lipofuscin with specific subcellular components or injurious agents may provide clues to neuropathogenesis. In Alzheimer's disease, lipofuscin is associated with oxidized mitochondrial DNA.[10] In a documented case of human mercury poisoning with psycho-organic symptoms, elevated mercury in brain localized in lipofuscin 17 years after exposure.[11]

Lipofuscin is experimentally-induced by strong pro-oxidants such as iron[12] or kainic acid.[13] In animals, lipofuscin forms initially in hippocampus, and later in cortical brain.[14] In animal experiments, lipofuscin deposition is retarded by supplementation with vitamins C and E[15] or carnitine,[16] and measurable brain activity is inverse to lipofuscin content.[17]

Edith Lopez-Hurtado and Jorge Prieto found greater lipofuscin in areas of autistic cortical brain concerned with language and communication, deficits of which are integral to the diagnosis of autism. After age-seven, in comparison to controls, greater lipofuscin was measured in autistics in Brodmann area[22] (Wernicke's, speech recognition), area[39] (reading) and area[44] (Broca's, language production).[18] (See Figure 1.)
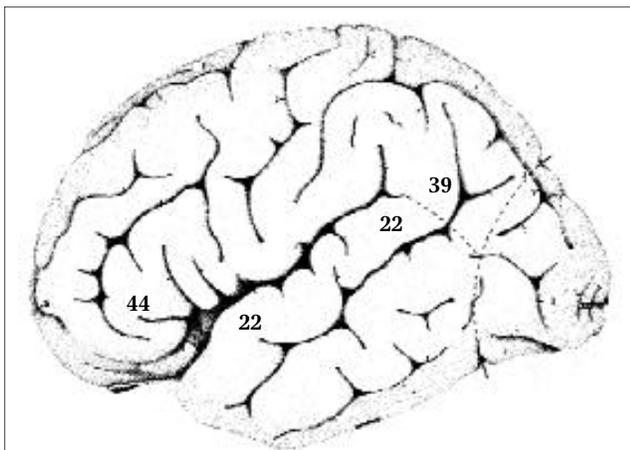

FIGURE 1 Greater Lipofuscin in Autistic Brain

Greater lipofuscin, a biomarker for oxidative injury, is found in areas of autistic cortex serving language and communication.[18]

In both autistic and control subjects, lipofuscin was always more prominent in Brodmann area[44] at all ages. Analysis by cortical layers showed that the number of cells (both pyramidal and non-pyramidal neurons) containing lipofuscin was larger in layers II and IV. A significant decrease in neuronal cell numbers was found in layers II and IV of autistic cortex, compared to controls.[18] Greater lipofuscin also has been reported in Rett syndrome,[19] on the autistic spectrum.

Retina, a virtual extension of the brain, is very sensitive to oxidative stress. Greater oxidative stress is associated with flattened electroretinograms and increased retinal lipid peroxides in animal experiments.[20] In autism, abnormal retinograms with flattened b-waves[21-22] suggest oxidative retinal injury. Retinographic response to antioxidants has not been tested in autism.

Data implying greater oxidation of biomolecules in autism are summarized in Table 1.

TABLE 1 Oxidized biomolecules in groups of autistic children versus controls	
Elevation / abnormality	**Reference**
Red-cell lipid peroxides by TBARS	(5)
Serum lipid peroxides	(6)
Urinary isoprostanes	(7)
Lipofuscin in cortical brain	(18)
Abnormal retinograms	(21)(22)

INDIRECT MARKERS ARE CONSISTENT WITH GREATER OXIDATIVE STRESS

Indirect markers for greater oxidative stress in autism include: 1) lower endogenous antioxidant enzymes and glutathione, 2) lower antioxidant nutrients, 3) higher organic toxins and heavy metals, 4) higher xanthine oxidase and cytokines, and 5) higher production of nitric oxide (NO·), a toxic free-radical.

Lower levels of antioxidant enzymes and glutathione in autism (Table 2) may stem from lesser production or greater consumption, and imply greater vulnerability to oxidants. Lower antioxidant nutrients (Table 3) may attribute to lower intake or absorption and/or greater oxidative depletion. A substantial literature documents increased oxidation of biomolecules and cell injury in relevant nutrient-deficient states.[29]

TABLE 2 Lower antioxidant enzymes and glutathione in groups of autistic children versus controls	
Lower in autism	**Reference**
Red-cell GSHPx	(23)(24)
Plasma GSHPx	(24)
Red-cell SOD	(24)
Platelet SOD	(23)
Red-cell catalase	(5)
Total plasma glutathione	(25)
Plasma GSH/GSSG	(25)

TABLE 3 Lower antioxidant nutrients in groups of autistic children versus controls	
Nutrient	**Reference**
Plasma vitamins C, E, and A	(26)
Red-cell activated B6 (P5P)	(27)
Red-cell B6 acitivity by EGOT	(26)
Red-cell magnesium	(26)
Red-cell selenium	(26)
Plasma zinc	(28)
Red-cell zinc	(26)

Nutrient levels affect the status of glutathione and antioxidant enzymes. The glutathione-boosting effect of vitamin C and vitamin E supplementation is well-known. Marginal deficiency of vitamin B_6 is associated with lower glutathione peroxidase (GSHPx) and glutathione reductase.[30] All forms of GSHPx contain selenium, and strong correlations exist between low and low-normal blood selenium levels and GSHPx activity.[31]

Organic toxins[33-40] and heavy metals[35] are strongly pro-oxidant. These may accumulate (Table 4) due to impaired detoxification, which is demonstrated in autism.[41] Toxins incite the production of oxidative species by various mechanisms. The volatile organic compounds and insecticides stimulate nitric oxide synthase (NOS).[42] Copper catalyzes the production of potent hydroxyl radical (OH·), especially when catalase is insufficient.[32] Mercury is known to increase oxidative stress by blocking mitochondrial energy production and depleting glutathione.

Circulating cytokines[40] and xanthine oxidase (XO)[5] are greater in autism, and both generate free radicals. XO actually results from oxidative alteration of xanthine dehydrogenase. Cytokines and XO can be both cause and effect of oxidative stress.

TABLE 4 Higher pro-oxidants in groups of autistic children versus controls

Parameter	Reference
Plasma perchlorethylene, hexane, pentane	(26)
Red-cell mercury, lead, and arsenic	(26)
Higher provoked urinary mercury	(35)
Plasma copper	(36)
Plasma *nitrite + nitrate*	(37)(38)
Red-cell *nitrite + nitrate*	(39)
Circulating cytokines	(40)
Red-cell xanthine oxidase	(5)

HIGHER FREE-RADICAL PRODUCTION IN AUTISM

NO·, which is short-lived, is measured indirectly as total *nitrite + nitrate*, which are stable derivatives of NO·. In autism, red-cell[39] and plasma[37,38] total *nitrite + nitrate* is elevated, and plasma *nitrite + nitrate* correlates positively with TBARS.[39] Excess NO· production is suspected to play a role in other neurobehavioral disorders, including schizophrenia,[43] Alzheimer's disease, Down's syndrome,[44] and multiple sclerosis.[45]

It's unknown whether production of excess NO· in autism is localized to specific organs or tissues. Cytokine-producing cells anywhere can stimulate NO·. If excess NO· localizes in autism, the brain and gut are plausible sources, as both are often abnormal in autism to gross and microscopic inspection, and behavioral and gastrointestinal symptoms predominate.

Excess NO· in brain would be a serious matter, as it increases apoptosis,[46] leaky blood-brain barrier (BBB),[47] neurodegeneration,[48] and demyelination.[49] Such mechanisms might effect neurodevelopment in autism.

Decreased activity of oxidation-sensitive receptors is found in autistic brain, and it is possible that this may relate to local NO·, or more generally to greater oxidative stress. Cholinergic receptor activity is decreased in autistic cortex,[50] and cholinergic receptors are sensitive to NO· toxicity.[51] Gamma aminobutyric acid (GABA) receptors, generically sensitive to oxidative stress,[52] are reduced in autistic hippocampus.[53] It is conceivable that the GABA-polymorphism that is associated with autism may lead to an increase in the sensitivity of this receptor to oxidative stress.[54]

In the existing literature, lesser cerebellar Purkinje cell numbers and smaller neurons in the entorhinal cortex and medial amygdala are consistent findings in autism[55] with marked Purkinje cell loss described as the most consistent finding.[56] "Stunted" pyramidal neurons and decreased complexity and extent of dentritic spines are found in hippocampus.[57] These findings are unexplained. Current technology would allow quantification and localization of specific oxidative (and nitrosative) biomarkers in autistic brain, and possible elucidation of microscopic pathology.

TABLE 5 Gut abnormalities in subgroups of autistic children

Abnormality		Autistic Sub-group	Reference
High intestinal permeability	42%	Asymptomatic	(58)
Reflux esophagitis	69%	Abdominal symptoms	(59)
Chronic gastritis	42%	Abdominal symptoms	(59)
Chronic duodenitis	67%	Abdominal symptoms	(59)
Ileal lymphonodular hyperplasia	89%	Regressed, gut symptoms	(60)
Colitis	88%	Regressed, gut symptoms	(60)

The autistic gut is inflamed (Table 5), and there appears to be a mutually amplifying positive feedback loop between gut inflammation and NO·. Pain, constipation or diarrhea, gastroesophageal reflux,[61] and increased intestinal permeability[58] are common. Variably, chronic inflammation ranges from esophagus to colon. Inflammation of the distal ileum with adenopathy is prominent.[60,62] In other clinical conditions, inflammation of the gut associates with greater increased NO· production. Plasma *nitrite + nitrate* is elevated in childhood colitis.[63] In chronic diarrhea, urinary *nitrite + nitrate* levels correlate with leaky gut.[64] In probability, the inflamed autistic gut produces more NO·.

NO· is potently antimicrobial.[65] Certain viruses and bacteria provoke massive local production of NO· in gut[66] and brain.[67] Unfortunately, massive NO· also oxidizes host tissue.[66,68] Thus, in gut, excess NO· is known to increase inflammation and permeability.[69] The young gut is uniquely sensitive to damage by NO·, particularly the ileum.[70,71] Too much NO· can deplete the antioxidant defense, depressing levels of reduced glutathione (GSH).[72,73] Low GSH, in turn, increases NO·.[74] Nitrite binds GSH.[75]

Excess NO· leads to increased formation of peroxynitrite (ONOO⁻), which savages biomolecules. ONOO⁻ is formed by reaction of NO· with superoxide (O⁻), and is much more reactive than its parent radicals. Known targets of ONOO⁻ attack, with possible relevance to autistic pathophysiology, include: tyrosine groups (as in glutamine synthetase and glutathione reductase inhibition), sulfhydryl (-SH) groups, superoxide dismutase (SOD), neurofilaments, ceruloplasmin (releasing pro-oxidant copper), membrane

receptors, ion channels, G-proteins, and methionine. ONOO⁻ depletes antioxidants, peroxidizes lipids, and breaks DNA.[31,76]

NO· is too ephemeral for distant transport. But hypothetically, excess NO· in one tissue may cause damage in a more distant site via higher circulating nitrite and nitrate. For example, experimental intravenous nitrite injures the BBB.[77] Higher levels of nitrite in autism may link chronic gut and brain injury. Inflamed gut may thus damage brain.

Or conceivably, distant NO· production may raise levels of circulating stable products of NO· (nitrite, nitrate, and S-nitrosohemoglobin), which may in turn lead to greater gut NO·, and resultant inflammation of the gut.[78-79] Nitrite and nitrate are selectively removed from the circulation by the gut.[80,81] Various bowel flora convert nitrite and nitrate to NO· by enzymatic reduction,[82,83] which is catalytically favored by low oxygen tension,[84] as found in gut. NO· from distant production also circulates as S-nitrosohemoglobin, and the release of NO· in the bowel from this carrier protein is facilitated by low oxygen tension and the presence of sulphides produced by certain bacteria.[78] As modulated by flora, excess production of NO· from anywhere in the body—including brain—might serve to inflame the gut.

BRAIN AND BBB SENSITIVITY TO OXIDATIVE STRESS

The brain is inherently sensitive to oxidative stress due to higher energy requirement, higher amounts of lipids and iron and autooxidizable catecholamines, and lower levels of certain endogenous antioxidant molecules.[85,86] The protective BBB also is relatively sensitive to oxidative damage.[87] Clinical and laboratory findings suggest a leaky BBB in autism. (See Table 6.)

TABLE 6 Leaky Blood-Brain Barrier in Autism?	
Clues and Predisposing Factors	**Reference**
High antibodies to brain proteins	(88)(89)(90)(91)
Sleep disturbance in autism	(59)(92)
Perivascular lymphocytic cuffing	(38)
Higher NO· / nitrite	(37)(38)(39)
Lower zinc	(26)(28)
Higher circulating cytokines	(40)
Higher heavy metals	(26)(35)

Rapid behavioral response to treatment with GSH hints of a leaky BBB in autism. In healthy animals with intact BBB, brain penetration by GSH is practically nonexistent. Yet, clinicians report immediate behavioral improvement in some autistic children simultaneous with GSH infusion,[93] suggesting a direct effect on the central nervous system.

In animals, experimental oxidative injury to the BBB preferentially injures the reticular formation.[94,95] In autism, widely reported difficulty falling asleep or staying asleep[92] suggests the possibility of reticular formation dysfunction. The specific nature of rapid eye movement (REM) abnormalities found in autistic sleep disturbance is more typically associated with neu-

rodegenerative diseases in which oxidative stress has been documented.[96] Other than melatonin, which has proven effective in autistic sleep disorders,[97] the effect of antioxidants on autistic sleep disturbance has not been investigated.

Laboratory observations suggest leaky BBB in autism. Perivascular lymphocytic cuffs reported in three of seven autistic brains,[38] are sentinel, though nonspecific. High autoimmune titers to central nervous system proteins in autism[88-91] suggest abnormal exposure of the immune system to brain antigens via leaky BBB.

The autoimmune response to brain antigen also may be promoted by oxidative generation of neoepitopes, which occurs via oxidative alteration of host proteins.[98] If they co-exist, autoimmune and oxidative mechanisms in the autistic brain may be mutually reinforcing, as NO· production is significantly increased in central nervous autoimmune disease.[67]

Conditions which have been documented in autism are associated with porous BBB in animals. Higher levels of circulating cytokines,[99] heavy metals,[100] NO·,[101] and nitrite[102] produce leaky BBB in animals. Lower zinc status in autism[27,29] may be relevant. Zinc at physiological concentrations protects the BBB from injury,[101] and zinc deficiency increases BBB permeability, particularly in conjunction with oxidative stress.[103]

Intriguingly, preliminary data find overgrowth of gram-negative aerobes in autistic throat and rectal cultures.[104] These organisms produce endotoxin, renowned for permeabilization of the BBB.

Investigation of the autistic BBB is warranted. Enhanced magnetic resonance imaging demonstrates BBB leaks,[105-106] and scanning electron microscopy visualizes BBB injury, including luminal protrusion, endothelial craters, vacuolation, inclusion bodies and necrosis, though such lesions may be sparse.[100]

GREATER OXIDATIVE STRESS AND THE GUT

Ischemia/reperfusion studies demonstrate that the gut is very sensitive to oxidative injury.[31,107] Ingested toxins (peroxidized fats, electrophilic food contaminants) and microbial metabolites present a large oxidative burden to the intestinal epithelium.[108] Sufficient quantities of GSHPx (to reduce peroxides), GST (to reduce electrophiles), and GSH (to facilitate both GSHPx and GST), are required to protect the gut from oxidation.

As indicated earlier, ileal inflammation and adenopathy are conspicuous in autistic children with gastrointestinal symptoms. Ileum appears more vulnerable to oxidative injury. In animals, GST is 36-fold lower in the distal ileum than proximal intestine.[109] Double knock-out genes for gastrointestinal GSHPx result in mucosal inflammation of the ileum, but not other parts of the intestine.[110] In human inflammatory bowel disease, NOS expression is most prominent in the ileum, and ileum is most sensitive to NO·-dependent oxidative injury.[111]

Excess NO· is a plausible mediator for autistic gastrointestinal symptoms. (See Table 7.) NO· degrades mucin, which protects the gut from a wide variety of irritants.[108] Excess NO· increases intestinal permeability,[112] prevalent in autism.[58] In

TABLE 7 Autistic Gut Abnormalities Possibly Mediated by Excess NO˙

Abnormality in autism	Reference
Inflammation	(66)(68)(70)(71)
Increased intestinal permeability	(69)
Low esophageal sphincter tone	(113)
Poor gall-bladder contraction	(105)
Slow-transit constipation	(70)

excess, NO˙ relaxes the esophageal sphincter,[113] and two-thirds of autistic children with gastrointestinal symptoms have reflux esophagitis.[59] Excessive NO˙ inhibits gallbladder contraction,[114] perhaps accounting for lighter-colored stools observed by parents and clinicians in many autistic children. Poor bile flow impairs nutrition and limits delivery of protective GSH to the gut mucosa.

Excess NO˙ also mediates slow-transit constipation.[70] Many autistic children are constipated, some with very large caliber stools. It is possible that malabsorption and floral overgrowths belie a tendency to constipation in even larger numbers of autistic children.

OXIDATIVE STRESS, LOW ENERGY PRODUCTION, AND EXCITOTOXICITY

Oxidative stress, impaired energy production and excitotoxicity are dynamically related. For instance, energy-producing mitochondria are sensitive to oxidative injury,[86, 115-120] and injured mitochondria leak more oxidants.[121-123] Also, impaired energy production predisposes to activation of excitatory receptors, decreased intracellular calcium buffering, increased oxidizing species, and apoptosis.[86,124]

Overstimulation of excitatory receptors results in oxidative neuronal injury,[125,126] and greater oxidative stress increases release of glutamate and subsequent stimulation of excitatory receptors.[127,128] Subcellular anatomy correlates with this functional relationship: excitatory glutamate receptors and NOS in brain and gut[129] are co-localized.

As a general rule, oxidative biochemistry adheres to the following construct, which is both consistent and useful:
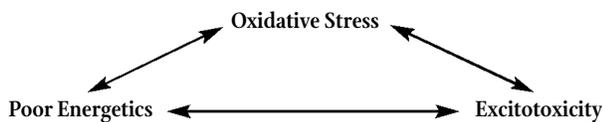
Accordingly, greater oxidative stress in autism implies possible problems in energy production and excitotoxicity.

IMPAIRED ENERGETICS IN AUTISM

Magnetic resonance imaging has demonstrated decreased ATP levels in autistic brain.[130] Higher lactate,[131-132] higher pyruvate,[133] higher ammonia, and lower carnitine[134] are documented in autistic children as a group, although not all autistic children have lower measurements in some or all of these parameters.

Collectively, these differences do suggest impaired mitochondrial function in autism, and in fact, mitochondrial abnormalities are reported in autistic case studies.[135-136]

Excess NO˙ in autism may impair energy production, directly or via ONOO⁻. Excess NO˙ reduces oxidative phosphorylation, lowering ATP and increasing lactate.[137] NO˙ directly inhibits complex IV, causing leakage of superoxide and inhibition of GSHPx.[31] ONOO⁻ selectively damages complexes I and III.[138] NO˙ inactivates coenzyme A (CoA), depriving mitochondria of this precious "energy currency."[78] (See Figure 2.)
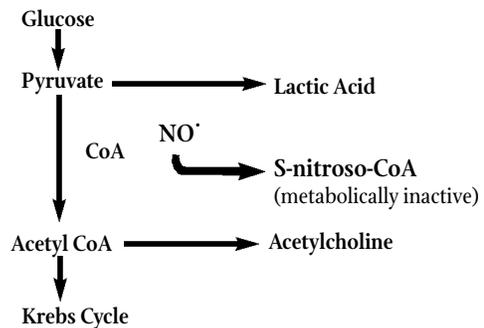

FIGURE 2 Impaired Energetics in Autism

Excess NO˙ inhibits CoA by conversion to metabollically inactive S-nitroso-CoA,[78] with resultant reduction of acetylcholine and ATP production. Lower ATP,[130] higher lactate,[131,132] and pyruvate[133] are found in autism.

EXCITOTOXIC MARKERS IN AUTISM

Higher extracellular glutamate in the brain is associated with excitotoxicity, especially if energy metabolism is compromised.[139] Glutamic acid decarboxylase (GAD) converts glutamate to GABA, and GABA lessens excitotoxicity. A decrement in brain GAD favors excitotoxicity, by increasing glutamate and decreasing GABA.

There is ample suggestion that GAD is deficient in autism. The quantity of GAD in post-mortem autistic brain is decreased by half.[140] Peripheral measurements are consistent with GAD impairment. Red-cell GAD binding affinity is lower,[26] plasma glutamate higher,[133,141] and plasma glutamine lower[141] in autism. GAD,[142] glutamine synthetase,[143] the glutamate transporter,[144] and inhibitory GABA receptors[9] are sensitive to oxidative stress.[52] (See Figure 3.)

Whether cause or effect of greater oxidative stress in autism, greater excitotoxicity is a reasonable hypothetical and clinical concern. Excitotoxicity can also be aggravated by oral ingestion
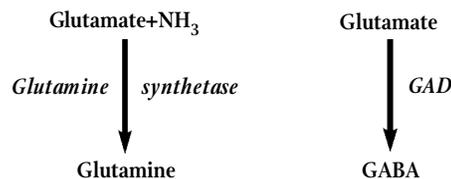

FIGURE 3 Excitotoxicity in Autism?

Higher extra-cellular glutamate and lower GABA increase excitotoxicity. GAD quantity[140] and activity[26] are lower in autism. GAD,[142] glutamine synthetase,[143] and the glutamate transporter[144] are sensitive to oxidative stress.

of excitotoxins.[145] The author joins other clinicians in advising autistic patients to avoid excitotoxic flavor enhancers such as MSG and aspartame in food and drink.

IMPAIRED CHOLINERGICS IN AUTISM

Laboratory and clinical observations suggest a significant cholinergic deficit in autism. Cholinergic receptor activity is lower in autistic cerebral cortex.[50] Treatment with cholinergic agonists[146-147] or precursor (deanol) for acetylcholine,[148] is associated with improved behavior in autism.

Response to bethanecol, a specific agonist for the muscarinic subtype of cholinergic receptors, invokes muscarinic impairment in autism. Oral bethanecol (2.5-12.5 mg b.i.d.) normalizes dilated pupils, increases bowel motility, and improves sleep pattern and behavior in many autistic children. Occasionally, sudden behavioral improvement is reported with the first dose of bethanecol,[146] and the author confirms this observation.

Neuroimaging reveals cerebral hypoperfusion in autism,[149-150] worsening with age,[151] and vasodilatation of cerebral microvessels is the province of muscarinic receptors.[152-153] A possible explanation for the rapid bethanecol response is sudden improvement in cerebral perfusion. Bethanecol may stimulate readily accessible muscarinic receptors in the small blood vessels which perfuse the brain. This hypothesis may be simple to test.

Muscarinic impairment in autism may potentiate greater oxidative stress. Experimentally, muscarinic signals are neuroprotective, shielding cells from oxidative stress and apoptosis.[154] Muscarinic receptor numbers are decreased by oxidative stress.[155]

Muscarinic receptors are sensitive to NO˙ toxicity,[156] and relative to other receptor subtypes, muscarinics are preferentially sensitive to inhibition by ONOO⁻ [157] and other oxidants.[158] As discussed earlier, excess NO˙ in autism may depress CoA. Besides its importance in energy production, CoA is a necessary precursor for the cholinergic neurotransmitter, acetylcholine. (See Figure 2.)

Insufficient CoA renders cholinergic neurons more vulnerable to a variety of toxic insults, including excess NO˙.[51] Low CoA does appear to play an important role in other encephalopathies.[54]

ANTIOXIDANT NUTRIENTS IN THE TREATMENT OF AUTISM

High-dose vitamin C

A double-blind, placebo-controlled university trial utilized 8 grams per 70 kg body weight per day of oral vitamin C in two or three divided doses in institutionalized autistic children.[159] Some of the cohort had been on doses of up to 4 grams of vitamin C prior to the trial. The cross-over design, comprised of three 10-week periods, included treatment of all subjects with vitamin C for the first 10 weeks. In the second and third phases of the trial, half the cohorts received placebo and then vitamin C. The other half received vitamin C and then placebo.

Psychometric testing was performed after each 10-week phase of the study, but not prior. Total scores on the Ritvo-Freeman (RF) scale, which rates 47 social, affective, sensory, and language behaviors, demonstrated improvement in the group going from placebo to vitamin C, and worsening in the group going from vitamin C to placebo (P=0.02).

Pacing, flapping, rocking, and whirling behaviors, in particular, corresponded to vitamin C manipulation, and a group of "strong responders" were described as "obvious" to the investigators. No serious side effects were reported in this study, but clinicians report excessive stool softening can limit vitamin C dosing in some autistic children.

Vitamin C is strongly antioxidant. This suggests—but does not prove—an antioxidant mechanism for its therapeutic effect. Antioxidant effects of vitamin C do seem neatly tailored to autism. Vitamin C provides good protection against NO˙ and ONOO⁻.[31] Vitamin C is known to protect neurons from glutamate neurotoxicity,[160] as glutamate re-uptake involves exchange for vitamin C.[161] Vitamin C blocks the inhibition of glutamate transport by NO˙,[162] an effect seen particularly in the presence of copper,[163] which is higher in autistic blood.[28]

Carnosine

Carnosine, a naturally occurring amino acid found in high concentrations in the brain, is a strong antioxidant and neuroprotectant.[164-166] A double-blind, placebo-controlled 8-week trial of carnosine 400 mg by mouth twice daily produced significant improvement in autistic children compared with placebo. Psychometric testing demonstrated improvements in vocabulary (P=0.01), socialization (P=0.01), communication (P=0.03), and behavior (P=0.04).[167] Side effects were inconsequential: sporadic hyperactivity responded to lowering the dose of carnosine, and no child had to discontinue the study due to side effects.

Possible physiological mechanisms for the carnosine effect in autism include its prevention of NO˙toxicity,[168] the binding of free radicals and reactive hydroperoxides, and the ability to complex with metals such as copper.[169] The copper:carnosine complex demonstrates antioxidant, SOD-like activity *in vitro*.[170]

Vitamin B$_6$

Any mechanistic hypothesis for autism should accommodate the successful application of high-dose vitamin B$_6$ pioneered by Bernard Rimland. Multiple controlled trials demonstrate that in combination with magnesium, B$_6$ improves behavior in many autistic children.[148,171-172] While serum B$_6$ levels usually are normal, B$_6$ activity, as reflected by erythrocyte glutamic oxaloacetic transaminase (EGOT) assay, was significantly lower in a group of autistic children than in controls.[26]

Pyridoxal kinase, which converts B$_6$ to its active form, pyridoxal-5-phosphate (P5P), can be impaired in autism. A preliminary study suggests very poor binding affinity of pyridoxal kinase in autistic red cells, as reflected by high K$_m$ (Michaeli's constant).[26] P5P activity in blood is below normal in over 40% of autistic subjects.[27]

Pyridoxal kinase impairment in autism is unexplained. Lower zinc[26,28] and energy status in autism are attractive explanations, as pyridoxal kinase requires ATP-facilitated release of zinc

from metallothionein for activation.[173] Inhibiting agents also should be considered. The strongest pyridoxal kinase inhibitors are the carbonyl agents, which are exogenous chemicals such as hydrazine, from jet fuel.[174] Endogenous carbonyls are potential inhibitors. They result from oxidative alteration of bodily lipids, proteins, and sugars, and are broadly elevated in clinical conditions associated with excess $NO^{\cdot}$.[22]

While the cause of poor B_6 function in autism is uncertain, we can be sure that B_6 impairment is an oxidative influence. As discussed earlier, even marginal B_6 deficiency is associated with lower GSHPx and gluathione reductase activity, lower reduced/oxidized glutathione ratios, and higher lipid peroxide levels.[30]

Mitochondrial decay results from B_6 deficiency, and is associated with increased oxidative stress.[175-176] P5P is required for the synthesis of key mitochondrial components: iron-sulfur crystals (for complex I, II, and III), heme (for complex IV),[177] and coenzyme Q10.[178] Experimentally, P5P protects neurons from oxidative stress, apparently by increasing ATP production and stemming extracellular glutamate.[179]

Lagging B_6 function lowers the excitotoxic threshold. P5P is a necessary cofactor for GAD, impairment of which can increase glutamate receptor activation, $NO^{\cdot}$, and oxidative stress.[180] P5P protects GAD, which is sensitive to oxidative impairment,[142] from inactivation.[181] (P5P also protects gastrointestinal GSHPx by complex formation.)[182] Predictably, P5P administration to animals increases brain GAD activity.[183]

Thus, high doses of B_6 may benefit autistic patients by increasing energy production, lessening excitotoxicity, increasing GABA, and reducing oxidative stress. Treatment with B_6 also may relieve a state of functional B_6 deficiency caused by excess oxidants. The B_6 vitamers are highly vulnerable to damage by oxidative species such as hydroxyl ($OH^{\cdot}$) and singlet oxygen ($^1O_2$).[184-186] Oxidative impairment of B_6 could impair myriad enzymes and neurotransmitters in autism.

Magnesium

In animal experiments, magnesium deficiency increases $NO^{\cdot}$,[187] increases lipid peroxides,[188] and lowers plasma antioxidants.[189] Lower magnesium is clearly pro-oxidant. Magnesium supplementation lowers oxidative stress experimentally in animals with higher oxidative stress.[190]

As a group, autistic children have lower magnesium, as measured sensitively in red cells.[26] Double-blind trials demonstrate behavioral improvement and normalization of evoked potential recordings in autistic children receiving combined high-dose B_6 and magnesium, but no significant improvement with high-dose B_6 or magnesium alone.[191] The synergism may be a cofactor function. For instance, B_6-dependent kinase, which affects diverse muscarinic and GABA-nergic functions, requires both B_6 and magnesium.[139]

Magnesium also protects against oxidative stress via functions unrelated to B_6.

Production of NADPH, for reduction of glutathione, requires magnesium. ATP synthase, which catalyzes energy production by oxidative phosphorylation, is magnesium-sensitive.[192]

In brain, magnesium normally blocks overactivity of excitatory receptors by modulating calcium channels.[193]

Zinc

Lower zinc status in autism is clearly established. Red-cell zinc, a sensitive indicator of zinc sufficiency, is significantly lower in the autistic group,[26] and in individual cases may be as low as half the lower limit for age-matched controls.[194] Plasma zinc is sub-normal in 40% of autistic children.[28]

Low zinc potentiates oxidative stress. In animals, zinc-deficient diet decreases total glutathione, vitamin E, GST, GSHPx, and SOD levels, while increasing lipid peroxides and free radicals in tissue, mitochondria, and cell membranes.[195-198] In elderly adults, zinc supplementation decreases lipid peroxides.[197] In diabetics with retinopathy, zinc supplementation increases GSHPx and decreases lipid peroxides.[200]

Zinc status affects the intestine. Zinc deficiency in animals increases gastrointestinal NOS and susceptibility to gastrointestinal infection.[201] Conversely, supplemental zinc decreases intestinal lipoxidation[202] and lessens intestinal permeability.[203]

Clinicians increasingly appreciate zinc as a mainstay in the treatment of autism. William Walsh, who has organized zinc and copper data on more than 3,500 autistic children at the Pfeiffer Treatment Center, finds that high doses of zinc (2-3 mg/kg body weight/day, as highly absorbable zinc picolinate) are often needed to normalize zinc levels and achieve optimal clinical response.[204]

Periodic measurement of plasma zinc is used to assure that zinc is not pushed above the normal laboratory range. Zinc is withheld on the day of testing to avoid artifact. Zinc supplementation lowers copper. Serum copper monitoring is used to avoid sub-normal levels.[205]

Copper excess is evident in autism. Higher total serum copper,[36] lower ceruloplasmin,[6] and higher unbound serum copper[205] are found in groups of autistic children. Copper, especially unbound, is highly pro-oxidant. Supplemental copper is rarely needed in autism, and even small doses of copper have been observed to produce negative behavioral effects.[205]

Higher serum copper/plasma zinc ratios (in autism, mean 1.63 v 1.15 in controls, $P<0.0001$),[36] are significantly correlated with systemic oxidative stress in neurodegenerative disease.[206] Sufficient zinc supplementation normalizes copper/zinc ratios.[205]

High zinc dosing can suppress manganese. A balancing dose of manganese, administered separately from zinc at approximately 5 mg manganese/30 mg of zinc, is often beneficial, and serum manganese levels also may be monitored to avoid excess.[205]

The antioxidant function of zinc is prodigious. There are several important mechanisms:

• *Zinc protects –SH groups against oxidation*, as for example, in the protection of the key antioxidant enzyme, GSHPx.[195] The initial event in experimental zinc deficiency is loss of membrane –SH groups, with consequent membrane fragility.[207]

• *Zinc competes with pro-oxidant metals* such as copper and iron for bindings sites, preventing metal-catalyzed free-radical formation.[200] Copper-containing enzymes are inherently prone to autooxidation, which is prevented by zinc.[198] Copper-induced membrane oxidation is prevented by zinc.[208]

• *Zinc is an essential constituent of copper-zinc* SOD, a key antioxidant enzyme. Even marginal zinc deficiency in humans decreases SOD activity.[209] Zinc-deficient SOD becomes pro-oxidant, catalyzing biomolecular attack by $ONOO^-$.[148] Zinc-less SOD is neurotoxic.[210]

• *Zinc induces the synthesis of metallothionein (MT)*,[211] an effective scavenger of free radicals (including $ONOO^-$)[212] and sequestrant for copper and other heavy metals.[213,214] In animals, high-dose zinc induces measurably higher gastrointestinal MT levels.[215] Moderate zinc deficiency in animals, in which overt negative health effects are not manifest, is associated with significant reduction of retinal MT.[213]

MT normally increases as a protective response to oxidative stress, but actually decreases in response to oxidants when zinc is deficient.[214]

MT blocks copper toxicity, but this protective effect is lost in the presence of excess $NO^\cdot$, which releases copper from MT, causing lipid peroxidation and apoptosis.[46] In brain, MTIII, a neuronal growth inhibiting factor, is particularly sensitive to copper displacement by oxidants.[216] Such a mechanism could be related to greater gross brain size in younger autistic children.[204]

• *Zinc exerts physiological glutamate receptor blockade*,[139] lessening excitotoxicity.

Diverse biomolecules are protected from oxidation by zinc. By complexing with phospholipids,[217] zinc blocks oxidation of fatty membranes.[209] Zinc blocks peroxidation of polyunsaturated fat unbound to membrane.[218] Zinc generally inhibits the oxidation of enzymes and other proteins,[198] including those with functional $-SH$ groups vulnerable to mild oxidative conditions: Na,K-ATPase, Ca-ATPase, aquaporin, the voltage-gated calcium channel, and the NMDA-calcium channel.[207]

Hypothetically, oxidative stress may decrease clinical zinc retention. At a molecular level, oxidants (including $NO^\cdot$), displace zinc from proteins, including MT.[197,216,219,220] Research is needed to determine if this phenomenon extrapolates to lesser whole-body zinc retention under greater oxidative stress. Schizophrenics do have diminished urinary zinc loss in response to high doses of B_6,[22] possibly associated with anti-oxidant effects of B_6.

Selenium in autism

Mean red-cell selenium levels are lower in autistic children[26] and this may contribute to reported lower levels of GSHPx.[23-24] As stated previously, GSHPx activity correlates with low-normal and borderline selenium levels.[31] Clinicians frequently treat autis-

tic children with oral selenium, 50-300 mcg daily.

GSHPx is irreplaceable in the antioxidant defense, especially for protection of mitochondria, which do not contain catalase for protection from peroxide.[222] In addition, GSHPx confers sole protection from organic hydroperoxides, which sustain the devastating lipoxidation chain reaction.[85,222]

Lower GSHPx activity in frank selenium deficiency is associated with peroxidative damage and mitochondrial dysfunction.[29] The physiological effects of selenium deficiency can be compensated partially by administration of vitamin E.[31]

GSHPx is sensitive to inactivation by copper[182] and mercury.[223] Human mercury exposure is associated with decreased GSHPx activity and increased lipid peroxides.[224] In animals, GSHPx is protected by P5P[223] and zinc supplementation.[225]

Lesser GSHPx in autism favors greater membrane lipid peroxidation, which is known to impair receptor and enzyme function, presumably due to conformational changes and altered binding.[226] Lipid peroxidation has been shown to inhibit muscarinic, adrenergic, serotonergic, and insulin receptors, as well as Na,K-ATPase and glutamine synthase.[227]

Reduced glutathione in the treatment of autism

In an open-label trial, daily intravenous GSH improved patients with early Parkinson's disease.[105] Likewise, intravenous GSH improves behavior in many autistic children, including very rapid extinction of perseverative behaviors such as hand-flapping. Rarely, apparent histamine-mediated reactions (sneezing, coughing, pruritic eyes) are noted.[93]

Oral GSH, up to 30 mg/kg body weight/day in divided doses, has been beneficial in some children with cystic fibrosis, a high-oxidant state.[126] The author finds similar doses of oral GSH helpful in some autistic children. Reversible adverse behavioral reactions to oral GSH have been reported in children with low plasma zinc levels. The adverse reactions may result from rapid induction of metallothionein by GSH, with temporary zinc depletion.[228]

Oral GSH is well-absorbed. In animals, plasma GSH doubles within 2 hours of a large oral dose, mostly from absorption of intact GSH.[229] Increased animal organ levels of GSH are attributable to absorption of intact GSH.[230] In healthy humans, a 15 mg/kg oral dose of GSH increases plasma GSH levels by 2- to 5-fold.[229]

Intestinal mucosal demand for GSH can exceed synthetic capacity when demand is high,[229] as would be expected in autism. Intestinal mucosa imports intact GSH from both the intestinal lumen[231-232] and the plasma[231] to combat oxidation. In the normal physiology, biliary excretion of GSH represents a significant portion of total hepatic GSH production, and bile regularly bathes the intestinal mucosa with GSH.

Severe degeneration of the epithelium of the small intestine and colon, with mitochondrial swelling and degeneration, results from experimental GSH deficiency; these changes are prevented by the administration of oral GSH, which is associated with increased mucosal GSH levels.[233] In animals, mucosal GSH levels increase rapidly and significantly after oral GSH, but less in the ileum than elsewhere.[234] Oral GSH may lower

oxidative stress in the autistic gut.

Strong *in vitro* anti-viral properties of GSH[235] have been noted.

An oxidative perspective on emerging new treatments

Subcutaneous vitamin B_{12} injections, as preservative-free methylcobalamin 1,250-7,500 mcg weekly to daily, reportedly improve behavior in autistic children.[236] One B_{12} intermediate, cob(I)alamin, is exquisitely sensitive to oxidative inaction,[237] so functional B_{12} deficiency may result from greater oxidative stress in autism.

$NO^{\cdot}$ and nitrite elevations in autism send up a B_{12} red flag. An intermediate form of B_{12} reacts specifically with $NO^{\cdot}$,[238-240] and nitrite inactivates methylcobalamin.[241] $NO^{\cdot}$ binds B_{12} to impair enzyme function, as with *in vivo* inhibition of methionine synthase by $NO^{\cdot}$ at physiological concentrations.[242] Large parenteral doses of B_{12} may scavenge and reverse the physiological effects of excess $NO^{\cdot}$.[243]

Oral folinic acid supplementation improves subnormal glutathione and GSH / GSSG ratios in autistic children,[25] thus questioning functional folate status in autism. Tetrahydrofolate is very sensitive to oxidation,[241,244] and degradation may be significant under conditions of greater oxidative stress.[245] Folate deficiency (which may be aggravated by B_{12} impairment) is known to decrease ATP levels and increase reactive oxygen species and excitotoxicity.[246]

Amino acid supplementation may be useful in autism. Plasma cysteine levels were much lower in a series of 286 supplement-naïve autistic children.[236] Cysteine is produced endogenously from methionine, and provides a full third of the substituent molecules in glutathione and metallothionein.

Oral n-acetyl-cysteine (NAC), as a source of cysteine, is generally well-tolerated in autistic children, while direct supplementation with cysteine is not. Intravenous NAC (150-600 mg NAC + 1000-2000 mg vitamin C + 1 ml sodium bicarbonate) treatments reportedly improve behavior in autistic children.[236]

The Pfeiffer Treatment Center follows generous zinc loading with a proprietary oral supplement[247] which includes the amino acid constitutents of MT. Initial data suggest that this so-called "Metallothionein Promotion" formula increases levels of MT.[37] Some parents report improvement in autistic behavior coincident with greater exposure to natural sunlight. Ultraviolet radiation induces rapid induction of metallothionein,[248] so may be of benefit if zinc is sufficient.

Thiamine tetrahydrofurfuryl disulfide (TTFD) via rectal suppository improves behavior and increases heavy metals clearance in autistic children.[249] TTFD provides high cellular levels of thiamine, which boosts three mitochondrial enzymes known to be especially sensitive to oxidative stress.[120,250] One of these, α-ketoglutarate dehydrogenase complex (KGDHC), is a rate-limiter in energy metabolism and is inactivated by $NO^{\cdot}$, both directly and via $ONOO^{-}$.[120]

Casein- and gluten-free diet improved behavior in autistic children, possibly by reducing excess central opioid effects.[251] Higher peripheral opioid peptides from casein and gluten are

demonstrable in autistic urine,[252] possibly due to oxidative inhibition of the enzyme needed to complete digestion of dietary casein and gluten.[253] In addition, a shift towards oxidation in the redox environment is known to strengthen opioid binding, and GSH to weaken it.[254]

Fatty acid supplementation is beneficial in autism.[255] Lower concentrations of highly unsaturated fatty acids in plasma[256] and red-cell membranes[8,257] suggest oxidative depletion of these key membrane building blocks and prostaglandin precursors. Depletion of omega-3 and omega-6 polyunsaturated fatty acids is also seen in schizophrenia, and these changes are associated with increased lipid peroxide levels.[258]

Eicosapentaenoic acid (EPA, an omega-3) is lower in red-cell membranes of autistic children generally, arachidonic acid (AA, omega-6)lower in the regressed subgroup.[8] Fish oil, high in EPA, suppresses production of $NO^{\cdot}$ and other free radicals[30,259] and increases expression of GST and mitochondrial SOD.[259] Brain levels of $NO^{\cdot}$ and lipid peroxides are less in animals on diets supplemented with fish oil.[260]

Administration of fish oil to even marginally B_6-deficient animals can result in increased tissue lipid peroxide levels.[261] Prior administration of vitamin B_6 and other antioxidants is suggested in autism, to avoid generation of toxic lipid peroxides.

Ongoing administration of fish oil to autistic children is associated with significant lowering of red-cell membrane dihomo gamma linolenic acid (DGLA).[8] DGLA is an essential ω-6 precursor for prostaglandin-1, which tightens leaky gut and boosts immunity. Accordingly, autistic children receiving fish oil may benefit from a balancing dose of evening primrose oil, which provides gamma linolenic acid (GLA), DGLA precursor.

Clinical and laboratory assessment helps titrate fatty acid dosing. After antioxidant loading, many autistic children do well on an initial dose of 3 grams fish oil and 1 gram evening primrose oil.[262] Optimal doses vary individually, and over time.

LABORATORY ASSESSMENT OF OXIDATIVE STRESS

The use of oxidative biomarkers in the clinical management of autism is just beginning. Various blood, urine, stool, and breath assays[263] may prove useful in determining optimal doses and combinations of nutrients and other interventions.

Some available assays include lipid peroxides, 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), isoprostanes, levuglandin adducts, nitrotyrosine, oxidized nucleic acids, protein carbonyls, advanced glycation end-products, cellular apoptosis, nutrient and antioxidant enzyme concentrations, total *nitrite + nitrate*, enzyme-binding affinities, and luminal $NO^{\cdot}$ by rectal catheter. Ten-fold higher levels of neopterin,[264] a marker for upregulation of $NO^{\cdot}$ synthesis,[76] suggest a possible clinical utility of this measurement.

In the research arena, autistic brain and gut should be examined for very specific oxidative and nitrosative markers. Conventional pathologic assessment of autistic brain tissue may not detect neuronal loss due to apoptosis, a marker for oxidative stress,[265] since removal of apoptotic cells can be rapid.[266]

FUTURE DIRECTIONS

This article has outlined data and concepts which suggest that greater oxidative stress in autism may be important in the expression of autistic symptoms, and perhaps the pathogenesis of autism. If oxidative stress proves important in autism, then the nutritional management of autism,[267] because it modulates oxidative stress, presumably gains importance, too.

Ultimately, in order to treat or prevent autism, we may need to reconsider some ingrained living habits. Consumption of free radicals via foods fried in polyunsaturated oils[268] may need to be curbed. Ingestion of excitotoxic flavor enhancers, chlorine, nitrite, nitrate, and copper in water may need reassessment. Pro-oxidant[269,271] and antioxidant[272,275] drug profiles may become more pertinent.

While oxidative stress may be a persistent and treatable problem, its impact may begin *in utero*. We may need to consider how oxidative influences during pregnancy alter development to produce relevant post-partum effects. Gestational zinc deficiency, for instance, produces oxidative DNA damage in newborn primates.[276] The ubiquitous flavor enhancer and excitotoxin, monosodium glutamate, traverses the placenta and causes fetal neurotoxicity in rodents.[277]

Higher $NO^{\cdot}$ is a fact of autism which may provide clues to specific etiologies, aggravants, and treatments. Viral infections can increase greatly $NO^{\cdot}$ production in brain and other tissues, so higher $NO^{\cdot}$ production in autism makes systematic examination of autistic brain and other tissues for viral antigen more urgent than ever.

Higher $NO^{\cdot}$ in autism might productively focus attention on treatment with antioxidants with specificity for $NO^{\cdot}$. Vitamin C is a good $NO^{\cdot}$-quencher,[278] and so are melatonin and uric acid. Melatonin effectively scavenges both $NO^{\cdot}$ and $ONOO^{-}$.[279] Melatonin has excellent potential for relief of oxidative stress in both brain and gut,[280-281] increases expression of GSHPx,[282] and has proven effective in autistic sleep disorders.[97]

Uric acid normally represents up to 60% of total plasma antioxidant capacity.[283] It effectively binds transition metals and reactive species, and is especially effective quenching $NO^{\cdot}$[31] and $ONOO^{-}$.[284] Careful upward titration of uric acid levels with oral inosine, a uric acid precursor, may be of benefit in high-$NO^{\cdot}$ states such as multiple sclerosis,[45] and autism.

Testing and treating mitochondrial function to improve energy production probably merits a higher priority in autism. Acetyl-L-carnitine (ALC) and -lipoic acid (ALA) enhance mitochondrial function and reduce oxidative stress in senescent animals.[119] Oral administration of the mitochondrial metabolite, L-carnitine, has improved behavior in children with Rett syndrome,[285] and initial testing of high carnitine doses in autism is underway.

One university center often treats patients referred for suspected mitochondrial disease with a combination of CoQ10, vitamin E, and balanced B vitamins.[286] CoQ10, in combination or alone, is an attractive potential intervention in autism. It facilitates ATP production by carrying electrons and protons in the electron transport chain, and also acts in its reduced form to protect mitochondria by quenching oxidants.[287] Vitamin B_3 is crucial to mitochondrial energy production and effective in the high-oxidative state of schizophrenia,[288] but has received little attention in autism.

The clinical relevance of enzyme, receptor, G-protein, and vitamin cofactor sensitivity to oxidative stress (Table 8) is uninvestigated. Glucose-6-phosphate dehydrogenase (G-6-PD) activity, given its central role in the reduction of GSH, is but one of many oxidant-sensitive functions which deserve scrutiny in autism.

TABLE 8 Sensitive to oxidative / nitrosative degradation	
Enzyme or Co-Factor	**Reference**
Glutamic acid decarboxylase	(142)
Glutamate transporter	(144)
Glutamine synthetase	(143)(227)
GABA channels	(289)
B6 vitamers	(184)(185)(186)
Pyridoxal kinase by carbonyl inhibition	(174)
B6-dependent enzymes by carbonyl inhibition	(290)
Tetrahydrofolate	(241)(244)
Methionine synthase	(291)
B12 vitamers	(237)(238)(239)(240) (241)
Glucose-6-phosphate dehydrogenase	(292)
Coenzyme A	(78)
-KGDHC	(31)(250)(251)
Na,K-ATPase, Ca++ channels, Aquaporin	(207)(227)
Catalase	(293)
Glutathione peroxidase	(182)

OVERVIEW

The data demonstrate greater oxidative stress in autism. The clinical response to antioxidant nutrients suggests that oxidative stress is important in the expression of autistic symptoms. The question is whether oxidative stress is very important mechanistically.

Antioxidant therapeutic trials, which measure oxidative biomarkers, could help elucidate the importance of an oxidative mechanism. While we wait for this research, clinicians and parents are advised to implement safe nutritional interventions—sooner, rather than later. It would not seem premature to start applying laboratory biomarkers for oxidative stress to optimize doses and combinations of nutrients.

The preliminary lipofuscin data are potentially very important, and replicative and expansive studies should be performed as soon as possible. It is conceivable that lipofuscin analysis will identify specific toxic or infectious etiology. At the very least, lipofuscin is a strong hint that neurodevelopment in autism may be altered by oxidative influences.

In this respect, chronic neonatal vitamin E deficiency may help us understand the potential effect of excess oxidative stress

on neurodevelopment. Vitamin E deficiency is a neurological disease which clearly results from low antioxidant protection from birth.[294,295] Lipofuscin deposition is prominent.[294,296] Neurological symptoms—gait disturbance, abnormal ocular movements— present at 18-24 months,[294] the same chronology as autistic regression.

Beyond analogy, vitamin E may have concrete implications in autism or in healthy children prior to regression. Neurological complications of vitamin E deficiency are seen in patients with common variable immune deficiency (CVID) and enteropathy, and vitamin E screening has been recommended for patients with these conditions.[297] The immune profile in autism approximates CVID,[296] and there is no question about enteropathy. Preliminary data suggest lower plasma vitamin E levels in autistic children.[26] We need more vitamin E assessment, including functional testing by red-cell hemolysis.

Optimistically, it is noted that oxidative damage to biomolecules is often at least partially reversible. Oxidative inactivation of enzymes, for instance, is reversed when sufficient antioxidant is provided.[174] Even structural elements such as cytoskeleton can undergo restoration by GSH.[299]

If we learn that oxidative stress is an important mechanism in autism, then our search for the genetic and environmental causes becomes much more focused. From the oxidative wounds, our science may more rapidly deduce the cause, treatment, and prevention of autism.

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CME Test Questions*

oxidative stress in autism

In the following questions, only one answer is correct.

1. Direct evidence of greater oxidation of biomolecules in autistic children includes all of the following except:
   A. Higher vitamin C levels
   B. Higher red-cell lipid peroxides by TBARS
   C. Higher urinary isoprostanes
   D. Greater brain lipofuscin

2. Lower blood concentrations of antioxidant enzymes are reported in autism for:
   A. Catalase
   B. Glutathione peroxidase
   C. Superoxide dismutase
   D. All of the above

3. One of the following would be expected to contribute to greater oxidative stress in autism:
   A. Higher antioxidant enzymes and glutathione
   B. Lower organic toxins and heavy metals
   C. Lower antioxidant nutrients
   D. Lower cytokines

4. Greater production of a free-radical, nitric oxide (NO˙), is
   A. Marked by lower levels of the stable derivatives, nitrite + nitrate
   B. Known to primarily originate from autistic brain
   C. Injurious to host tissue in excess, but also potently antimicrobial
   D. Associated with lesser production of extremely reactive peroxynitrite (ONOO⁻)

5. Oxidative stress
   A. Is both cause and effect of excitotoxicity
   B. Is both cause and effect of impaired energy production
   C. Neither A or B
   D. Both A and B

6. Impaired mitochondrial function in autism is suggested by
   A. Higher lactate
   B. Higher pyruvate
   C. Lower carnitine
   D. All of the above

7. Greater excitotoxicity in autism
   A. Is suggested by central and peripheral laboratory markers
   B. Presumably lowers oxidative stress in autism
   C. May stem from lower oxidative stress in autism
   D. Suggests a possible benefit from monosodium glutamate or aspartame

8. In the double-blind, placebo-controlled trial of oral vitamin C in autism
   A. Significant side effects were reported
   B. A dose of 8 grams per 70 kg body weight per day was utilized
   C. A and B
   D. None of the above choices

See page 92 for Self-Assessment answers