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Oxidative Stress and Dietary Interventions in Autism: Exploring the Role of Zinc, Antioxidant Enzymes and Other Micronutrients in the Neurobiology of Autism

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Additional information is available at the end of the chapter

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1. Introduction

Autism Spectrum Disorders (ASD) consist of a set of complex neurodevelopmental disorders characterised by impaired communication and social behaviour, and repetitive or stereotyped pattern of behaviour. The prevalence of ASD has increased in recent decades to 0.6-1% [1-3]. Broadening of the diagnostic criteria and increased awareness of autism among parents and health professionals likely contribute to the prevalence increase. However, the reason for the increase is not completely understood. Alterations of developmental processes and gene expression profiles have been identified in ASD but neuronal mechanisms and perturbations of neuronal networks underlying the ASD phenotype are unclear. ASD varies in severity and the clinical phenotype reflects multifactorial background [4]. Co-morbidity with some genetic syndromes and autism exist [5]. Affected males outnumber females roughly 4:1 [6-8]. The family studies imply that the autism has a strong genetic basis but no single high risk genes for ASD are identified [9]. Hundreds of de novo mutations with extreme locus heterogeneity have been identified in genes encoding protein network ranked for autism candidate genes [10]. Recent studies suggest that environmental factors could play a much larger role in susceptibility to ASD than earlier expected [4]. Since the population's genetic inheritance is relatively constant over longer periods, the increased incidence rate of autism during last decades could indicate that there is an important environmental component in the etiology of autism [11].

The symptoms of ASD appear in childhood and persist throughout the person's lifetime. Since there is evidence that an initiating event for autism may appear early in intrauterine life, an

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early diagnosis and treatment could be possible. However, the early diagnostic uncertainties and the nature of deficits unfortunately often delay the diagnosis. Besides limited social and communication skills, behavioural and emotional symptoms, abnormal sensory responses and activity levels often seen as attention deficits are common in ASD individuals [12-14]. Intellectual disability associates often with autism (30-60%) and epilepsy is seen in about 25% of individuals diagnosed with ASD [1, 8, 15]. Many autistic children exhibit behavioural and sleep problems and aggression towards others or self as symptoms of their condition.

Unfortunately, there is no cure for autism and symptomatic treatment optimal for autistic individuals without major side effects is lacking. Improvement in patient care – both treatment and rehabilitation – directly influence the prospects of individuals with neurological disorders, including their abilities to integrate to the society and need for life-time support. Behavioural problems in ASD increase stress of people who take care of autistic children and dealing with these problems can be extremely challenging. The child's symptoms might result from an overload of demands (allergens, infectious agents, toxins, psychosocial stresses, inflammation, oxidative stress) in combination with weakness or susceptibilities, which impaired ability to respond to the demands (impaired energy production, inherited enzyme weakness, nutritional deficiencies, osteopathic disorders, sleep deficits, hormone imbalances, etc.) and increased vulnerability. There is evidence that many interrelated environmental factors may act as risk factors to development of autism [16].

2. Evidence for the role of oxidative injury in autism and the rational use of antioxidant therapy in autism

There is compelling evidence that cumulative damage by oxidative species play a role in many diseases including autism [17]. Reactive oxygen species (ROS) are unstable and aggressive molecules, which have the tendency to give their unpaired electron to other cellular molecules or snatch electrons from other molecules to attain stability [18]. ROS can be neutralized by antioxidant defence systems, including antioxidant enzymes and antioxidant compounds. Dismutation of the superoxide species, which is catalyzed by superoxide dismutases (SOD), leads to the formation of hydrogen peroxide. This can in turn be metabolized to water by catalases or peroxidases. Superoxide and hydrogen peroxide also undergo a series of iron-catalyzed reactions to yield hydroxyl free radicals (OH-). These are highly toxic themselves and can also generate more free radicals by reaction with other biomolecules, such as proteins or membrane fatty acids.

Antioxidants are compounds that reduce the production of free radicals and ameliorate the oxidative injury. In contrast to the rapid production of toxic oxygen species, the capacity of antioxidant systems, such as the enzyme SOD, catalase, glutathione (gamma-glutamylcysteinylglycine, GSH) peroxidase, and vitamins C and E, is limited, and there is a lag time in their adaptation. Dietary antioxidants and micronutrients in the diet, such as zinc, influence the development and function of immune cells, the activity of stress-related proteins and antioxidant enzymes, and help to maintain genomic integrity and stability [19, 20]. All these

physiological functions occur through the action of proteins involved in the regulation of zinc homeostasis such as metallothioneins which bind zinc with high affinity but, at the same time, release free zinc ions in response to oxidative/nitrosative stress that modulates the expression of zinc-dependent genes, activates antioxidant enzymes and has impact on immune response [21]. Zinc may induce the synthesis of metallothionein that act as a scavengers of metals and free radicals [22, 23]. The release of zinc from metallothioneins represents an intracellular response to stress. Biochemical modification of stress-related proteins might represent a useful target to influence zinc homeostasis and related mechanisms in autism.

A number of studies have implicated disturbed zinc metabolism to the neurobiology of autism. Many children with ASD are shown to suffer from zinc deficiency and excess copper levels [24, 25]. Low levels of zinc during development can adversely affect learning, memory, and attention [26]. Zinc deficiency has also been shown to associate with a behavioural syndrome characterized by reduced activity levels and slower response times [27]. Zinc is an important nutrient for the immune system, and supplementation with this mineral has been shown to reduce the duration of the common cold by suppressing the viral inflammation in the respiratory tract [28-30]. Zinc deficiency can result in a weakened intestinal immune system, which makes the digestive tract more prone to infection with certain parasites [31]. It is also reported in maldigestion and/or malabsorption that often associate with autism [32, 33]. There is evidence that zinc is required for intestinal wound healing and zinc is necessary to maintain the health and integrity of epithelial cells that line the intestines.

A variety of environmental factors that affect brain development during embryonic and perinatal periods may play a part in autism. These risk factors could be influenced by genetic mutations in genes involved in the inflammatory response such as TNF-alpha and interleukin 6 (IL-6) and in the maintenance of zinc homeostasis such as metallothioneins [34]. Il-6 has been associated with neurodegenerative disorders and autism [35, 36]. In genetic studies, measurable differences associated with genes that encode enzymes and other proteins impacting the methylation cycle, the folate metabolism and the glutathione system are reported between children with autism and healthy controls [37]. In particular differences in allele frequency and/or significant gene-gene interactions for genes encoding the reduced folate carrier (RFC), transcobalamin II (TCN2), catechol-O-methyltransferase (COMT), methylenetetrahydrofolate reductase (MTHFR), and one of the glutathione transferases (GST M1) are found. These genetic results, combined with the biochemical observations of dysfunction in the methylation cycle, strongly suggest that variations in genes associated with this cycle and its related biochemistry are involved in the genetic predisposition to developing autism.

How genetic mutations contribute to autism is not clearly understood. A hypothesis for treatment of a genetic form of autism with intellectual disability and epilepsy caused by BCKDH (Branched Chain Ketoacid Dehydrogenase Kinase) mutations by dietary amino acid supplementation was recently put forward [38]. Mutations inactivating a protein called BCKD-kinase prevent the breakdown of branched-chain amino acids. Normally, the amino acids are transported across the blood-brain barrier by special transporters. Since plasma amino acids compete with each other for transportation into the brain, the brain amino acid concentration will be substantially changed by low levels of branched-chain amino acids that affect the

expression of transporters. The amino acids serve as precursors for neurotransmitters like dopamine and serotonin, which play a role in mood and pleasure-seeking, and whose activities are likely associated with autism.

Recent studies have associated mitochondrial dysfunction with autism [39, 40]. Defects and malfunction observed in the mitochondria of autistic children suggest that oxidative stress in mitochondria could influence the onset of autism and explain the immunological anomalies present in autistic children. While many inherited genetic mitochondrial disorders occur in the mitochondria of all cells in the body, some are limited to specific cell sites, such as the brain cells which rely largely on mitochondria for energy [41, 42].

3. Potential targets for treatment to modulate oxidative stress status

Selenium (Se) is a micronutrient and mineral. It is a structural component and a co-factor of the antioxidant enzyme glutathione peroxidase. For this reason, supplementation with selenium can modestly increase glutathione levels in persons who are selenium deficient. Selenium has an antagonistic action on mercury and other toxic metals. No association of autism with hair concentrations of selenium was found in a recent meta-analysis [43].

Zinc (Zn) Zinc is an important cofactor for metabolism relevant to neurotransmitters, prosta-glandins, and melatonin, and indirectly affects dopamine metabolism. It is necessary for 100 different metalloenzymes and metal–enzyme complexes [44], many of them in the central nervous system. It contributes to structure and function of the brain [45]. Zinc is considered to be an important mineral for children. It is suggested that infants need more zinc for growth and development than older children and that that lack of zinc early in life may be linked with the development of autism. Zinc deficiency has been implicated in hyperactivity and jitters [46]. Children with autism have been shown to have lower zinc/copper ratios than normally developing peers and disturbed zinc homeostasis is suggested as a risk factor for neurodegenerative diseases [24, 25, 47].

N-Acetyl-L-Cysteine (NAC) NAC is sulphur-containing (sulfhydryl) amino acid which is present in many proteins, and is in the same class as the amino acid methionine. NAC is a naturally occurring amino sugar and is a form of cysteine which has been demonstrated to facilitate the short term cellular detoxification of alcohol, tobacco smoke, acetaminophen poisoning and environmental pollutants in several *in vitro* studies. NAC supplementation over long periods associates with modest increases in serum glutathione, but has not proven particularly useful in the treatment of chronic, long-term intracellular glutathione deficiencies. Furthermore, therapeutic levels of NAC are relatively toxic. At therapeutic doses, oral NAC supplementation can cause significant side effects. Cerebral symptoms, nausea, blurred vision, and vomiting are associated with NAC supplementation.

Vitamin A and E Retinol, the most useful form of vitamin A, (along with retinal and retinoic acid) and vitamin E are lipid-soluble. They can build up in the body and cause toxicity in excess amounts. Vitamin A is believed to improve sensory perception, language, and attention [48].

Autistic children could have a vitamin A deficiency because of gastrointestinal inflammation caused by leaky gut syndrome, allergies or viral infections. Lower levels of vitamin E are reported in ASD patients than in healthy controls [49, 50].

Vitamin B_6 and B_{12} Some forms of epilepsy are linked with deficiency of B vitamins. Lowered concentrations of B vitamins have been linked with cognitive decline and dementia in older adults. No statistically significant differences were found in plasma B_{12} levels between ASD cases and controls in meta-analysis [51]. Beneficial effects from high dose supplementation of vitamin B_6 with magnesium are shown in a subgroup of ASD individuals. Magnesium is combined to the treatment to prevent hyperactivity that can be caused by vitamin B_6 taken alone [52]. Peripheral neuropathy is a rare side effect of high dose vitamin B_6 treatment which generally disappears when supplementation is finished.

Folic acid Folic acid, the synthetic form of folate or vitamin B_9, during the first month of pregnancy may reduce child´s risk of autism [53]. Folate, vitamin B_6 and vitamin B_{12} are important coenzymes of the homocysteine-degrading remethylation and transsulfuration pathways [54] and their deficiencies can lead to an elevated serum concentration of homocysteine (hyperhomocysteinemia). In addition, B vitamins play a crucial role in the reduction of oxidative stress and in the methylation of different proteins [55]. Serum and plasma levels of folic acid are not affected in children with ASD when compared with control subjects and homocysteine show no association with ASD [51].

Vitamin C Higher or not abnormal plasma levels of vitamin C have been reported in individuals with ASD when compared with controls [49, 50]. There is evidence that vitamin C brings about significant improvement in people with autism [56]. Vitamin C softens stools and can help in constipation by making the stools easier to pass.

Magnesium (Mg) Plasma magnesium levels are shown to be lower in autistic than control children [57]. Magnesium is usually combined with vitamin B_6 supplement in ASD [52]. The efficacy of this treatment in ASD remains to be verified. Magnesium has been helpful for many autistic children who suffer from constipation. Magnesium is a smooth muscle relaxant, and it helps to pass stools by promoting rhythmic contractions of the intestinal smooth muscle. High magnesium supplementation can cause diarrhea as a side effect and the dose of magnesium should be increased gradually until the desired effects are achieved.

Phenol sulfotransferase (PST) Phenol sulfotransferase is an enzyme involved in liver detoxification. Researchers have proposed that PST is compromised in autistic children. A study [58] demonstrated that the PST enzyme system was functioning at sub-optimal levels in more than half of the autistic children tested. Since the deficiency of sulfur in the bloodstream and impairment of the PST system interferes with the body's ability to process and eliminate phenols, this may explain why many children with autism are so sensitive to phenols ingested via certain foods. Low levels of plasma sulphate are reported in autistic children when compared with age-matched control children [59].

Coenzyme Q_{10} Classical mitochondrial diseases associate with a subset of autism cases. Both nuclear and mitochondrial genes can underlie mitochondrial dysfunction that is associated with autism [60]. Coenzyme Q10 administration in rats increases mitochondrial concentra-

tions, extended survival times, and exhibited neuroprotective effects [61]. In human, coenzyme Q10 has also been shown to be beneficial in patients with mitochondrial disease [62].

Uric acid Urate is the final oxidation product of purine metabolism. A subset of autistics display higher uric acid excretion to urine than controls [63].

Amino acids There is evidence that children with ASD are likely to have abnormalities in amino acid metabolism. Gastrointestinal problems and selective eating may contribute to these changes. In several studies, an increased plasma level of glutamate that is the major excitatory neurotransmitters in the central nervous system is a consistent finding [64]. The level of glutamine and proteins that are involved in transforming glutamate to GABA, an inhibitory neurotransmitter in brain, are shown to be decreased in children with autism [65]. In addition, the levels of essential amino acids are reduced in urine of ASD individuals. Raised glutamic acid levels and reduced plasma glutamine are also found in individuals with Asperger syndrome and their siblings and parents [66].

L-Arginine Arginine is a semi-essential amino acid because the human body does not always manufacture an adequate supply. Arginine deficiency may result from digestive [67]problems or selective eating in ASD. It has been found that autistic subjects have more essential amino acid deficiencies than controls [67] and high plasma levels of arginine were reported in a recent mass spectroscopy study of high-functioning ASD males [68].

Lithium Lithium appears to be an essential mineral in small quantities for good mental health. Low levels of lithium have been reported in the hair of children with autism and their mothers when compared with not autistic children and their mothers in Arizona.

Prostaglandins (PGs) Accumulation of the very long chain fatty acids (VLCFAs) in the membrane of red cells of autistic individuals [69] indicates involvement of peroxisomal beta oxidation in the pathophysiology of ASD. Peroxisomes are cellular organelles which are important in the biotransformation of endogenous compounds in lipid metabolism, including fatty acids, steroids, and prostaglandins They are pivotal for the formation of myelin, neurotransmission, detoxification of exogenous compounds and xenobiotic [70]. Defects in peroxisomal beta oxidation [71] may link disturbances in endocrine, gastrointestinal, and immune systems as well as cytochrome P450 enzyme nitric oxide synthase (NOS) and nitric oxide formation (NO) in ASD [69]. Plasma prostaglandin E2 (PGE2) and leukotriene levels have been shown to higher in ASD than control individuals [49].

Cytokines Inflammation and immune system dysfunction are implicated in neuropsychiatric disorders such as ASD, attention deficit hyperactivity disorder, and schizophrenia. In fact, there is a significant overlap in the pathogenic factors, structural and functional abnormalities of brain, and clinical manifestations. Altered immune responses in ASD children are seen as decreased responsiveness of peripheral blood mononuclear cells to mitogen stimulation, reduced number of T lymphocytes, elevated levels of interleukin 1 receptor antagonist and elevated production of tumor necrosis factor (TNF)-α and interleukin-1β (IL-1β) by blood mononuclear cells. An emerging area of research in autism is the role of prenatal exposure to inflammatory mediators during critical developmental periods. Epidemiological data have revealed significant correlations between prenatal exposure to pathogens, including influenza,

and the occurrence of autism [72]. It was suggested that the cytokine interleukin-10 (IL-10) could play a key role in the mechanisms that lead to alterations in the adaptive immune response in individuals with autism [73]. Croonenberghs et al. [74] found elevated levels of interleukin-12 and gamma interferon in autistic patients. They reported that proinflammatory cytokines may induce some of the behavioral symptoms of autism, including social withdrawal, resistance to novelty and sleep disturbances.

3.1. Defects of antioxidant system in fragile X syndrome, a variant of ASD

Fragile X syndrome (FXS) is a common cause of inherited intellectual disability and the most common monogenic cause of autism [75]. Neurobehavioral symptoms of FXS include restricted repetitive and stereotyped patterns of behavior, hyperactivity, defects in sensory integration, and communication difficulties. About 30% of FXS males fulfill the standardized criteria of autism [76-78]. Improved understanding of the FXS etiology has facilitated clinical trials to identify targeted treatments with potential to reverse or improve behavioral and cognitive deficits in FXS. Studies of the animal models of FXS suggest that an imbalance in gamma-aminobutyric acid (GABA)/glutamate transmission is involved in the pathogenesis of behavioural defects.

Defects of antioxidant system is seen as altered levels of components of the glutathione system and higher levels of reactive oxygen species, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activation, lipid peroxidation and protein oxidation are found in the murine model of FXS [79]. These findings have led to research to treat FXS individuals with antioxidants. High doses of alpha-tocopherol have been shown to reduce hyperactivity, anxiety, and corticosterone levels in *Fmr1* knockout mice, the mouse model for FXS [80]. Similar beneficial effects have been shown experimentally with melatonin which is a sleep hormone in addition to its effects as an anti-oxidant [81]. GABA-B was identified as a drug target in treatment of FXS and autism. Recent studies show that arbaclofen and STX209, an oral selective GABA type B (GABA-B) receptor agonist, have the potential to normalize the deficient inhibitory neurotransmission in FXS and autism [82-84]. In addition, clinical trials with metabotrobic glutamate receptor antagonists and donepezil in FXS are on-going [82]. Furthermore, beneficial effects of minocycline and lithium on the functional defects of FXS individuals remain to be properly evaluated.

3.2. Antioxidant properties of zinc

Several mechanisms can be involved in antioxidant function of zinc. First, zinc may protect protein sulfhydryl groups from oxidative modification by influencing the conformation and reducing potential of thiol groups. Since the sulfhydryl groups are required for the catalytic activities of several enzymes, zinc protects the enzyme's activity from oxidative inactivation. Second, zinc may antagonize the activity of transition metals such as iron and copper. Zago and Oteiza [85] showed that zinc may compete with copper and iron ions and prevent transition metal mediated oxidative modifications. Third mechanism for the antioxidant property of zinc is that zinc may reduce oxidative damage indirectly by modulating antioxidant defence including (a) enzymes which catalytically remove free radicals and reactive

species, like superoxide dismutase, catalase, and glutathione peroxidase; (b) proteins which minimize the availability of pro-oxidants, like transferrins, ceruloplasmin and metallothioneins; (c) low-molecular-mass ROS and RNS scavengers, like glutathione, ascorbic acid, uric acid, and alpha-tocopherol.

3.2.1. Zinc deficiency

Inadequate zinc intake has been implicated in many diseases; however, no laboratory test can clearly distinguish zinc deficiencies [86, 87]. Most at risk of zinc deficiency are young children, teenage girls, and people over age 71. Although zinc deficiency is largely uncommon in the developed world, it has been estimated to affect about 2 billion people worldwide. Elderly people, those with lower incomes, and those with less education may be apt to consume inadequate amounts of zinc. Zinc deficiency can be caused by insufficient dietary intake of the mineral and also by some underlying conditions including malabsorption syndrome, liver and renal disease, diabetes, sickle cell disease and other chronic illnesses [88, 89]. Appropriate absorption of nutrients depends on an intact intestine and intestinal injuries can lead to zinc deficiency. This may cause appetite loss and diarrhea which speeds the downward spiral of zinc loss and tissue damage [90]. Supplementation with 12.5-50 μM zinc has been shown to enhance epithelial cell restitution, the initial step of wound healing. Zinc has been found to help healing of intestinal lesions that associate with inflammatory bowel disease that is a serious intestinal disorder.

Improved neurophysiologic performance, positive growth response, and significantly reduced mortality and morbidity with zinc supplementation have been observed in Chinese children [91]. Zinc deficient animals display an increased susceptibility to exogenous oxidative stress such as endotoxin exposure and hyperoxia [92] and zinc is thought to protect macromolecules such as proteins, lipids and DNA from oxidative damage. Mice defective in CuZnSOD develop neurological damage and cancer at an accelerated rate as they age [16]. Zinc depletion affects the expression of metallothioneins that are low-molecular-weight proteins with high cysteine content and high affinity for metal ions [93]. In addition, zinc depletion decreases α-tocopherol and ascorbate levels in liver and lung [94], but may not change α-tocopherol level in testes [95]. Moreover, plasma uric acid concentration has been shown to be elevated occasionally in zinc-deficient rats [96].

3.2.2. The role of zinc in reversing synaptic deficits in autism

There is evidence that zinc deficiency plays a role in autism [24] and the zinc-dependent mechanisms underlying the neurobiology of autism are under active investigation. Zinc plays important roles in nucleic acid/protein synthesis, cell replication, tissue growth and repair. Zinc finger proteins (ZNF81 and ZNF182) are the largest class of DNA binding proteins encoded in the human genome [97] and mutations involving genes encoding these proteins have been shown to associate with mental retardation [98]. Zinc is a regulator core component of the postsynaptic density (PSD), an active zone at the synapse. A low postsynaptic availability of zinc has been shown to affect the activity dependent increase of proteins of the ProSAP/Shank family which are linked to ASD [99]. Mice with acute zinc deficiency were shown to

display behavioural abnormalities such as over-responsivity and hyperactivity-like behaviour whereas prenatal zinc deficiency caused ASD-related behaviour such as deficits in vocalization and social behaviour. Furthermore, low zinc levels were shown to increase the incidence of seizures, hypotonia, attention deficits, and hyperactivity in patients with Phelan-McDermid syndrome, an ASD caused by haploinsufficiency of a member of ProSAP/Shank family.

In the brain, zinc is in its ionic form (Zn^{2+}) within synaptic vesicles of glutamatergic nerve terminals or bound to metalloproteins and intracellulary mobilized by oxidative stress. $Zn^{2+}$is thought to be released from the nerve terminals in an activity-dependent manner synaptically and it may be a key modulator of neuronal activity and survival. Extracellular $Zn^{2+}$is involved in the regulation of the balance of excitation and inhibition, and exogenously applied Zn^{2+}have effects on the activity of glutamate, $GABA_A$, and glycine ionotropic receptors.

Zinc homeostasis in the brain is maintained by the blood-brain and blood-cerebrospinal fluid barriers. Researchers are beginning to understand zinc's role in maintaining the structural integrity of the endothelia, which line the blood vessels, and the epithelia, which line the gastrointestinal tract. In atherosclerosis, arterial endothelial cells are destroyed by oxidating fatty acids and inflammatory immune factors. Zinc deficiency magnifies the defect. Supplementation with zinc has recently been found to protect the integrity of the blood vessel cell lining which helps maintain immune function in the elderly [100]. Within two months of zinc supplementation, resistance improves, and the chances of surviving an infection increase [101]. The critical role of zinc to immune function is consistent with its beneficial effects against infection. Supplement of zinc, as well as vitamin C, may enhance the activity of natural killer cells. Deficiencies of zinc, as well as vitamins A and D, conversely reduce natural killer cell function [102].

3.3. Copper-Zinc and Cadmium-Zinc Imbalance

Many metallic elements play an important role in the maintenance of human health and an imbalance in trace elements may be a significant factor in a wide variety of physical and psychiatric conditions. Copper and zinc are regarded as neurotransmitters and they are both found in high concentrations in the hippocampus of brain. Elevated copper and depressed zinc levels have been associated with hyperactivity, attention deficit disorders, depression, and ASD [103]. Also, many individuals with ASD or paranoid schizophrenia have elevated blood copper levels combined with other biochemical imbalances [19,20].

The actions of selenium, zinc, and copper are all intermingled in the regulation of detoxifying and antioxidant enzymes. Zinc may induce a decrease in intracellular cadmium accumulation and the sequestration of cadmium by cadmium-induced metallothionine [104]. However, the actual activity of cadmium/zinc-metallothionine-whether protective or damaging-is believed to depend on various parameters governed by the extracellular and intracellular environment [105].

3.4. Reduced glutathione

Glutathione is present in two forms in the body; in a "reduced" or an "oxidized" form. The reduced form or "L" type glutathione is the most active form and is found in healthy cells. Reduced glutathione is responsible for all vital biological activity /function of glutathione in the body. In normal healthy cells the oxidized glutathione is quickly recycled back to its active reduced state and the majority of glutathione in the body is present in its reduced form. Reduced glutathione is a tripeptide composed of the amino acids glutamine, cysteine, and glycine (gamma-glutamylcysteinylglycine, GSH).

GSH is synthesized sequentially by glutamate-cysteine ligase (GCL) and GSH synthase (GS) [106]. The cystine/glutamate antiporter controls the biosynthesis of GSH by transporting cystine, the rate-limiting precursor of GSH synthesis, into the cell in exchange for glutamate [107]. Methylmercury (MeHg) administration induces oxidative stress in cortex which could be antagonized by riluzole induced GSH synthesis through activation of glutamate transporters (GluTs) [108].

GSH is the major cellular antioxidant and plays an important role in the protection of cells against damage from free radicals and other electrophils and also influences cellular radiosensitivity, cellular response to hyperthermia, and cytotoxicity to some kinds of chemotherapeutic agents. It protects the body against the damage caused by exposure to toxins and is a powerful detoxifier of heavy metals. Early studies showed the role of GSH in inflammation [18]. GSH delivery to the central nervous system (CNS) is limited due to its poor stability and low bioavailability.

3.4.1. Application/role of reduced glutathione (GSH) in autism

The impact of glutathione in autism has been described [109]. Children with autism have been shown to have low plasma levels of metabolites in the pathway of glutathione redox metabolism [110] suggesting that children with autism have a more oxidized extracellular GSSG. If dietary GSH is insufficient, oxidative stress, toxicity and cell damage may occur to mucosal cells in the small intestine. The elimination of fat-soluble compounds, especially heavy metals like mercury and lead are dependent upon adequate levels of glutathione.

In autism the methylation cycle was found to be blocked at methionine synthase, which is the step of homocysteine methylation for formation of methionine [11, 111]. A significant decrease in the level of plasma methionine and lowering of the ratio of S-adenosylmethionine to S-adenosylhomocysteine are two effects of this blocking. The latter change results in a decreased capacity to promote methylation reactions. In addition, the flow through the transsulfuration pathway was also reduced leading to lower plasma levels of cysteine and glutathione and a lowered ratio of reduced to oxidized glutathione. The lowered ratio of reduced to oxidized glutathione reflects a state of oxidative stress [112]. The block in the methylation cycle and alterations of glutathione were found to be linked, since supplements used to restore the normal function of the methylation cycle (methylcobalamin, folinic acid and trimethylglycine) also restored the levels of reduced and oxidized glutathione [111].

3.4.2. The role of GSH-enzymes in the metabolism of arachidonic acid

The tissue content of GSH is normally very high, in some tissues concentration up to 5 mM is found. The function of GSH is often tissue protective, and GSH plays a central role as a cofactor in numerous enzyme reactions. GSH-peroxidase (GSH-Px) is one of the GSH-enzymes located in the circulation almost exclusively in the red cells, various GSH-transferases that have peroxidise-like activity and bind chemicals, and γ-glutamyl transferase that reflects the liver function and is involved in the transport of amino acids across the cell membrane. GSH is also consumed by some cytochromes, most notably cytochrome P-450. Several steps in the metabolism of arachidonic acid may be normally regulated by GSH-enzymes [113]. An early observation was that GSH may function as a chemical cofactor or coenzyme in the formation of some PGs, particularly PGEs [114].

The findings of several studies indicate that alterations of cellular methylation capacity, antioxidant defence, and oxidative stress contribute to the pathophysiology of autism. An imbalance in intracellular levels of GSH and GSSG could provide a biochemical explanation for multisystem issues, such as increased frequency of infections, gastrointestinal pathology, impaired detoxification and neurologic pathology, associated with both autism and glutathione depletion.

The abnormal metabolite levels in pathways of methionine, folate, and glutathione metabolism observed in autism may reflect subtle changes in gene products that regulate activity in these pathways [37, 110]. Even small variations in the gene expression and enzyme activity, when expressed chronically, could have a significant impact on downstream metabolism. Many autistic children have been shown to exhibit a threefold reduction in the ratio of "active" GSH to "inactive" glutathione (GSSSSG). Cysteine, another substance needed for GSH synthesis, was also significantly reduced, suggesting that the building blocks for GSH synthesis are insufficient in ASD [11].

3.5. Gluten vs. Glutamate (glutamic acid) vs. Glutamine

Glutamic acid is the major excitatory neurotransmitter that increases the firing of neurons in the central nervous system. It is converted into glutamine and in GABAergic neurons to GABA, an inhibitory neurotransmitter. Glutamic acid and its neurologically inactive sibling, glutamine, are amino acids. Glutamic acid is found in most foods but it is particularly abundant in gluten grains (wheat, barley, rye), soy/legumes/peanuts, dairy products, nuts, seeds, meats and the gluten-grain substitutes (quinoa, amaranth, tapioca as well as the non-gluten grains millet, flax and sorghum). Cells lining the intestinal tract convert glutamate to glutamine, which in turn is used by the villi to maintain the health and integrity. The conversion of glutamate to glutamine happens also in liver and kidneys, which is fortuitous because many food substances that are rich in glutamic acid – namely gluten grains, casein (from dairy), and soy – are three of the four food substances that damage the villi and their ability to make the conversion. Glutamine is converted to glutamate by a mitochondrial enzyme, the phosphate-activated glutaminase.

3.6. N-Acetyl-L-Cysteine and glutathione

N-Acetyl-L-Cysteine (NAC) is an antioxidant that helps increase glutathione synthesis which, in turn, helps the body defends against harmful toxins. NAC is the acetyl derivative of L-cysteine. While L-cysteine plays important metabolic roles as a key antioxidant, a gluta-thione precursor and a natural source of sulfur for metabolism, it is unstable and can become degraded during absorption. NAC on the other hand, is more stable than L-cysteine. Taken orally, NAC converts into L-cysteine after being absorbed, and raises blood and tissue cysteine levels.

4. Summary and conclusion

It is generally accepted that complex diseases such as autism is influenced by genetic alterations at multiple and variable sites that interact to reach a threshold of toxicity that triggers the disease expression. When expressed chronically even small variations in the gene expression and enzyme activity caused by genetic changes and environmental factors could have a significant impact on downstream metabolism leading to development of autism. A metabolic imbalance can promote chronic oxidative stress and impaired methylation capacity which results in alterations of normal developmental maturation of neurologic and immunologic systems associated with autism. The production of free radicals is critical in the regulation of many biological functions, cellular damage, and the pathogenesis of disorders affecting central nervous system. Oxidative stress is shown to play a role in many neuropsychiatric disorders, including ASD. Understanding of functional connections of autism-associated genes and the impact of environmental risk factors on cellular responses linked to ASD phenotype will allow to distinguish disease-related pathological as well as compensatory processes and to identify targets for treatment of different features associated with ASD. The human genetic heterogeneity increases the complexity of the effects of environmental factors. New biomarkers are desired to support clinical trials which are the final way to find out the efficiency of new types of interventions.

Abbreviations

ASD, autism spectrum disorders; FXS, Fragile X syndrome, GABA, gamma-aminobutyric acid; GSH, gamma-glutamylcysteinylglycine; IL-6, interleukin 6; NAC, N-Acetyl-L-Cysteine; PST, phenol sulfotransferase; ROS, reactive oxygen species; SOD, superoxide dismutases; VLCFA, very long chain fatty acids

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