Lead Article

Nutritional interventions for autism spectrum disorder

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Autism spectrum disorder (ASD) is an increasingly prevalent neurodevelopmental disorder with considerable clinical heterogeneity. With no cure for the disorder, treatments commonly center around speech and behavioral therapies to improve the characteristic social, behavioral, and communicative symptoms of ASD. Gastrointestinal disturbances are commonly encountered comorbidities that are thought to be not only another symptom of ASD but to also play an active role in modulating the expression of social and behavioral symptoms. Therefore, nutritional interventions are used by a majority of those with ASD both with and without clinical supervision to alleviate gastrointestinal and behavioral symptoms. Despite a considerable interest in dietary interventions, no consensus exists regarding optimal nutritional therapy. Thus, patients and physicians are left to choose from a myriad of dietary protocols. This review, summarizes the state of the current clinical and experimental literature on nutritional interventions for ASD, including gluten-free and casein-free, ketogenic, and specific carbohydrate diets, as well as probiotics, polyunsaturated fatty acids, and dietary supplements (vitamins A, C, B6, and B12; magnesium and folate).

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairment in social skills and repetitive behaviors, as well as speech and nonverbal communication. The prevalence of ASD has steadily increased over the past decades.[1,2] Although the mechanisms underlying the etiology and manifestations of ASD are not well understood, genetic[3] and environmental[4,5] factors appear to contribute to disease expression. Recent evidence suggests that up to 40%–50% of ASD symptom variability may be determined by environmental factors.[4]

Although there is no consensus on ASD causation, multiple studies, carried out in populations across the world, have documented deficits in antioxidant and methylation metabolites.[6–8] Thus, levels of glutathione (GSH), the primary intracellular antioxidant and detoxifying agent, and *S*-adenosylmethionine (SAM), a methyl donor for hundreds of metabolic reactions, are significantly lower in ASD. Impaired methylation is particularly relevant for ASD because methylation of DNA and histones provides crucial epigenetic regulation of gene expression during neurodevelopment. Notably, oxidative stress and methylation metabolite measurements can distinguish persons with ASD from neurotypical subjects with up to 97% accuracy.[9–11] Because metabolic pathways providing GSH and SAM are supported by specific nutritional factors (eg, sulfur

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amino acids cysteine [CYS] and methionine [MET], folate, vitamins B12 and B6), they are critical for ASD diets.

The gut-brain axis theory proposes that nutritional programing in early life, reaching as far back as the gestational period, may influence cognitive function and predispose to ASD in genetically susceptible individuals.[6,7] In several animal studies, researchers have attempted to elucidate the role of nutrition in the development of ASD. For example, maternal obesity has been proposed to lead to fetal brain inflammation.[8] The nutritional composition and balance of the diet also appear to play a role, with polyunsaturated fatty acid (PUFA) deficiency during pregnancy being implicated with reduced learning and memory and reduced cognitive functioning if not corrected in early development.[9] In addition, prominent activation of microglia and astrocytes as well as severe chronic inflammation in multiple brain areas have been noted in individuals with ASD.[10] Interestingly, microbiota-devoid mice have impaired microglia maturation and function, as well as improper myelin formation.[12] These abnormalities were attributed to changes in cytokine and neuropeptide production and gene transcription, as well as a lack of microbiota-derived metabolites that include short-chain fatty acids and lipopolysaccharides. This gut-brain signaling appears to be bidirectional, with central nervous system disturbances leading to changes in intestinal permeability, motility, secretion, and microbiota composition[11] (Figure 1). Perturbations in gut microbiome composition in ASD have been documented in numerous studies.[6,12–15] Accordingly, the gut-brain axis theory has been expanded to the microbiota-gut-brain axis to account for the influence of microbial organisms on cognitive development and functioning.

Gastrointestinal (GI) disorders are a common comorbidity in patients with ASD, and although intestinal dysbiosis is increasingly documented in ASD, the causality remains unelucidated. However, in preclinical models, gut symptom severity appears to be strongly correlated with ASD symptom severity, anxiety, and sensory conditions that are modulated by the intestinal microbiome.[15] Abdominal pain, diarrhea or constipation, and gastric reflux are among the comorbid GI symptoms commonly observed in individuals with ASD. Patterns of selective eating and nutrient deficiencies as well as alterations in the integrity of the intestinal epithelial membrane and subsequent increased intestinal permeability are also reported in ASD.[16,17]

Because of an increasing amount of evidence implicating the intestinal microbiome not only in normal development and functioning of the nervous system but also as a possible causative or facilitative agent in neuropsychiatric diseases such as ASD, considerable interest exists in nutritional interventions. Of note, greater than 80% of parents of children with ASD reported using some form of dietary intervention,[18] although controversy exists in the literature regarding the effectiveness, implementation criteria, and possible adverse effects of elimination or restrictive diets. In this review, we summarize the current clinical and experimental literature on nutritional interventions in ASD, including gluten-free and casein-free diets, ketogenic diets, specific carbohydrate diets, probiotics, PUFAs, and dietary supplements.

GLUTEN-FREE AND CASEIN-FREE DIETS

Gluten-free diets involve the dietary exclusion of gluten, a mixture of proteins found in wheat and related grains such as barley and rye as well as in many processed and prepackaged food products. Gluten-free diets are the first-line treatment for celiac disease, but in recent years, they have also gained popularity in cases of non-celiac gluten sensitivity and wheat allergy. Casein-free diets focus on the dietary exclusion of casein, a protein found in dairy products, and are implemented traditionally in patients with galactosemia or cow-milk allergy. These diets are often combined to create a gluten-free and casein-free (GFCF) dietary protocol and have been used for several years as a nontraditional treatment approach for patients with ASD.

The rationale for the use of GFCF diets in ASD largely stems from the effects of opioid peptides, released by digestion of both gluten and casein. As first demonstrated by Lazaro et al.,[19] gluten- and casein-derived opioid peptides, as well as the prototypical opioid morphine, decrease uptake of CYS by cells and, because CYS is rate limiting for GSH synthesis, their activity results in decreased GSH levels. Furthermore, opioid peptides decreased the methylation index (ie, the ratio of the methyl donor SAM to its inhibitory product *S*-adenosylhomocysteine), leading to altered patterns of DNA methylation and gene expression. Within the GI tract, inhibition of CYS uptake by epithelial cells can adversely affect the systemic availability of CYS, restricting GSH production, with potential epigenetic consequences. Moreover, lower GSH levels in the GI tract can promote inflammation and contribute to symptoms of GI discomfort and dysfunction. Thus, a GFCF diet may provide clinical benefit by improving CYS absorption and GSH levels, particularly in individuals with low GSH levels, as is the case in ASD.

Among dairy cows, there are 2 naturally occurring genetic variants of the gene for β-casein that affect the extent of opioid peptide release during digestion. A single nucleotide polymorphism (SNP) in A1 β-casein

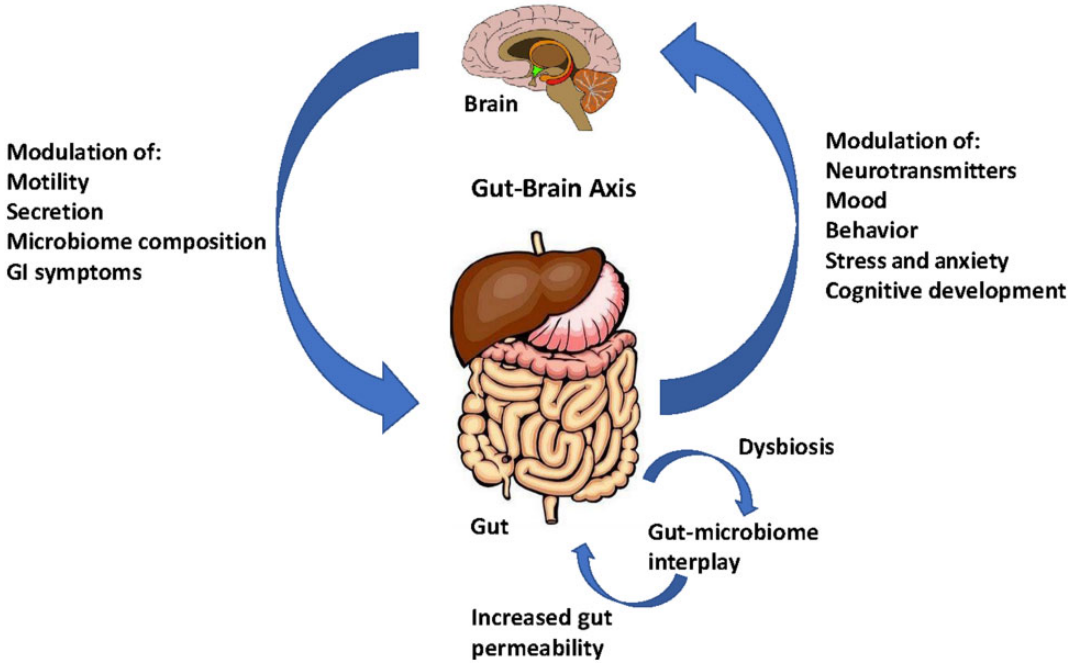

Figure 1 **A schematic representation of interaction between gut-brain axis.** There is a bidirectional communication between gut-brain axis that can modulate GI and central nervous system functions. Gut microbiota that can be influenced by nutritional intervention play an important role in governing this bidirectional signaling. *Abbreviation:* GI, gastrointestinal

facilitates release of β-casomorphin-7 (BCM7), whereas BCM7 release from A2 β-casein is limited.[20] Indeed, in a double-blind, crossover study in which consumption of milk containing a mixture of A1 and A2 β-casein was compared with only A2 β-casein, researchers reported that although both types of milk increased plasma GSH levels, consistent with their CYS-rich whey protein content, the GSH level was almost twice high with consumption of A2-only milk.[21] Thus, opioid peptide release from casein appears to exert a significant inhibitory effect on CYS absorption, limiting systemic GSH levels. Assuming that opioid peptides released from gluten have a similar effect, a GFCF diet may be beneficial in ASD, because of augmented antioxidant status. The action of BCM7 to restrict CYS absorption applies not only to absorption of casein-derived CYS but also to CYS derived from other protein sources ingested along with milk.[20]

The activity of casein- and gluten-derived opioid peptides is terminated upon their hydrolysis by dipeptidyl peptidase IV, which is present on the surface of intestinal epithelial cells and in various strains of gut bacteria.[22] Bacterial species commonly found in the intestine of infants (eg, strains of *Bifidobacterium*) exhibit particularly high dipeptidyl peptidase IV activity,[22] suggesting the bacteria may limit BCM7 activity during early development. Notably, human β-casein is of the A2 type, implying that BCM7 release from breastfeeding may be relatively low in comparison with formula from A1 or mixed A1/A2 cows.[23] Additional studies are needed to evaluate whether this difference contributes to the benefits of breastfeeding. Studies have reported an association between decreased breastfeeding and autism,[24,25] although no association was found in a study based on the US National Survey of Children's Health.[26]

Besides opioids and β-casomorphin activity, it has been demonstrated that persons with ASD have elevated levels of antibodies to a series of dietary proteins including gluten/gliadin and milk proteins such as α-gliadin (eg, immunoglobulin [Ig] A and IgG), deamidated gliadin peptide (deamidated gliadin peptide–IgA and IgG), total specific gliadin IgG (all fractions: α, β, γ, and ω), and casein IgE.[27] In addition, intestinal permeability was increased in 25.6% of children with ASD compared with 2.3% of healthy children. Interestingly, a GFCF diet helped decrease the levels of some of these antibodies to dietary proteins. It was suggested that gluten and casein stimulate the immune system, leading to increased production of antibodies to dietary proteins in a subset of persons with ASD. A better knowledge about the antibody titers to food antigens will help identify persons with ASD who can benefit more from a GFCF diet, compared with nonresponders.

Oxidative stress associated with low levels of GSH promotes the release of pro-inflammatory cytokines and can thereby contribute to GI inflammation.[28] The actions of casein- and gluten-derived opioid peptides,

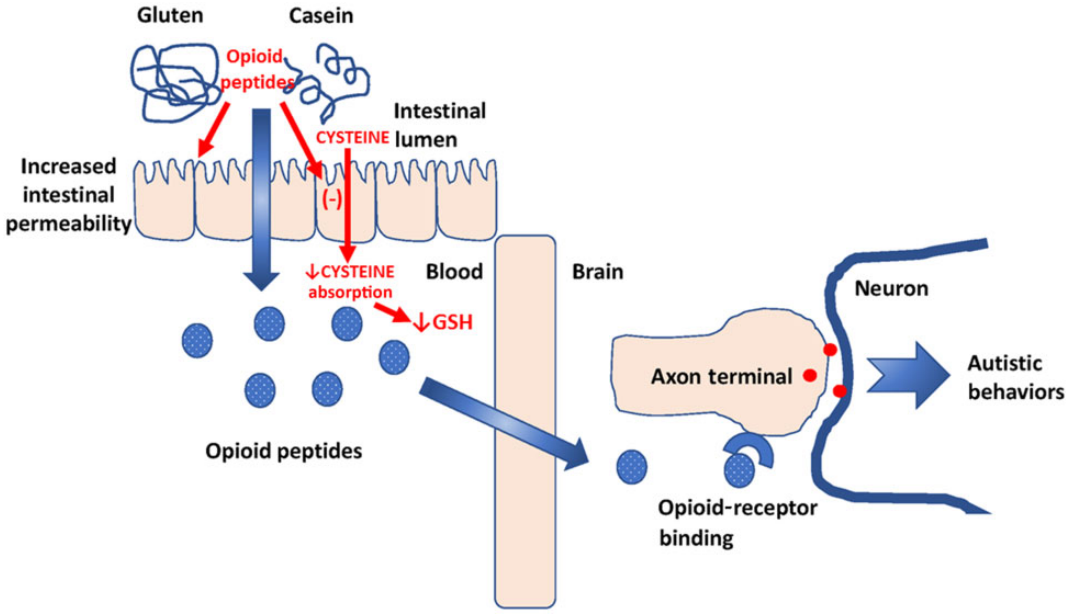

Figure 2 **Topographic representation of opioid-excess theory of autism.** According to opioid-excess theory, there is a production of abnormal peptides from incomplete breakdown of gluten and casein diets. These peptides can pass through permeable intestinal membrane, eventually cross the blood-brain barrier, and bind to opioid receptors in the brain, affecting neurotransmission. *Abbreviation:* GSH, glutathione.

therefore, potentially can promote inflammation of the GI tract, which is a common feature of autism, and a GFCF diet may ameliorate GI inflammation. Importantly, GI inflammation is associated with hyperpermeability (ie, leaky gut),[29] increasing the opportunity for opioid peptides to exert systemic effects.

Systemic opioid effects can occur if the opioids can pass into the general circulation[19] (Figure 2). Emerging evidence indicates that, indeed, opioid receptors may be involved in regulating aspects of social behavior and may contribute to the pathogenesis of ASD.[20] However, the theory has been criticized because of the inability of studies to find abnormal levels of opioids in the plasma or central nervous system of children with ASD.[19] Exogenously, these opioid-like peptides are thought to be derived from the incomplete breakdown of certain foods like gluten-containing cereals and dairy products, possibly due to a deficiency in peptidases.[6] According to the leaky-gut hypothesis, these gluten- and casein-derived peptides are then thought to enter the circulation, due to increased intestinal permeability, which is often seen in patients with ASD along with comorbid GI symptoms.[11,16,17,21,22] Interestingly, gluten sensitivity has been implicated with other neuropsychiatric conditions, including schizophrenia, ataxia, and attention-deficit/hyperactivity disorder.[23]

The literature on GFCF diets is conflicting. A recent systematic review of 4 randomized controlled trials (RCTs) on GFCF diets found a 50% efficacy rate of treatment.[23,24] Interestingly, the studies in which the researchers found a significant effect had a duration of 12 months, whereas those studies in which significance could not be established had a duration of 12 weeks.[24–28] This suggests the benefits of a GFCF dietary intervention may not be apparent until a more prolonged treatment is implemented. It is highly plausible that the gut dysbiosis, inflammation, and perturbations in intestinal motility and permeability encountered in many patients with ASD take a substantial time to normalize on a treatment diet after years of dysfunction. In a small, open-label, 12-week RCT, researchers found no significant differences between treatment and control groups[29] However, this study had a small sample size and short duration, which may have contributed to the lack of significant effect. In contrast, in a recent RCT with a larger sample size of 80 patients, researchers found that a gluten-free diet alone was effective at improving GI and behavioral symptoms after 6 weeks.[30] No adverse events were reported and no nutritional deficiencies were identified in any of the RCTs in which these metrics were investigated—addressing a common concern regarding special diets.[25,27,29] In another recent RCT, a gluten-free, casein-free, and soy-free diet for 12 months, with additional supplements, was effective at improving nutritional status, nonverbal intelligence quotient, autism symptoms, and other symptoms in most individuals with ASD.[31] With the broad nature of the intervention, however, it is difficult to establish the main factor contributing to the observed improvements.

A different approach was taken in an RCT in which the effect of gluten and casein supplementation on

maladaptive behavior and GI symptoms was investigated.[32] The researchers found no effect after 7 days of casein and gluten supplementation on behavioral or GI symptoms.[32] However, the study participants did not appear to be eating a GFCF diet before the intervention and thus were likely already exposed to gluten and casein, which may explain the lack of difference between supplemented and placebo groups. Similarly, a RCT was conducted to explore the effect of gluten and casein supplementation on the behavior and intestinal permeability of patients with ASD.[33] No significant differences were observed between the placebo and experimental groups, but the authors noted the study was underpowered to detect small changes in the measured outcomes.[33]

Although inconclusive results were reported in systematic reviews focusing on interventional studies, authors of a review article sought to provide a more comprehensive discussion of the evidence regarding GFCF diets, including review articles, group experimental intervention studies, randomized clinical trials, case reports, and group observational studies published between 2005 and 2015.[34] Of the 10 review articles examined, nearly all cited the current evidence as inconclusive to recommend GFCF diet as a standard therapy. In the 7 group experimental studies examined, results were again heterogeneous, with multiple studies reporting improvements in parent-rated or subjective behavioral or communication measures, but only 1 study showing statistically significant improvement using blinded assessment.[34] Of note, this was the only study that lasted longer than 3 months, with an intervention-diet duration of 24 months. This same time dependency for detection of treatment effects was also seen in the RCTs discussed previously. In a recent case-control study in which the effect of GFCF or ketogenic diets on ASD was examined, researchers found significant improvement after 6 months of following either interventional diet.[35] Of the 5 case studies examined, 4 reported significant improvement of cognitive, behavioral, and language symptoms after the intervention. Moreover, in 3 studies, improvements were noted in communication skills and cognitive scores to such an extent that the children no longer fit diagnostic criteria for ASD.[34] Interestingly, in the 4 studies showing improvement in children receiving a GFCF diet, the children had preexisting GI symptoms. Finally, according to the 4 observational studies included in the review, treatment for longer than 3–6 months and the presence of preexisting GI symptoms led to larger treatment response.[34]

Overall, conclusive evidence on the efficacy of a GFCF diet for ASD is lacking despite the widespread use and popularity of the diet among patients and clinicians. Nonetheless, the current literature indicates identifying specific subsets of patients, such as those with preexisting GI symptoms, can improve treatment response. However, the treatment may have to be maintained for several months before observable improvements are seen and thus patients' families should be cautioned against expecting a "miracle cure." It is important to recognize the lack of uniform outcome measurements when comparing different dietary interventions studies, including GFCF diets, as a real challenge when interpreting results. More studies with objective measures of improvement are warranted to identify the patient populations that can stand to benefit from a GFCF dietary intervention. Care should also be taken to ensure adequate nutritional intake when excluding food items, especially when many patients with ASD already exhibit selective eating patterns and limited variety in their diets.

KETOGENIC DIET

The ketogenic diet (KD) is rich in fat, sufficient in protein, and low in carbohydrates, resulting in the body using fat metabolism as a primary fuel source. KD has also been proposed as a nutritional intervention to ameliorate behavior symptoms associated with ASD.[36,37]

A systematic review of literature examining the interplay between KD and ASD reveals encouraging findings, particularly from animal studies. The most promising of these reports come from investigations with $BTBR^{T\ +\ tf/j}$ (BTBR) mice, which closely resemble the ASD behavioral phenotype.[36,37] These studies indicate the KD may be an effective intervention for ASD, because the dietary intervention significantly improved sociability and communication in the mouse model while also reducing self-directed repetitive behaviors. In a second study with BTBR mice, improvement of deficits related to myelin formation, white matter development, and neurotransmitter signaling pathways also was noted when mice were fed a KD.[38] Importantly, this study lends credence to the more recent, unifying gut-brain axis theory of linkage between the brain and microbiome, signifying potential connections in ASD.

With this theory in mind, the impact of KD on the gut microbiome of the BTBR mouse model of ASD has been examined. One such study demonstrated that KD promoted gut microbiome remodeling in the BTBR model, opening the door to an ASD therapeutic mechanism by which KD might alter the gut microbial composition and thereby ameliorate neurological symptoms.[39] In addition, high-resolution intracortical microstimulation was used in another study to demonstrate that KD reversed abnormal basal sensorimotor

excitation/inhibition pathways in the BTBR mouse model of ASD.[40]

Interestingly, in a study of KD in BTBR mice, a KD significantly reduced levels of *O*-linked-*β*-*N*-acetyl glucosamine (O-GlcNAc) in the livers of juvenile BTBR mouse model of ASD, but no differences were observed in their brains.[41] Protein O-GlcNAc is a post-translational modification to Ser/Thr residues that integrates energy supply with demand. The abnormal patterning of O-GlcNAc is evident in many neurological disorders, including ASD.[41] It was concluded that a KD exerts its beneficial effect independently of O-GlcNAc–related pathways.

Other murine models have yielded mixed results. For example, the effects of KD in the MeCP2 mouse model of ASD, which exhibits similar symptoms to patients with Rett Syndrome (a syndrome with a similar phenotype to ASD), revealed improvement in motor behavior and anxiety. The same results were found, however, with MeCP2 mice subjected to caloric restriction, leading to the conclusion that the beneficial effects may have been caused by the caloric restriction rather than the KD.[42] Meanwhile, in another study, a KD increased social exploration in wild-type Long-Evans male mice, though no conclusive statement was made about the effect of a KD specifically on ASD.[43] Finally, in a study in which the effect on ASD of maternal immune activation due to viral or bacterial infection during the first trimester in pregnant C57BI/6 mice was examined, the KD partially or completely reversed all abnormal behavior induced by maternal immune activation.[44]

The limited human studies and case reports for KDs have shown similar mixed results. One case report of a 4-year-old girl demonstrated an increase of approximately 70 points in the intelligence quotient, which correlated with improved cognitive and language functions. In addition, improvements in social skills, increased calmness, and a resolution of stereotypies (CARS) after treatment with a GFCF diet and customized KD (ratio of CARS before KD to CARS after KD was 49:17) was observed.[45] Another case report of a 9-year-old girl with classic autism and adenylosuccinate lyase deficiency type II demonstrated a 95% reduction in seizure frequency after the implementation of a KD.[46]

In larger cohort studies, KDs (as administered and reported by parents) were believed to substantially improve seizures and result in noticeable improvements in learning, social behavior, speech, cooperation, stereotypy, and hyperactivity.[47,48] More recently, a modified ketogenic, gluten-free diet regimen with supplemental medium-chain–triglycerides oil, given because of primary and secondary mitochondrial dysfunction in the ASD population, was given to 15 children with ASD and demonstrated significant improvements over 3 months in social affect and overall Autism Diagnostic Observation Schedule–2 scores. In addition, parents of the treated children self-reported improvements in traditional ASD behaviors such as eye contact, language, focus, and hyperactivity.[49] This might be representative of a smaller subtype of ASD, but having multiple dietary interventions in parallel might sometimes be more beneficial as compared with individual ones by themselves.

It should be noted that despite encouraging results, human ASD studies involving KDs have several important limitations. These limitations include smaller sample sizes; difficulty adhering to the KD; discrepancy between the length and composition of KD administration; behavioral rigidity in patients with ASD, leading to KD unpalatability and, therefore, high dropout rates; and finally, nutritional deficits. In addition, the KD is associated with its own risks, including higher risk of inflammation and mitochondrial dysfunction, and the adverse effects of constipation, reflux, and other comorbidities.[50,51] Additional studies are warranted to understand the effects of KD in individuals with ASD.

PROBIOTICS SUPPLEMENTATION

The administration of probiotics to individuals with ASD is an emerging field of interest and study. With regard to ASD, there are currently very few studies on the effects of probiotics in individuals with ASD. Compounding this is the fact that there are many different probiotics and very little information about adverse effects and therapeutic actions or appropriate dosing and strains suitable for persons with ASD. However, probiotics interact with gut microbiota and may down-regulate GI inflammation and intestinal permeability.[52]

The results of a study in which probiotics were given to children aged 2–9 years with ASD suggested reduction in intestinal inflammation and restoration of abnormal GI microbiota.[53] These findings were corroborated by several other studies in which improvement in GI and ASD symptoms was reported.[54,55] Probiotic supplementation was identified in another study as potentially reducing the risk of neuropsychiatric disorder development later in childhood (possibly) by mechanisms not limited to gut microbiota composition.[56]

Several theories underlie the potential mechanisms behind probiotics and their interplay with ASD. The first is the opioid-excess theory inherent in the effectiveness of the GFCF diet, suggesting that probiotics may help process gluten products without causing as much damage to intestinal permeability, while also preventing peptide leakage and strengthening the blood-brain barrier.[57–60] The second theory, involving dietary protein

intolerances, also supports this conclusion. This theory suggests the anti-inflammatory properties of probiotics could counter the inflammatory immune responses in individuals with ASD, even improving behavioral problems observed in ASD.[61,62] The final theory relies on probiotics being used to correct gut dysbiosis and decreasing the production of endotoxins, which, therefore, would reduce gut inflammation and permeability, thus preventing the ability of these endotoxins to affect the central nervous system.[52] Several studies have supported this theory while also demonstrating a correlated finding of improved behavioral outcomes.[52,63–65] On par with these findings, a decrease in the number of *Lactobacillus* spp. and *Clostridium* spp., followed by increase in an aggressive form of *Candida* spp. (pseudohyphae presenting), has been reported in persons with ASD compared with neurotypical control subjects, suggesting gut dysbiosis.[66] In addition, a significant correlation between intestinal dysbiosis and ASD disease severity (CARs score) was observed.[66] Increased gut permeability was also observed that was attributed to the decrease in number of *Lactobacillus* spp., because these probiotic strains contribute to the maintenance of tight junctions in intestinal epithelia.

On the basis of the beneficial effects of probiotics, questions of maternal probiotic supplementation to prevent ASD (and other diseases) have been raised. Because there is evidence linking gut health to human diseases, it is reasonable to speculate that the maternal administration of probiotics may prove to be a prominent avenue for exploration in the prevention of human diseases, including ASD.[67] Additional studies are warranted to determine whether probiotic supplementation can be useful for pregnant mothers as preventive tool for ASD.

Despite these theories and findings, reaction has been cautiously optimistic. Although the need for additional research has been identified in several reviews of related topics, 1 group recently stated there is currently no clear evidence of positive effects of oral probiotics in ASD.[68–70] Others, by contrast, have advocated for probiotic supplementation as an adjuvant therapy.[54] Additional studies are warranted to decipher the role of probiotics as a treatment modality for ASD.

SPECIFIC CARBOHYDRATE DIET

The Specific Carbohydrate Diet (SCD) is a restrictive diet first introduced in the 1920s by Sidney Haas for the treatment of celiac disease. It was later popularized by Gotschall,[71] the mother of a patient with inflammatory bowel disease who was treated by Haas. The aim of the SCD is to reduce symptoms of carbohydrate malabsorption and growth of pathogenic intestinal microbiota by restricting intake of complex carbohydrates (ie, starch and disaccharides, including lactose, maltose, and sucrose). Instead, the diet recommends monosaccharides (eg, glucose, fructose, galactose), including fruit, vegetables, honey, certain legumes, nuts, meats, and eggs. Restricted foods include starches such as grains, potatoes, most dairy, sugar, and processed foods.

The rationale for the use of SCD for autism stems from studies indicating dysbiosis as well as impaired carbohydrate digestion and absorption in individuals with ASD.[14,72–75] These microbiota perturbations are thought to contribute to the comorbid GI symptoms and perhaps also to behavioral symptoms. However, despite being a widely used dietary intervention with and without clinical guidance, there is a profound lack of published evidence for the efficacy or safety of SCD in patients with ASD. The literature search for this review revealed only 1 published case report to date.[76] The authors examined the implementation of SCD for management of GI issues in a patient with ASD and fragile X syndrome. The dietary intervention was well tolerated and improvements were noted in GI symptoms, nutrient status, and behavioral domains.[76] Despite the promising findings, concerns for nutritional deficiencies that may result from restrictive dietary interventions still exist, especially when not clinically supervised or when patients exhibit selective eating patterns and already restricted dietary diversity. On the basis of these results, it was concluded that use of the SCD protocol in patients with ASD warrants additional investigation.

POLYUNSATURATED FATTY ACIDS

PUFAs have been identified as a key factor in normal brain growth and development, and have been implicated in such areas as synapse and memory formation and cognitive function development. Several studies have specifically identified arachidonic acid (AA; ω-6), eicosapentaenoic acid (EPA; ω-3), and docosahexaenoic acid (DHA; ω-3) as essential, raising the theory that PUFA deficiency may serve as a mechanistic pathway for the development of ASD.[77–80] In fact, from results of a study of lipid metabolic pathways, researchers concluded that impairment of these pathways may contribute to the development of autism.[81] This theory is supported by the identification, in several studies, of altered phospholipid–fatty acid compositions in plasma and red blood cells from patients with ASD as compared to matched control participants.[82,83] A limited investigation into fatty acid levels in 11 Canadian children with autism revealed significantly lower levels of red blood cell DHA, EPA, AA, and ratios of ω-3 to ω-6, in addition to lower serum DHA, AA, and linoleic acid levels.[84]

Given the general acceptance by the scientific community that PUFAs may be implicated as a pathophysiologic pathway for the development of ASD, the question naturally arises as to whether PUFA supplementation could benefit patients with ASD. To this point, however, results are mixed. In a recent pilot trial of ω-3 and ω-6 fatty acid supplementation, clinically significant improvements in ASD symptoms and related behaviors (parent reported) were found for children born preterm and showing early signs of ASD.[85] Similarly, an analysis of the effects of orally supplemented ω-3 and vitamin B12 in a propionic acid–induced rodent model of autism demonstrated a positive therapeutic effect.[81] Specifically related to language development, a pilot study of ω-3 and ω-6 supplementation for 3 months in preterm toddlers with ASD symptoms demonstrated an increase in gesture use, but not word production, among the treatment group more than the placebo group, indicating the possible effectiveness of supplementation for treating delayed language development.[86]

Meanwhile, a secondary analysis on the effects of dietary DHA, EPA, and γ-linolenic acid supplementation in preterm toddlers with ASD symptoms revealed a statistically insignificant, but medium to large, change in baseline Infant/Toddler Sensory Profile scores, indicating support for a larger randomized trial.[87] Similarly, a randomized trial of 68 children and adolescents with ASD treated with ω-3 supplementation revealed significant improvement in social motivation and social communication subscale scores with a moderate to large effect. However, there was no treatment effect, indicating that supplementation with ω-3 PUFAs may be better suited for study as an adjunct to behavioral therapies in ASD.[88]

The results of these trials are largely inconclusive; they were hampered by different variables, though mostly by sample size. Thus, several authors have conducted systematic reviews and meta-analyses on supplementation to find a more definitive conclusion. As may be expected, however, these meta-analyses also have been largely inconclusive. In a recent analysis of studies examining the relationship between ω-3 long-chain PUFA supplementation and ASD, authors concluded that, compared with placebo, ω-3 long chain PUFA supplementation significantly improved social interactions and repetitive and restricted interests and behaviors, though all studies were limited by small sample sizes and potentially inadequate study durations.[89]

Similarly, authors of another systematic review examining the link between ω-3 and ASD concluded there may be a small, but not significant, benefit to supplementation in children with ASD. The authors analyzed 3 RCTs. First, from results of an internet-based RCT of 57 children assigned to 6 weeks of treatment, authors concluded there was a nonstatistically significant change in hyperactivity, as defined by the Aberrant Behavior Checklist and reported by parents and teachers, in the treatment group as compared with the control group.[90] The second RCT was a 16-week study evaluating the efficacy of supplementation of arachidonic acid and DHA in 13 participants with ASD who ranged in age from 6 to 28 years. The results suggested improvement in social interaction in participants.[91] Interestingly, different conclusions were revealed in the third study. After 6 months of treatment with DHA supplementation in 48 children aged 3–10 years who had ASD, the researchers observed no improvement in the core symptoms of ASD nor in associated behaviors.[92] Given these conclusions, the systematic review's authors called for larger, higher-quality RCTs to examine the role of ω-3 PUFAs, while also advocating for restraint in recommending ω-3 PUFAs as an alternative to behavioral therapies, instead recommending their potential use as a complement.[93]

Authors of a meta-analysis of 6 trials ($n = 194$) noted that ω-3 supplementation may improve hyperactivity, lethargy, and stereotypy in children with ASD, but they also were careful to point out that these studies were limited by size and demonstrated small overall effects, ultimately preventing the researchers from drawing formal conclusions.[94] Most interesting, however, may be the findings of a fourth systematic review and meta-analysis, which is the only 1 in which that authors drew definitive conclusions from the limited data. This analysis of 5 RCTs ($n = 183$) presented the limited data currently available as suggesting that ω-3 supplementation does not enhance the performance of children with ASD.[95]

Ultimately, it is clear from the encouraging results of several of these studies, and the paucity of information, that additional testing is warranted. However, all these studies have wide discrepancies in important factors, including, but not limited to dose used, duration of supplementation, measurement of ASD, the wide variety of ASD presentations, and the fatty acid supplemented. Additional research into these variables is suggested before definitive conclusions can be made.

DIETARY SUPPLEMENTS (VITAMINS A, C, B6, AND B12; MAGNESIUM AND FOLATE)

A variety of additional dietary deficiencies have been identified as potential triggers for the pathophysiology of ASD, including vitamins A, C, B6, B12, folate, and D; and ferritin. As a result, these potential deficiencies have spurred various trials of supplementation to treat the symptoms of ASD.

Vitamin A

Similar to PUFAs, several studies have demonstrated that vitamin A deficiency is a common finding among children with ASD.[96] In fact, a national survey of children with ASD in Chongqing, China ($n = 154$), demonstrated that children with ASD had low rates of serum vitamin A deficiency that were negatively correlated with CARS scores ($P = 0.021$).[97] Meanwhile, authors of other studies have called for establishing normative values for vitamin A in pregnant women and newborns to help provide references while investigating potential pathologies related to vitamin A deficiency for ASD, specifically those related to the oxytocin-CD38-vitamin A axis in pregnant women.[98]

Given this background, researchers have tried to demonstrate that vitamin A deficiency may induce ASD, as convincingly demonstrated in a recent study of rats.[99] Corroborating these findings, researchers demonstrated in the same study that vitamin A supplementation improved autistic-like behaviors in pups with gestational vitamin A deficiency.[99] In this study, the proposed mechanism linked gestational vitamin A deficiency to suppress retinoic acid receptor β signal transmission and, therefore, decreased CD38 expression in offspring.

In children with ASD, studies of vitamin A supplementation have been less common or have been flagged with a warning. Authors of a pilot study concluded vitamin A supplementation led to significant improvement in autism symptoms, but they cautioned that vitamin A supplementation may only be suitable for a subset of children with ASD.[96] Similarly, in another study, researchers cautioned that the mechanism underlying the link between vitamin A and ASD warranted additional investigation before making definitive conclusions.[97] Interestingly, in a longitudinal study of dietary supplementation in children with ASD ($n = 288$), researchers demonstrated that vitamin A supplementation actually led to excess vitamin A intake, which may be undesirable.[100] These data were corroborated by those from another study in which excess consumption of vitamin A by specific age groups of children with ASD was found.[101] Finally, on the basis of results of a study of vitamin A supplementation on gut microbiota, authors suggested that although vitamin A may play a role in autism biomarkers, there was no clear correlation among vitamin A supplementation, concentration, and ASD symptoms.[102]

Ultimately, given these findings, it is likely there is a mechanism linking vitamin A deficiency to ASD. Perhaps the most promising potential mechanism is the oxytocin-CD38-vitamin A axis, or the retinoic acid receptor β–CD38-oxytocin axis, as discussed earlier in this section, which warrants additional investigations. In current studies, though, correction of vitamin A deficiency via supplementation has not proven very effective as a treatment for ASD symptoms.

Vitamin C

Most of the literature regarding vitamin C and ASD comes from the perspective of nutritional deficiency, given the propensity for individuals with ASD to have feeding difficulties and nutritional inadequacy. A patient with impactful food selectivity that resulted in dramatic vitamin C deficiency was the subject of a case report,[103] as was the case of a 10-year-old boy who had scurvy disguised as osteomyelitis.[104] Interestingly, scurvy has not been an uncommon presentation in children with ASD; 7 patients with developmental disorder presented with scurvy at Boston Children's Hospital.[105] There are similar reports among other studies.[106–112] Similarly, a comparative study of nutritional intake in 63 children with ASD and 50 typically developing children revealed that a significantly greater proportion of ASD children were below the average threshold for vitamin C intake.[113] These results were further corroborated by a case-control study of male children and adolescents with ASD, and results of another comparative study in Valencia, Spain.[114,115]

Interestingly, in the longitudinal study of dietary supplementation in children with ASD ($n = 288$) mentioned in the section titled Vitamin A,[100] results demonstrated that supplementation resulted in excess vitamin C among children aged 2 to 3 years with ASD. It was concluded that many children with ASD are erroneously given micronutrients as supplements, leading to excess intake, which appears to be the case for vitamin C in this study.[100] In the Chinese survey of the correlation between nutrition and ASD symptoms, vitamin C was not studied.[97]

Mechanistically, it has been theorized that these elevated vitamin C levels actually may be beneficial, based on targeting of oxidative stress. In a randomized, double-blind, placebo-controlled vitamin and mineral treatment study, children with ASD treated with vitamin C had significant reductions in oxidative stress (indicated by the ratio of oxidized glutathione [GSSG] to reduced GSH). It was suggested that these increased levels of vitamin C were beneficial in significantly reducing the oxidative stress in these patients.[116]

Methylation and transsulfuration micronutrients (folate, vitamins B6 and B12). Normal neurodevelopment reflects programed changes in gene expression, in which methylation-dependent epigenetic regulation (ie, methylation of DNA and histone proteins) plays a

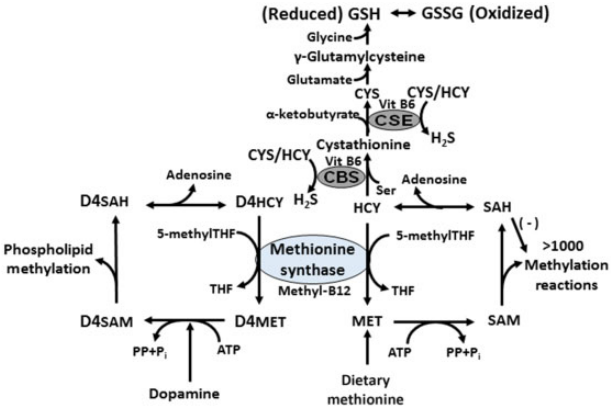

Figure 3 **Methylation and transsulfuration metabolic pathways.** The 4-step MET cycle (lower right) supports a multitude of methylation reactions, including methylation of DNA and histone proteins, providing epigenetic regulation. MET is activated to the methyl donor SAM, whereas SAH, the product of methyl transfer reactions, is an inhibitor of methylation. HCY can either be converted to MET by methionine synthase, with methyl-B12 and 5-MTHF serving as cofactors, or it can be converted by transsulfuration to CYS, supporting GSH synthesis, in vitamin B6–dependent reactions. HCY and CYS can also be converted to H_2S. Methionine synthase also supports a 4-step cycle of phospholipid methylation mediated by a methionine residue in the D4 dopamine receptor (D4Met). *Abbreviations:* ATP, adenosine triphosphate; CBS, cystathionine-β-synthase; CSE, cystathionase; CYS, cysteine; GSH, glutathione; GSSG, oxidized glutathione; H_2S, hydrogen sulfide; HCY, homocysteine; MET, methionine; methyl-B12, methylcobalamin; MTHF, methylene-tetrahydrofolate reductase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; ser, serine; THF, tetrahydrofolate; vit, vitamin

central role.[117,118] Deficits in methylation status have been reported in ASD in conjunction with low levels of GSH and oxidative stress, providing a rationale for dietary supplementation of micronutrients that support methylation.[6–9] As illustrated in Figure 3, the MET cycle of methylation is linked to GSH synthesis by the transsulfuration pathway, which converts homocysteine (HCY) to CYS. Folate, in the form of 5-methyltetrahydrofolate (5-MTHF), is an essential cofactor for conversion of HCY to MET by MET synthase (MTR), along with vitamin B12 in the form of methylcobalamin (methyl-B12). Vitamin B12 is highly vulnerable to oxidation during the catalytic cycle of MTR as it awaits replenishment of its methyl group from 5-MTHF.[119] The presence of oxidative stress increases inactivation of MTR, resulting in HCY accumulation, which can be shunted to the transsulfuration pathway, increasing synthesis of the antioxidant GSH. In this relationship, therefore, vitamin B12 serves as a biosensor of cellular redox status, making methylation activity and its epigenetic manifestations sensitive to oxidative stress. Thus, the well-documented occurrence of oxidative stress in ASD is likely to interfere with epigenetic-regulated neurodevelopment.

Naturally occurring genetic variants (ie, SNPs) can adversely affect the efficiency of different components of the transmethylation cycle and transsulfuration pathway, increasing the impact of micronutrient deficiencies in vulnerable subpopulations possessing 1 or more SNPs. Perhaps the best studied example is lower production of 5-MTHF due to the C677T SNP in 5,10-methylene-tetrahydrofolate reductase (MTHFR), which increases thermolability of the enzyme. An association of C677T alleles with ASD has been reported in several studies.[7,120–123] Other methylation-related SNPs have also been linked to ASD, including the reduced folate carrier (RFC G80A), transcobalamin (TCN2 G776C), catechol-*O*-methyltransferase (COMT G472A), MTR (MTR A2756G), and MTHFR A1298C.[120,124]

Vitamin B6 is a cofactor for the transsulfuration pathway enzymes cystathionine-β-synthase (CBS) and cystathionine-γ-lyase, the latter of which is also known as cystathionase (CSE). Expression of both CBS and CSE is promoted by vitamin D.[125–127] Importantly, evidence indicates CBS expression is restricted to astroglial cells, suggesting the transsulfuration pathway may not function in neurons,[128] Brain CSE activity in humans is low, especially in younger years, but increases starting in the fifth decade of life, as reflected by lower levels of cystathionine.[129,130] CSE expression is induced by the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α),[131] whose levels increase with age.[132] Young patients with ASD have decreased brain levels of cystathionine,[130] consistent with premature CSE expression induced by TNF-α, cerebrospinal fluid levels of which are higher in patients with ASD.[133] CBS and CSE also convert HCY and CYS to hydrogen sulfide (H_2S), which inhibits mitochondrial respiration and decreases reactive oxygen species formation. Because H_2S is a diffusible gas, its production by astroglial cells can exert effects on nearby neuronal cells. Thus, CBS and CSE activities combine to augment antioxidant production (ie, increase GSH synthesis) and decrease antioxidant demand (ie, decrease reactive oxygen species' production).

Vitamin B6 and magnesium. Historically, studies of vitamin B6 in ASD primarily examined the effects of its combination with magnesium (Mg) and, as with almost all supplementation studies, results were mixed.[134–137] From a mechanistic perspective, it has been proposed that some ASD symptoms may reflect neurotransmitter system deficiencies and that vitamin B6 may enhance these systems because of the role it plays in neurotransmitter synthesis.[116] However, in a 2017 review of 7 RCTs investigating B6 and Mg supplementation, authors concluded supplementation was not helpful in

improving ASD symptoms.[138] Similarly, in a systemic review of trials from 1993, 1997, and 2002, authors concluded no recommendation could be made regarding B6 and Mg supplementation as a treatment for ASD.[134,137,139,140] The same authors, in a follow-up meta-analysis, similarly concluded there was not sufficient evidence to demonstrate treatment efficacy given the small amount (and poor quality) of studies available.[141]

When discussing vitamin B6 and supplementation with vitamin B6 and Mg, it is important to note that "the American Psychiatric Association and the American Academy of Pediatrics have stated that megavitamin treatment for learning disabilities and autism is not justified."[142] It is also suggested that these statements may have contributed to the paucity of investigations of their use (as well as an explanation for why the vast majority of research is not recent).[142] Results of a recent study, however, have indicated plasma glutamine levels may predict vitamin B6 responsiveness in ASD, although these results have been presented with caution because the study was limited by sample size and a low proportion of B6 responsiveness to ASD.[143] As with all nutrients, however, children with ASD have demonstrated low levels of B6 intake.[101]

Vitamin B12

There is very little literature about the role of B12 in ASD, although findings have been encouraging. It also should be noted that when discussing B12 in the context of ASD, generally it is methyl-B12 being referred to, because it is the only member of the B12 family with the ability to directly stimulate the MET/HCY biochemical pathway, which is responsible for forming the universal methyl donor SAM.[144] Cyanocobalamin is less effective for B12 supplementation because it requires liver metabolism for activation.

As with all nutrients, children with ASD have demonstrated low levels of B12 intake.[116] ASD has been associated with simultaneous B6, B9, and B12 deficiencies that lead to a block of cystathione formulation and, therefore, resultant accumulation of HCY, ultimately leading to lower methyl-group availability and decreased urine levels of MET and SAM. It has been suggested that the well-known simultaneous deficiency of these vitamins may indicate that intestinal dysbiosis may be the true underlying cause of reduced absorption, although the potential role of genetic mutations has not been ruled out.[145]

Vitamin B12 is a key cofactor in MET transmethylation/transsulfuration metabolism (Figure 3). Because reduced methylation and antioxidant capacity has been observed in persons with ASD,[6,7] there has been an increased interest in B12 supplementation. In 1 study, oral dietary supplementation substantially increased levels of glutathione and SAM.[116] SAM levels, in particular, improved to near-normal, but, as the primary driver of methylation, the SAM increase may have been influenced by improvements in adenosine triphosphate, a cofactor for the conversion of MET to SAM. Similarly, glutathione levels improved and contributed to the reduced oxidative stress referenced along with vitamin C supplementation. It was suggested that this reduction was likely due to stabilizing levels of NADPH, as the major cofactor for the recycling of GSSG to GSH. The results of this study indicate oral B12 may be an alternative to subcutaneous methyl-B12, which is interesting, given the absorption pathway of oral B12 involving intrinsic factor.[116]

In a postmortem brain tissue study, levels of methyl-B12 were decreased by greater than 10-fold over the lifespan in normal individuals, but levels in young persons with ASD were significantly lower compared with tissue from age-matched control individuals, indicative of a premature acceleration of the normal age-dependent decrease. Lower methyl-B12 level was accompanied by decreased activity of MTR, for which vitamin B12 and 5-MTHF are required cofactors.[130] Because impaired methylation interferes with epigenetic regulation of gene expression, which is crucial for normal brain development,[7] there is a clear rationale for methyl-B12 supplementation in ASD.

On the other hand, observed conflicting results have been reported in some studies. A survey of children with ASD found no correlation between B12 concentration and CARS score.[97] Interestingly, though, authors of another study concluded that extremely high maternal plasma folate and B12 levels at birth were associated with ASD risk, although the researchers were careful to state that this study did not "question the importance of consuming adequate folate and vitamin B12 during pregnancy."[146]

The effects of treatment with methyl-B12 administered by subcutaneous injection was examined in 2 RCTs.[144,147] In the initial study, it was demonstrated that, similar to B6, methyl-B12 provided substantial symptom improvement for a subgroup of children with ASD (30%), although a significant effect was not observed for the overall group.[144] Notably, the responder group also showed significant increases in GSH and GSSG levels. A follow-up RCT ($n = 50$) in 2016 demonstrated that methyl-B12 supplementation significantly improved Clinical Global Impressions–Improvement scores but not 2 other ASD rating scores.[147] Behavioral improvement was associated with improved MET metabolism and cellular methylation capacity. Because methyl-B12, vitamin B6, and 5-MTHF are all essential

cofactors for transmethylation/transsulfuration pathway function, combined deficiencies may be more deleterious. Concomitant functional deficits in vitamins B6, B12, and 5-MTHF have been documented in ASD.[145] Under such conditions, RCTs examining supplementation of only a single cofactor would be limited in their ability to demonstrate clinical improvement. Indeed, significant improvement in ASD symptoms and intelligence quotient were reported with the use of a multicomponent vitamin and mineral supplement.[31] Most recently, however, results of a study into a propionic acid–induced rodent model of autism indicated supplementation of ω-3 and vitamin B12, alone or in combination, could result in a positive therapeutic effect.[81]

As mentioned, an association between childhood ASD and SNPs has been reported in genes related to B12 and folate metabolism.[148,149] Given that B vitamins and folate are key cofactors of 1-carbon metabolism, it is reasonable to speculate that genetic variations to key enzymes like MTHFR, MTR, MTR reductase, reduced folate carrier, dihydrofolate reductase, and transcobalamin (TCN2), among others, could be indicated in childhood ASD. Interestingly, though, a study of genotypes of TCN2, MTR, MTR reductase, and MTHFR in the Han Chinese population showed no association between childhood ASD and its severity.[148] Similarly, in another study, individuals with at least 1 copy of the MTHFR 677 T allele had 4 behaviors that were more common and problematic: direct gaze, current complex body movement, history of self-injurious behavior, and current over reactivity, though it should be noted that no differences were found in the Peabody Picture Vocabulary Test, Third Edition; Ravens Colored Progressive Matrices; or the Vineland Adaptive Behavior Scales.[149] Ultimately, it is reasonable to hypothesize that SNPs related to 1-carbon metabolism may be implicated in ASD, although considerably more research is warranted.

FOLATE

ASD has been connected to abnormalities in folate metabolism leading to several mechanistic hypotheses for causes and symptomatic treatment in ASD.[150] Improper folate metabolism and impaired, folate-dependent, 1-carbon metabolism and transsulfuration pathways could provide the basis for biochemical testing for ASD with high sensitivity and specificity, according to a study.[151] These results provide a most exciting recent development at the intersection of metabolite disorder and ASD. It has been suggested that folic acid supplementation in pregnancy may significantly reduce the risk of ASD.[152–155] Interestingly, however, there may also be an association between excess folic acid supplementation during pregnancy and ASD.[156]

Folic acid supplements must be converted to the active 5-MTHF form to support MTR and methylation reactions, with the methyl group arising from mitochondrial formic acid production. High-dose supplementation with folinic acid (also known as 5-formylTHF and leucovorin), which has an equivalent activity as folic acid due to its conversion into 5-MTHF, resulted in improved verbal communication in patients with ASD, especially if the patient was positive for folate receptor-α autoantibody (FRAA).[157] It has been suggested that FRAA exposure during fetal development and infancy may contribute to ASD, so identifying women who are FRAA positive and treating them with high-dose folinic acid may be a strategy for reducing the risk of ASD and other developmental deficits.[158] One such strategy may include high-dose folinic acid, corticosteroids, and a milk-free diet to provide adequate folate while also decreasing FRAA.[159] Given the importance of FRAA presence, it has been suggested that FRAA biomarkers should be considered for subgrouping children with ASD, especially when considering treatments.[160]

In another study, no correlation was found between serum folate concentrations and CARS scores.[97] In addition, similar to other nutrients, children with ASD have demonstrated significantly lower daily intake of folate, whereas supplementation in patients with ASD has led to excess folate.[100,113] Additional studies are warranted to clarify the optimal form and optimal dose of folate for possible prevention and treatment of ASD.

Folate may also be supplemented as L-methylfolate. A recent case study from Siscoe and Lohr described their administration of L-methylfolate to a patient with ASD who tested positive for the MTHFR C677TT allele; this was the first known report of L-methylfolate administration to such a patient.[161] It also should be noted that folic acid is more readily available than folinic acid in China, hence the preponderance of studies examining folic acid specifically.[162] More studies are warranted to decipher the role of L-methylfolate supplementation and folic acid in the pathophysiology of ASD.

CONCLUSION

With no current cure for ASD, treatments are centered around speech and behavioral therapies to improve social functioning and communication. However, given that many individuals with ASD suffer from GI disturbances and that these microbiota changes are thought to play a role in the expression of GI and non-GI symptoms, interest in nutritional interventions remains high among patients' families and clinicians. Although many

Table 1 **A summary of nutritional interventions for autism spectrum disorders**

Nutritional interventions	Clinical implications or advantages	Limitations or disadvantages
Gluten free casein free diet[24–30]	RCTs provided evidence of behavioral and GI symptom improvement; no reported adverse effects or nutritional deficiencies	Long treatment time required for response; adherence difficulty; may only be effective in subset of patients
Ketogenic diet[36–38,50,51]	Animal studies and limited cohort studies demonstrate potential for behavioral symptom improvement	Few existing studies, restrictive diet, and potentially unpalatable, limited sampling can cause nutritional deficits
Probiotics[54,55,68–70]	Significant potential for improvement of GI and ASD symptoms	Limited studies and unproven mechanistic theories; mixed reaction by parents and ASD community to current research
Specific carbohydrate diet[76]	Anecdotal reports of symptom improvement	Very few existing studies; no RCTs; very restrictive diet and difficult adherence
Polyunsaturated fatty acids supplementation[77–84]	Implicated as a pathophysiologic pathway for ASD; potential for combining with other therapeutic modality	Mixed results regarding supplementation; lack of consistent RCTs
Vitamin A supplementation[96,97,100–102]	Potential mechanism linking vitamin A deficiency to ASD pathophysiology	Correction of vitamin A deficiency via supplementation has proven ineffective and may lead to adverse effects associated with excess vitamin A intake
Vitamin C supplementation[97,100,103,104,113]	None	ASD nutritional deficiency has been linked to scurvy and other vitamin C–related adverse effects, but there is no apparent link between deficiency and pathophysiology or supplementation and therapy
Vitamin B6 and magnesium supplementation[138,139,142]	Proposed mechanism for therapy via supplementation	No conclusive data demonstrating therapy via supplementation; statements against supplementation from scientific bodies (American Psychiatric Association and American Academy of Pediatrics)
Vitamin B12 supplementation[81,97,138,144]	Encouraging early results may demonstrate improvement in ASD symptoms	Paucity of data and studies; ultimately, effects are inconclusive and warrant additional study
Folic acid supplementation[114,146,150–155]	Folic acid supplementation in pregnant mothers may prevent ASD; potential future in screening for ASD related to folate-dependent 1-carbon metabolism and sulfuration pathways	Results are only gestational, no evidence in therapy for ASD symptoms after birth

Abbreviations: ASD, autism spectrum disorder; GI, gastrointestinal; RCT, randomized controlled trial.

dietary interventions have been studied, there has been a lack of conclusive scientific data about the effect of therapeutic diets on autism, and as such, no definitive recommendation can be made for a specific nutritional therapy as a standard treatment for ASD (Table 1).[24–26,28–30,36–38,50,51,54,55,68–70,76–84,96,97,100–104,113,114,138,139,142,144,146,150–155]

Although there is no conclusive evidence of the benefit of nutritional therapies for persons with ASD, many parents or healthcare providers choose to continue dietary interventions and have observed promising results with regard to behavior and GI abnormalities. A major problem in interpreting the available evidence for dietary interventions is the variability in measurement of the therapeutic effect. Studies often explore the effect of nutritional therapies on differing aspects of ASD and rarely compare effectiveness with that of other interventions, making generalizability and comparison between treatment modalities less robust. Lack of control groups in many studies and high levels of parental or physician expectancy about the benefit of these interventions may bias the results of dietary studies. It is also relevant to highlight the lack of blinded studies to decrease bias in parents' or caregivers' perception as well as behavioral raters in these studies. An additional limitation in these studies is the variety of outcome measurements used to evaluate behavioral improvements that can range from parents or caregivers' questionnaires to the use of diagnostic instruments, such as the Autism Diagnostic Observation Schedule and CARS, not really designed to detect changes in behaviors but rather to differentiate individuals with ASD from those without ASD. This limitation poses a challenge when comparing different studies using similar interventions. Furthermore, dietary intervention studies frequently have a small sample size that, again, affects the generalizability of the results. It would be useful to better define and standardize what

aspects of ASD symptomology are targeted by these interventions, what outcomes are considered an improvement, and how these outcomes are measured.

Despite these methodological drawbacks, studies indicate that nutritional interventions can be used as an adjuvant treatment to usual first-line therapies, which include speech and language therapy, occupational therapy, applied behavioral therapy, and educational programs. More studies are warranted to determine which factors in these diets offer symptomatic improvement and which factors lead to the undesirable effects. Understanding the mechanisms for improvement provided by the various dietary protocols will help in designing interventions that maximize efficacy and ease of implementation. Another challenge lies in the observation that different ASD subpopulations seem to respond to different dietary interventions. The challenge remains to develop ways to identify specific biomarkers that can be associated with the behavioral improvements following nutritional intervention for ASD. In addition, there is a need to identify the subgroup of patients who will benefit from a specific therapy and to improve the ease of implementation of the therapies for patients' families.

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