



## Is the gluten-free and casein-free diet efficacious in the treatment of childhood autism spectrum disorder?

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### Abstract

Autism spectrum disorder (ASD) is a set of heterogeneous neurodevelopmental conditions, characterized by early-onset difficulties in social communication as well as repetitive and unusually restricted behaviors and interests. The treatment of ASD is based primarily on psychoeducational and behavioral interventions. Since the effectiveness of available treatments for ASD is limited, many families search for alternative therapies, such as the gluten-free and casein-free (GFCF) diet. Despite the popularity of the GFCF diet as a supplementary treatment in children with ASD, several rigorous evaluations have failed to confirm its effectiveness. The majority of the available studies examining the efficacy of the GFCF diet are seriously flawed and allow no firm conclusions. The available evidence regarding the effectiveness of the GFCF diet in the treatment of childhood ASD is very weak and cannot be considered promising. The GFCF diet should be used only if an allergy or intolerance to nutritional gluten or casein has been established. The identification of a hypothetical diet-related ASD phenotype may help in selecting children who could benefit from a GFCF dietary intervention. An important consideration is that potentially ineffective therapies may imply considerable opportunity costs, with other possibly more effective treatment approaches remaining unutilized.

**Keywords:** Autism spectrum disorder; Children; Gluten-free and casein-free diet; Opioid excess hypothesis; Treatment.

### 1. Introduction

Autism spectrum disorder (ASD) is a set of heterogeneous developmental conditions; the core features of ASD are early-onset social communication difficulties and repetitive, stereotypical and restricted sensory-motor behaviors (Kanner, 1943; Levy et al., 2009). While ASD was previously considered a rare and narrowly defined condition of childhood, it is viewed today as a lifelong condition with a spectrum ranging from very mild to severe. The disorder was grouped with neurodevelopmental disorders in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). Previously distinguished subtypes, such as Asperger's disorder and pervasive developmental disorder not otherwise specified, are now consoli-

dated under the diagnosis of ASD. Comorbid conditions, such as attention-deficit hyperactivity disorder and anxiety disorders, are common and have been reported in more than 70% of individuals diagnosed with ASD (Gotham et al., 2015; Simonoff et al., 2008). The prevalence of ASD has been estimated to be approximately 1% globally (Elsabbagh et al., 2012) and 1.5% in developed countries (Lyll et al., 2017). Prevalence estimates have risen significantly over the last two decades. While this rise in prevalence might reflect a concomitant rise in the incidence of ASD, changes in the concepts and diagnostic criteria of ASD have been suggested as alternative explanations (Fombonne, 2009). A wide variety of risk factors for ASD, such as prenatal/perinatal and maternal lifestyle and dietary factors, have been suggested (Lyll et al., 2014; Mandy and Lai, 2017). Genetic studies have identified risk patterns (Gaugler et al., 2014; Tick et al., 2016), and gene-environ-

ment interactions with risk-inducing environmental compounds have been discussed (Herbert, 2010). Neurobiological examinations have found early changes of brain development as well as of neuronal reorganization and connectivity in ASD (Bauman and Kemper, 2005; Ecker et al., 2015; Hazlett et al., 2017; Lewis et al., 2014; O'Reilly et al., 2017). However, genetic and neurobiological studies have as yet been unable to provide any clinical benefit or identify reliable biomarkers for routine use (Walsh et al., 2011). There are therefore no biological diagnostic tools available, and the diagnosis of ASD must rely solely on behavioral assessment (American Psychiatric Association, 2013).

There is no curative therapy for ASD. The treatment is primarily based on psychoeducational and behavioral interventions (Reichow et al., 2014), with medication used as an adjunct. While some studies using low-intensity interventions involving parent-child interaction have shown significant effects on children's social behavior and communication (Weitlauf et al., 2014), others have been unable to find any positive effects (Carter et al., 2011a). Meta-analyses of treatment studies using early comprehensive and targeted behavioral interventions (naturalistic developmental behavioral interventions) (Schreibman et al., 2015) have shown some positive effects on adaptive skills and language skills (Reichow et al., 2012; Weitlauf et al., 2014). However, only one trial was truly randomized, and no effects were found when these approaches were compared to other developmental interventions of equal intensity (Weitlauf et al., 2014). While drugs do not directly improve social communication in children with ASD, they may be able to reduce comorbid symptoms. Evidence-based pharmacotherapy of children and adolescents with ASD, using atypical antipsychotics (risperidone, aripiprazole), is limited to the treatment of co-occurring behaviors, such as agitation, irritability, aggression and other disruptive behaviors (Fung et al., 2016). It is important to note that the reactions of a child's family to the diagnosis of ASD affects the outcome as much as any treatment (Dykens et al., 2014).

Since the effectiveness of available treatments for ASD is limited, many families search for alternative therapies (Owen-Smith et al., 2015; Perrin et al., 2012). A potential role of nutrition in the etiology of ASD has been suggested. It has been reported that up to a third of parents of children with ASD conceal information on nutritional interventions from the physician responsible for treating their children (Trudeau et al., 2019). Moreover, up to a fifth of preschool children with ASD have been given some form of restriction diet (Rubenstein et al., 2018). In particular, the nutritional proteins gluten (from wheat, barley, rye and oats) and casein (from milk and other dairy products) have been hypothesized to be involved in ASD, and special diets free of gluten and casein have been suggested to be of value in the treatment of ASD (Lange et al., 2015). The present short review critically discusses the evidence regarding the therapeutic efficacy of the gluten-free and casein-free (GFCF) diet in ASD.

## 2. Rationale for the GFCF diet in ASD

The rationale for the administration of GFCF diets in people with ASD stems mainly from the effects of opioid peptides, released by the digestion of nutritional gluten and casein. The opioid excess hypothesis, first proposed in 1979, draws parallels between the acute behavioral effects of opiates and the symptoms of ASD and speculates that autism may be "an emotional disturbance arising from an upset in the opiate systems in the brain" (Panksepp, 1979). The opioid excess hypothesis postulates that opioid peptides produced through the metabolism of gluten and casein can

pass across an abnormally permeable intestinal membrane and, in consequence, are capable of exerting effects on neurotransmission by binding to opioid receptors (Panksepp, 1979). Opioid receptors may be involved in the regulation of social behavior and possibly also in the pathogenesis of ASD (Genius and Lobo, 2014). Gluten and casein have similar molecular structures and are metabolized to gluteomorphine and casomorphine, respectively. These peptides have been suggested to accumulate and to enter the blood circulation through an elevated permeability of the intestinal membrane ("leaky gut") in ASD (D'Eufemia et al., 1996). The opioid peptides formed during digestion might then permeate the blood-brain barrier and act directly on the brain by binding to opiate receptors and mimicking the effects of opiate drugs (Shattock and Whiteley, 2002). In doing so, they could cause an increase in the activity of the endogenous opioid system, which has been speculated to be linked to the behavioral symptoms of ASD (Panksepp, 1979). However, the opioid excess hypothesis has been criticized, since the available studies have been unable to reveal abnormal opioid levels in the plasma or brain of children with ASD (Lázaro et al., 2016).

Gastrointestinal problems, such as gastrointestinal inflammation, abdominal pain, diarrhea and chronic constipation, are commonly seen in children with ASD (Buie et al., 2010a; Buie et al., 2010b; Kang et al., 2014). These problems have been found to correlate with the severity of ASD (Adams et al., 2011) and may be partly due to digestive enzyme deficiencies, food sensitivities and small bowel inflammation (Kang et al., 2017). The gastrointestinal symptoms in children with ASD indicate a potential link between ASD and celiac disease or gluten sensitivity. Celiac disease is a gastrointestinal disease characterized by a gluten sensitivity, which is responsible for the immunogenic response and mucosal inflammation, intestinal villi atrophy and increased intestinal permeability associated with the disorder (Kelly et al., 2015). Since celiac disease is triggered by the consumption of food containing gluten (Elli et al., 2015), the only treatment currently available for celiac disease is to follow a strict lifelong gluten-free diet (Hill et al., 2016). Today, large numbers of individuals without celiac disease avoid gluten in the belief that a gluten-free diet is associated with health benefits, potentially resulting in the unnecessary consumption of gluten-free foods (Gaesser and Angadi, 2015).

A comorbidity between ASD and celiac disease has been suggested by several large-scale epidemiological studies (Atladóttir et al., 2009; Butwicka et al., 2017; Lebwohl et al., 2021). Furthermore, a predisposition towards autoimmunity has been suggested (Money et al., 1971). In comparison with controls, children with ASD have been reported to display elevated markers of innate and adaptive immune response (Jyonouchi et al., 2001) and, in particular, to have higher concentrations of proinflammatory cytokines following exposure to gluten and casein (Jyonouchi et al., 2002). The mechanism of an elevated immune reactivity to gluten observed in a subgroup of children with ASD has been suggested to be distinct from that in celiac disease (Lau et al., 2013). An increase in antigliadin antibody response and its association with gastrointestinal symptoms in these children has been interpreted as an indication of immunological or intestinal permeability abnormalities (Lau et al., 2013). These findings are the basis of investigations into a link between ASD and food allergies (Jyonouchi, 2009; Li et al., 2021).

The opioid excess hypothesis predicts elevated urinary levels of opioid peptides as potential biomarkers of ASD. As a therapeutic consequence, diets low in gluten and casein have been hypothesized not only to normalize the urinary peptide levels but also to ameliorate the behavioral symptoms of children with ASD (Reichelt et al., 1991; Shattock and Whiteley, 2002). Gluten-free

**Table 1. Hypothetical mechanisms of action of the GFCF diet in ASD**

Hypothetical mechanism	Findings
Excess opioid activity	Opioid overactivity leading to decreased social behavior in animal models of ASD (Panksepp, 1979). Possible involvement of opioid receptors in the pathogenesis of ASD (Genuis and Lobo, 2014). Increased (and also decreased) levels of opioid-like peptides, produced through gluten and casein metabolism and entering the blood circulation through a “leaky gut” (D’Eufemia et al., 1996), in the serum, cerebrospinal fluid or urine of individuals with ASD (Pellissier et al., 2018).
Increased autoimmunity	Elevated markers of innate and adaptive immune response (cytokines, cytokine tumor necrosis factor- $\alpha$ etc.) in children with ASD (with regression) (Jyonouchi et al., 2001; Money et al., 1971) leading to a hypothetical “autoimmune phenotype” with environmental stimuli triggering an immune response which exacerbates ASD features. Hypothetical knock-on effects of dietary triggers on molecular pathways with high levels of antibodies against gliadin, casein and the digestive enzyme dipeptidyl peptidase IV, which is involved in the processing of gliadin (Vojdani et al., 2003, 2004a, 2004b).
Oxidative stress and inflammation	Impaired antioxidant defense in the cerebellum of individuals with ASD (Gu et al., 2013). Problems in the metabolism of nitrous oxide leading to increased oxidative stress (Frye and Slattery, 2016; Wrońska-Nofer et al., 2012). Increased oxidative stress triggered by gliadin (Monguzzi et al., 2019). Increased oxidative stress demonstrated by elevated markers of oxidative stress in untreated children with celiac disease (Stojilković et al., 2007). Possible release of pro-inflammatory cytokines contributing to gastrointestinal inflammation (leading to “leaky gut”) due to oxidative stress associated with low glutathione levels (Elder et al., 2006).
Reactivity of antibodies to gluten products	Possible indication of gluten sensitivity in celiac disease and ASD by presence of antibodies against tissue-transglutaminase (Cervio et al., 2007), transglutaminase 6 (Hadjivassiliou et al., 2013; Józefczuk et al., 2018) and gliadin (Cade et al., 2000; De Magistris et al., 2013; Kawashti et al., 2006; Lau et al., 2013; Vojdani et al., 2003, 2004a, 2004b) Potential for antibodies and other irritants to travel along the gut-brain axis indicated by findings of inflammation and abnormal intestinal permeability in ASD (Ashwood et al., 2003; Ashwood et al., 2004; Jyonouchi et al., 2002; Souza et al., 2012; Torrente et al., 2002).
Gut microbiota perturbations	Possible link between abnormal gut microbiota composition and ASD (Finegold, 2011; Mülle et al., 2013; O’Mahony et al., 2015; Rosenfeld, 2015; Wang et al., 2013). Possible downregulation of gastrointestinal inflammation and intestinal permeability by interaction between GFCF diet and gut microbiota (Doenya, 2018b).

diets involve the dietary exclusion of gluten in grains and various processed food products, while casein-free diets exclude casein in dairy products. Numerous studies have examined the combination of these diets, the GFCF diet, as an alternative intervention in children with ASD in light of the limited effectiveness of the available therapies.

Studies examining the urinary profiles of children with autistic syndromes have found elevated concentrations of certain peptides (Cade et al., 2000; Israngkun et al., 1986; Knivsberg et al., 1990; Knivsberg et al., 1995; Reichelt et al., 1981; Reichelt et al., 1986; Reichelt et al., 1991). Furthermore, reductions in both these peptide concentrations and autistic symptomatology in children adhering to a GFCF diet have been reported (Cade et al., 2000; Knivsberg et al., 1990; Knivsberg et al., 1995), advancing the popularity of the GFCF diet in media reports (Schreck et al., 2013).

In addition to a hypothetical opioid activity of improperly digested gluten products, research on pathological interactions between gluten and ASD has focused on inflammation caused by oxidative stress and reactivity with anti-gluten antibodies (see Table 1). These hypotheses provide some explanation for the comorbidity found between ASD and celiac disease. The intestinal microbiome has been suggested to be a possible causative or facilitative agent in mental disorders, including ASD (Doenya, 2018a; Lange et al., 2020; Li et al., 2017; Vuong and Hsiao, 2017). ASD has been linked to an abnormal gut microbiota composition, with distinct increases or decreases in certain microbial groups being found in the stool of children with ASD (Finegold, 2011; Mülle et al., 2013; O’Mahony et al., 2015; Rosenfeld, 2015; Strati et al., 2017; van de Sande et al., 2014; Wang et al., 2013). However, other studies have found no compositional perturbations of gut microbiota in people

with autism (Son et al., 2015). The changes in gut microbial communities are frequently accompanied by strong food preferences and may contribute to the comorbid gastrointestinal symptoms and possibly also to the behavioral problems observed in children with ASD (McElhanon et al., 2014). However, reverse causation, with behavioral changes leading to microbiota perturbations, may also explain the associations between the gut microbiome and ASD. Interactions of probiotics and gluten-casein-free diets with gut microbiota may downregulate gastrointestinal inflammation and intestinal permeability (Doenya, 2018b).

### 3. GFCF diet in ASD

A systematic review of the available literature (Reissmann et al., 2020) identified 18 survey studies seeking to explore the popularity of complementary and alternative medicine (CAM) treatments among parents of children diagnosed with ASD. Parents reported the use of multiple CAM treatments, particularly dietary treatment forms. Dietary CAM treatments included vitamin or mineral supplementation and specific forms of diet (e.g. Feingold diet, GFCF diet, sugar-free diet), of which the GFCF diet was the most common (Green et al., 2006; Salomone et al., 2015; Smith and Antolovich, 2000). Higher levels of parental education, more severe ASD symptoms, comorbid disorders and younger age of children with ASD have been shown to be associated with the use of CAM treatments (Reissmann et al., 2020). The prevalence, reported by parents, of a previous use of one or more of various CAM treatments in their children with ASD was 54–81%, while the current



use of at least one CAM treatment was reported by 28–62% of parents (Reissmann et al., 2020). The current use of a GFCF diet was found in 8–32% and previous use in 20–55% of families. Up to 41–69% of parents reported positive effects of the GFCF diet on various aspects of their children's functioning (Carter et al., 2011b; Christon et al., 2010; Hanson et al., 2007; Hopf et al., 2016; Smith and Antolovich, 2000; Winburn et al., 2014). However, the core features of autistic symptoms may be other than those influenced most effectively by the GFCF diet. In a survey study from the United Kingdom, 20–29% of the parents reported a significant improvement in the ASD core dimensions (social interaction, communication, repetitive behaviors, restricted interests) (Winburn et al., 2014), 54% of parents reported significant improvements regarding gastrointestinal symptoms and 42% reported significant positive effects on attention and concentration in their children (Winburn et al., 2014). Significant effects of the GFCF diet on comorbid problems were also shown in another survey, which found more positive effects of a GFCF diet, as reported by parents, when their children presented with gastrointestinal symptoms or signs of allergy (Pennesi and Klein, 2012). These findings suggest that there may be a subgroup of children with ASD who could benefit from a GFCF diet. However, this should be further investigated and needs to be validated by clinical observations in addition to those of parents.

### 3.1. Intervention studies using the GFCF diet

First reports of the potential effectiveness of a GFCF diet in ASD were published in the 1990s. For example, a study comprising 15 individuals with autistic syndromes found a reduction in urinary peptides resulting from the metabolism of gluten and casein and an improvement in some behaviors associated with autism in follow-up assessments after one year (Knivsberg et al., 1990) and four years (Knivsberg et al., 1995). Similar behavioral results were reported in a follow-up study of a 5-month administration of a gluten-free diet in 22 children with autism and associated spectrum disorders, while no significant reductions in urinary peptide levels were found (Whiteley et al., 1999). However, major limitations of these studies included open methodology and a lack of randomization and blinding. Numerous investigations of variable scientific rigor have since been conducted to determine the effects of a GFCF diet on behavioral changes in children diagnosed with ASD. The effects of a GFCF diet on the symptoms ASD have been assessed in several interventional studies, some of which were reviewed in a Cochrane report (Millward et al., 2008). This report included only two small randomized controlled trials and reported mixed results regarding GFCF dietary effects. A more recent systematic review of the literature included dietary intervention trials and food challenge studies (Reissmann et al., 2020), with the classification of report strength and the judgment of quality indicators performed according to criteria proposed for the evaluation of intervention studies in ASD research (Reichow et al., 2008). The main findings of this systematic review will be presented briefly below.

Several case studies, including anecdotal case reports (Adams and Conn, 1997; Fields and Fields, 1976) and more scientific trials (Herbert and Buckley, 2013; Hsu et al., 2009; Knivsberg et al., 1999), have sought to establish a role of the GFCF diet in the relief of symptoms of autism. These studies found some evidence for positive effects of the GFCF diet for at least some of the measures assessed, such as symptoms of autism, cognitive skills and physical development. However, the findings of these reports should be considered as weak evidence at best, since none of them were conducted with adequate scientific rigor (see Reissmann et al., 2020).

The effects of GFCF dietary intervention were also examined in several group studies. Of six uncontrolled group trials assessing the elimination of both gluten and casein (Cade et al., 2000; Gemmell and Chambliss, 1997; Jyonouchi et al., 2002; Knivsberg et al., 1990; Knivsberg et al., 1995; Lucarelli et al., 1995; Pontino et al., 1998; Whiteley et al., 1999), all but one study (Gemmell and Chambliss, 1997; Pontino et al., 1998) found positive dietary effects on the core symptoms of ASD, cognitive deficits, gastrointestinal problems or comorbid symptoms. These trials lacked control procedures and had various other major methodological problems, resulting in only weak scientific evidence, which should be treated with caution (see Reissmann et al., 2020). In regard to five controlled group trials (Elder et al., 2006; Ghalichi et al., 2016; Johnson et al., 2011; Knivsberg et al., 2002; Knivsberg et al., 2003; Pedersen et al., 2014; Seung et al., 2007; Whiteley et al., 2010), the scientific rigor was adequate in all but one study (Knivsberg et al., 2002; Knivsberg et al., 2003). Two favorable evaluations of GFCF diet effects are at variance with the negative findings of two studies with diet adherence lasting six (Elder et al., 2006; Seung et al., 2007) to 12 weeks (Johnson et al., 2011). These negative results are supported by another small, randomized controlled study of the GFCF diet in young children with ASD (Johnson et al., 2011). A further GFCF diet study in children with ASD, conducted with adequate scientific rigor, failed to show positive effects on ASD symptoms, neurodevelopmental ratings or symptoms of attention and hyperactivity (Pedersen et al., 2014; Whiteley et al., 2010).

Another systematic review based on the findings of six randomized controlled trials updated the evidence of the therapeutic effectiveness of the GFCF diet in childhood ASD and concluded that there is little evidence supporting beneficial effects of the diet (Piwowarczyk et al., 2018). A recent systematic review and meta-analysis, based on six relevant randomized controlled trials investigating possible benefits of the GFCF diet in children diagnosed with ASD found no effects on clinician-reported autism core symptoms, parent-reported functional level or behavioral difficulties (Keller et al., 2021). The quality of evidence ranged from low to very low due to serious risk of bias, inconsistency and imprecision.

Taken together, the available dietary intervention studies have found highly divergent results and do not therefore allow for clear-cut conclusions regarding the effects of the GFCF diet in children with ASD (see Table 2). It is important to note that the majority of trials conducted without adequate scientific rigor found evidence of beneficial effects of the GFCF diet, while more rigorous scientific evaluations were unable to show a consistent pattern of results. Many trials are hampered by various methodological flaws, such as reliance on parental reports as the sole source of information on ASD symptoms as well as a lack of adequate control groups, rater blindness, control for additional treatments or assessment of treatment fidelity. Future studies on the efficacy of the GFCF diet would need to take these aspects into account. In particular, the ratings of ASD symptoms should be performed by uninvolved and blinded clinicians rather than parents.

Given that many positive findings following a GFCF diet in ASD have been reported by studies with longer rather than shorter follow-up periods (see Reissmann et al., 2020), longer follow-up periods involving multiple clinical assessments should be employed. Significant beneficial effects have been found in studies with a duration of 12 months, while studies with interventions lasting 12 weeks showed no significant effects (de Magistris et al., 2013; Elder et al., 2006; Gogou and Kolios, 2017; Gogou and Kolios, 2018; Hyman et al., 2016; Knivsberg et al., 2002; Whiteley et al., 2010). A prolonged administration may be required to reveal benefits of GFCF diets, since inflammation, dysbiosis and altered

**Table 2. Summary of findings of GFCF dietary intervention studies in children with ASD**

Highly divergent findings of GFCF diet intervention studies
Methodological flaws of many GFCF intervention studies: strong reliance on (unblinded) parental reports as the sole information source regarding ASD symptoms; frequent lack of control procedures (control groups, measurement baselines, control for additional treatments); frequent lack of assessment of treatment fidelity
Positive effects of GFCF diet adherence mainly found in studies conducted without adequate scientific rigor
No consistent pattern of findings following a GFCF diet in more rigorous scientific evaluations
More positive findings reported by case/group studies with longer follow-up periods
Summary: No clear-cut conclusions possible regarding GFCF diet effects in children with ASD

intestinal permeability found in many people with ASD may be normalized only following extended periods of dietary intervention.

### 3.2. Challenge studies with gluten and/or casein

On the basis of research on food allergies, several studies have examined the effects of challenges with gluten and/or casein in children adhering to some form of GFCF diet. In a case study, acute negative effects were reported in a child following the consumption of gluten-/casein-containing foods, particularly wheat products (O'Banion et al., 1978). An uncontrolled group study found that a challenge with casein and other food allergens in children with ASD on a casein-free diet was associated with an increase in behavioral symptoms, such as inappropriate emotional responses, motor disturbances as well as disturbed concentration and perception (Lucarelli et al., 1995). Other studies failed to show any clear effects of such dietary challenges (Bird et al., 1977; Hyman et al., 2016; Irvin, 2006; McCarthy and Coleman, 1979; Navarro et al., 2015). The most persuasive evidence stems from a small randomized double-blind placebo-controlled challenge study, in which children with ASD who had followed a GFCF diet regimen for two weeks before being randomly assigned to either a gluten/casein challenge or a placebo group (Navarro et al., 2015). The results of this study found no consistent effects of the gluten/casein challenge on gastrointestinal symptoms or behavioral problems, such as inattention, hyperactivity or inattention (Navarro et al., 2015). Taken together, the majority of the available challenge studies were unable to show clear effects of a gluten/casein challenge on ASD symptoms, gastrointestinal symptoms, comorbid behavior problems or cognitive functioning. However, the majority of challenge studies were conducted with inadequate scientific rigor, and the available evidence needs to be viewed with caution.

### 3.3. Potential harms of the GFCF diet

The available studies exploring potential harms of the GFCF diet in children with ASD have mainly assessed nutritional adequacy or physical development. Four studies have examined possible nutritional deficiencies of children with ASD on restriction diets in comparison with children with ASD on unrestricted diets or healthy control children (Arnold et al., 2003; Cornish, 2002; Hyman et al., 2012; Mari-Bauset et al., 2016). Three of these studies estimated nutritional adequacy from dietary records maintained by parents and found no evidence of more nutritional deficiencies following a GFCF diet than in the comparison groups (Cornish, 2002; Hyman et al., 2012; Mari-Bauset et al., 2016). The fourth study sought to estimate deficiencies from plasma-derived concentrations of

amino acids and found deficiencies in several essential amino acids, including the neurotransmitter precursors tyrosine and tryptophan (Arnold et al., 2003). Two studies of physical development following a GFCF diet examined bone development of children with ASD on a casein-free diet (with a low intake of calcium) with children on unrestricted diets (Hediger et al., 2008; Neumeyer et al., 2013). These studies reported that children with ASD generally displayed a decrease in bone density, with a significantly greater reduction in the children on a casein-free diet (Hediger et al., 2008; Neumeyer et al., 2013). These aspects require further consideration in future investigations of GFCF diets.

## 4. Future directions

The gluten-free diet is a well-established treatment for individuals with heightened sensitivity to gluten and gluten-related disorders, such as celiac disease, allergic reactions (wheat allergy), non-celiac gluten sensitivity and other immune-mediated disorders (e.g. gluten ataxia and dermatitis herpetiformis) (Sapone et al., 2012). However, the utility of the gluten-free diet in other conditions is not evidence-based (Jones, 2017).

Studies examining potential benefits of the GFCF diet in children with ASD need to address numerous methodological problems and use research designs with adequate control procedures (see Table 3). Other recommendations include an (extended) trial duration of at least 12 months and the use of a broader range of measures, which may provide insight into other relevant aspects, such as moderators and mediators of therapeutic effects as well as potential risks of the GFCF diet. Another important problem to be addressed is the reliance on parents as an information source. Parents as unblinded providers of treatment may be biased in their perception of dietary outcomes. Thus, future studies should implement clinician-administered rating procedures and use objective observational measures and standardized and ecologically valid assessment instruments. In order to identify potential responders to GFCF diets, a range of variables, including comorbid gastrointestinal symptoms (Díaz-Atienza et al., 2012), intestinal permeability (Boukthir et al., 2010) as well as gastrointestinal enzymatic and inflammatory activity (Wang et al., 2009), deserve further examination.

Future studies should include children with established disturbances in the metabolic breakdown of food proteins or those at risk for allergies to gluten or casein. A diet-related phenotype of ASD may be associated with biological aberrations linked to abnormal gut-brain axis functioning (Whiteley, 2015). The identification of such a diet-related ASD phenotype may help in selecting those children who might benefit from a GFCF dietary intervention.

The findings of a recent study suggest that an intervention including a wider variety of dietary elements may be effective in the

**Table 3. Methodological recommendations regarding GFCF studies in ASD (see also Reissmann et al., 2020)**

Randomized controlled trial with adequate control condition and control for attention effects (e.g. nutritionist counseling) in control group
Assessment of additional therapies
Sufficient trial duration (>12 months) with multiple follow-up assessments
Ensuring and maintaining treatment fidelity (diet adherence)
Blinded clinician ratings in addition to parent ratings of behavior
Multi-method/multi-rater assessment using standardized assessment tools: clinical measures, rating scales for parents and teachers, neuropsychological and cognitive tests, ecologically valid behavioral observations in natural settings
Sufficiently large sample size (>30) with control of attrition
Risk measurement regarding nutritional status, physical development, bone density etc
Assessment of potential moderating and mediating variables, such as food allergies, gastrointestinal symptoms and behavioral symptoms (e.g. attention, hyperactivity)

treatment of childhood ASD (Adams et al., 2018). The randomized, controlled, single-blind 12-month trial of a comprehensive dietary and nutritional intervention in 67 children and adults with ASD aged 3–58 years and 50 non-sibling neurotypical controls examined the effects of a special vitamin/mineral supplement and further treatments added sequentially, including essential fatty acids, Epsom salt baths, carnitine, digestive enzyme and a GFCF soy-free diet (Adams et al., 2018). In comparison with the non-treatment group, the treated group showed significant improvements in nonverbal intellectual ability as well as ASD symptoms and developmental age. According to parental reports, vitamin/mineral supplements, essential fatty acids and the gluten/casein/soy-free diet were the most beneficial (Adams et al., 2018). Furthermore, FODMAP food components (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) have increasingly been investigated due to their possible relation with extraintestinal medical conditions. In particular, diets low in FODMAPs might have beneficial effects on various mental conditions, including ASD (Aranburu et al., 2021). However, this requires further research.

## 5. Conclusion

Despite the popularity and widespread use of the GFCF diet as a supplementary treatment in children with ASD, several rigorous scientific evaluations have failed to confirm the effectiveness of this approach. While the available dietary studies do not confirm the conceptualization of ASD as a metabolic disorder or the predictions of the opioid excess hypothesis, the increasing evidence for associations between gut anomalies and the brain in children with ASD points to a role of immunological factors in the frequently reported gastrointestinal problems. Therefore, the consideration of dietary factors, including gluten and casein, should not be disregarded prematurely. Food-derived proteins could play a role in triggering allergic responses, which may influence brain development and neuronal functions. More scientifically rigorous and methodologically sound investigations are required to examine the importance of dietary factors in the etiology and treatment of ASD.

In summary, the available evidence in support of the effectiveness of the GFCF diet in the treatment of childhood ASD is very weak and cannot currently even be considered promising. Potentially ineffective dietary therapies may imply considerable opportunity costs, with other possibly more effective treatment approaches remaining unutilized.

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