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Review

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Efficacy of Gluten Free, Casein-Free (GFCF) Diet in the Treatment of Autism Spectrum Disorder – A Mini Review

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Abstract

Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects social interaction, communication, and behaviour. The underlying causes may vary among individuals, and one possible cause is genetic mutation. The opioid theory suggests that people with autism have an impaired ability to break down certain proteins, including gluten and casein, leading to the production of opioid peptides that affect brain function. This theory has inspired a therapeutic approach in which consumption of a gluten-free, casein-free (GFCF) diet may alleviate ASD-associated symptoms. The aim of this study was to review the current evidence on the GFCF diet, and its outcomes based on recent clinical trials. **Materials and Methods:** A comprehensive search was conducted using PubMed and Google Scholar databases to identify relevant articles. The search was limited to studies published between 2010 and 2022, using the following keywords to identify potentially relevant articles: "autism spectrum disorder" AND "autism" AND "gluten-free casein-free diet." From this search, 10 articles were selected for further review based on the inclusion criteria. **Results:** The results indicate that the GFCF diet was found to be effective in only 30% of the studies, while 70% of the outcomes showed no significant effect. **Conclusion:** Research on the effectiveness of the GFCF diet in treating autism remains limited, and the outcomes are subjective, with some studies showing no significant improvement in symptoms. While the opioid theory remains controversial, further research is needed to fully understand the relationship between autism, the GFCF diet, and the opioid theory.

Keywords: Autism, Autism Spectrum Disorder (ASD), gluten free diet, casein free diet, clinical trial, opioid theory

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INTRODUCTION

Autism spectrum disorder (ASD) is a neurological and developmental condition that impacts how people interact with others, express themselves, learn, and behave. Although autism can be detected at any age, it is classified as a "developmental disorder" since symptoms often arise between the ages of two and three years.[1] People with ASD have a wide range of abilities. Some individuals with ASD, for example, may have excellent speech abilities, while others may be nonverbal. Some people with ASD require significant assistance in their daily lives, while others can live and work with minimal to no support.[2]

In the Diagnostic and Statistical Manual of Mental Disorders—5th edition (DSM-5), the concept of a 'spectrum' ASD diagnosis was established by consolidating the discrete pervasive developmental disorder (PDD) diagnoses from DSM-IV, which encompassed autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder. Rett syndrome is no longer classified as an ASD in the DSM-5 since it is considered a distinct neurological condition. A specific social pragmatic communication disorder (SPCD) was defined for persons with social communication impairments but no repetitive, restrictive behaviours.[3]

According to the WHO, around 1 in 100 (1.0%) children globally are estimated to have ASD.[4] Centre for Disease Control and Prevention (CDC) reported that, 1 in 59 US children below the age of 8 (or approximately 1.68%) have ASD.[5] East Asia had a prevalence of 0.51% (95% CI: 0.06-4.22%), South Asia had a prevalence of 0.31% (95% CI: 0.14-0.65%), and West Asia had a prevalence of 0.35% (95% CI: 0.07-1.80%).[6] There is no official statistics available in Malaysia for the number of autistic children.[7] This is due to the fact that autism is classified as a learning disability alongside other cognitive and developmental impairments.[8]

Due to their unique characteristics, ASD individuals may need intense care, which reduces parents' productivity. Challenges in communication, behavioural variability, sensory sensitivities, difficulty with routine changes, social and emotional struggles, safety concerns, and the need for specialised education and therapies contribute to the demanding caregiving responsibilities. Parents frequently find themselves navigating a complex landscape, dedicating considerable time and effort to address the diverse needs of their child with ASD, which can limit their capacity for productivity in other aspects of life. Investigations to explore strategies that may alleviate ASD symptoms may be beneficial for them and their caretaker.[9]

Nutritional intervention for ASD has garnered increased attention due to the recognition of the gut-brain connection and the potential impact of the gut microbiome on neurological function. This interest is fuelled by the observation of nutrient deficiencies, such as vitamin D and omega-3 fatty acids, in some individuals with ASD, prompting efforts to address these imbalances through dietary changes and supplements. Additionally, the identification and elimination of food sensitivities or allergies, as well as the pursuit of anti-inflammatory and antioxidant properties in certain diets and nutritional supplements, contribute to the rationale behind these interventions.[10]

Various nutritional interventions and diets have been explored for children with ASD beyond the GFCF approach. These include the ketogenic diet, which promotes a high-fat, low-carbohydrate intake; the low oxalate diet, targeting potential issues related to oxalates and inflammation; the specific carbohydrate diet (SCD), which restricts certain carbohydrates to address gut health; the Feingold Diet, eliminating artificial additives and salicylates; and the Mediterranean diet, known for its overall health benefits. Additionally, supplementation with vitamin D, omega-3 fatty acids, and probiotics has been investigated for potential benefits. Each intervention aims to address specific aspects, reflecting the diverse nature of ASD.[11,12]

The gluten- and casein-free (GFCF) diet receives special attention in the context of ASD due to the observed higher prevalence of gastrointestinal (GI) symptoms in some individuals with ASD. The interplay between ASD and GI issues is intricate, and while not universal, addressing these symptoms is crucial for the

well-being of affected individuals. In addition to constipation, diarrhoea, abdominal pain, and gastroesophageal reflux disease, food sensitivities and allergies can contribute to discomfort. The potential link between ASD and GI symptoms has led to increased exploration of dietary interventions, such as the GFCF diet. The hypothesis driving this diet suggests that removing gluten and casein, proteins found in certain grains and dairy products, may alleviate GI symptoms and improve behavioural issues in individuals with ASD. However, scientific evidence supporting the diet's efficacy is inconclusive, with mixed results in studies.[13]

Immunological and inflammatory responses in the gut are complex and can be associated with conditions like increased intestinal permeability (leaky gut) and alterations in gut microbiota, which have been explored in the context of ASD. Including these aspects in the discussion would provide a more comprehensive overview of the potential mechanisms involved in the interplay between ASD and GI symptoms.[14]

This review assessed the relationship between ASD, GI symptoms, and nutrition. This paper focuses on GFCF diet therapy in autism and present our findings in a narrative summary.

LITERATURE SEARCH

The literature search was done using the database PubMed and Google Scholar from 2010 to 2022 with the following key terms: “autism spectrum disorder”; “autism” and “gluten-free casein-free diet”. The reference list and review were crossed search to get potential study journals. Only English-language materials were selected. Journals, reviews, and sources utilising experimental and observational study designs were included.

RESULTS

Gluten-free casein-free (GFCF) diet

GFCF diet is an elimination diet in which the proteins gluten and casein are removed from the regular diet.[15] Many studies have suggested that this particular diet contributes to a better course of the disease in people with ASD. The specific ways in which the diet may exert positive effects on the course of ASD vary and can include improvements in behaviour, communication skills, GI symptoms, and overall well-being, although the mechanisms are not yet fully understood and require further research for conclusive evidence. It was initially suggested in the 1980s that avoiding gluten and casein can cause autism-like symptoms by interference of brain functions.[16]

Research on children with ASD showed that casein, a type of protein that typically causes immune sensitivity in children to be among the proteins most frequently related to an immunological response. Eliminating casein from the diet is theorised to alleviate potential immune-related issues and, in turn, improve behavioural symptoms, communication skills, and overall well-being in some individuals with ASD.[17] Research done in 2005 found that children with ASD who followed the GFCF diet produced less tumour necrosis factor-α (TNF-α) than those who did not.[18] TNF-α is a cytokine, a type of signalling protein involved in the regulation of inflammation and immune responses in the body. The suggests a potential link between dietary interventions and immune system modulation.[18] Elevated levels of TNF-α have been associated with inflammatory processes and immune system dysregulation. The significance of reduced TNF-α production in children with ASD following the GFCF diet may imply a positive impact on the inflammatory and immune responses. However, the most widely accepted explanation for GFCF diet adoption is the relationship between ASD behavioural abnormalities and an excess of opioid receptor agonists.[19]

Opioid theory

The opioid theory in the context of autism is a hypothesis that suggests dysfunction in the endogenous opioid system may contribute to certain behaviours and characteristics observed in individuals with ASD. The underlying mechanism of the above-mentioned theory is inadequate hydrolysis of dietary proteins, in addition to abnormally high intestinal permeability and peptide absorption in autistic individuals. As a result, gluten and casein may create 'opioid-like' peptides that enter the bloodstream and penetrate the brain-blood barrier. These circulating peptides may cause a systemic inflammatory response before impacting the central nervous system.[20]

Effectiveness of GFCF diet in ASD

A study of 25 patients with ASD taking GFCF diet conducted by Knivsberg *et al.* in the 90s provided the first information on the benefits of GFCF in ASD. The researchers observed notable improvements in autistic behaviours, especially in the initial year of management, along with a decline in epilepsy episodes and urine peptide values brought on by the breakdown of gluten and casein.[21] Since then, interest in this area

has increased as more researchers published similar positive findings. However, the studies had significant methodological shortcomings such as an open design that is not randomised and small sample size.

In 2010, Knivsberg and Whiteley collaborated on the ScanBrit research, a significant study marked by its rigorous scientific methodology. This single-blind, randomised experiment featured a two-year follow-up period and boasted a larger sample size, encompassing a total of 72 participants. Over the 12 months of follow-up, the ScanBrit study discovered a substantial impact of the GFCF diet on cognitive function and behavioural difficulties, however, the effect stopped increasing after a year. Children between the ages of 7 and 9 were among the ideal age range to succeed with a GFCF diet since the age range of respondents has been found to be a successful predictor.[22] The age range of 7 to 9 years has been identified as an ideal period for the successful implementation of a GFCF diet for children with ASD. This success can be attributed to the fact that children within this age range are more likely to have a higher compliance with dietary interventions, as they are generally more open to trying new foods and are less likely to exhibit selective eating behaviours compared to younger children. Additionally, children in this age group may have a better ability to communicate any discomfort or adverse effects related to dietary changes, which can aid in the successful implementation of the GFCF diet. Furthermore, the study by Elder *et al*. (2015) noted that the effect of the GFCF diet on cognitive function and behavioural difficulties ceased to increase after a year.[23] Hence, the Elder study was stopped after a year.

The research by Elder *et al.* (2015) does however, have certain drawbacks, including an absence of a placebo and limited observation of concurrent therapies, which point to a limitation of scientific reliability.[23] Other research has indicated positive outcomes with the GFCF diet in the context of ASD, showing improvements in behaviour and a reduction in both gastrointestinal symptoms and behavioural disorders.[24,25] However, these studies showed limitations in terms of their small sample size and heterogeneity as well as a short duration of study as summarised in **Table I**.[18.19,20]

GFCF diet's ineffectiveness in ASD treatment: non-significant findings

While the benefits of a GFCF diet for individuals with ASD have been consistently reported, our investigation has also brought to light current studies that present less favourable outcomes and unremarkable improvement findings. These contrasting results are succinctly summarised in **Table II**. In 2010, Johnson and colleague performed a single clinical study and found that the GFCF group did not significantly enhance maladaptive behaviours or developmental outcomes. The ScanBrit study, conducted by Whiteley *et al*. in 2013, had a sample size of 78 children with ASD. The follow-up duration for this study was not explicitly mentioned in the provided references. However, their sample size (n=22) and follow-up duration (12 weeks) were significantly smaller and shorter than the ScanBrit study.[26]

Two double-blind placebo-controlled design study was done in 2015. The study conducted by Pusponegoro *et al.*[27] was a greater sample number, however, has a shorter follow-up time frame. The study did not find any significant improvement in gastrointestinal and behavioural symptoms.[27] Another study conducted by Navarro *et al*. [28] indicated that there was no significant difference between the behaviours of children on GFD

Table I: Studies with positive outcomes concerning the effectiveness of the GFCF diet in ASD .

Authors	Country	Sample Characteristics	Study Design	Follow-up Period	Outcomes
Whiteley et al., (2010)[20]	UK, Norway, Denmark	72 ASD 4 to 10 years	Single-blind, randomized	24 months	A significant benefit in behavioural problems and cognitive functioning for 12 months the effect stop after one year.
Ghalichi et al., (2016)[19]	Iran	76 ASD 4 to 16 years old	Randomized, single-blind	6 weeks	Showed a significant decrease in gastrointestinal symptoms and behavioural disorder.
El-Rashidy et al., (2017)[18]	Egypt	45 ASD 3 to 8 years old	Case-control	6 months	Significantly improve autistic manifestations.

GFCF- Gluten-Free and Casein-Free; ASD - Autism spectrum disorder

Table II: Studies with non-significant outcomes concerning the effectiveness of the GFCF diet in ASD .

Authors/year (Ref)	Country	Sample Characteristics	Study Design	Follow-up Period	Outcomes
Johnson et al., 2010 (10)	USA	22 ASD 3 to 5 years old	Single-blind, randomised	3 months	No significant benefit on behavioural symptoms or developmental improvement
Pusponegoro et al., 2015 (21)	Indonesia	74 ASD 4 to 6 years old	Randomised, double-blind, placebo-control	1 week	No significant change in gastrointestinal and behavioural symptoms
Navarro et al., 2015 (22)	USA	12 ASD 4 to 7 years old	Randomised, double-blind, placebo-controlled	4 weeks	No significant difference in behavioural scores between the intervention group and the placebo group
Hyman et al., 2016 (23)	USA	14 ASD 3 to 5 years old	Double-blind, placebo-controlled challenge	30 weeks	No significant effects on physiologic and behavioural problems or autism symptoms
Piwowarczyk et al., 2019 (24)	Poland	58 ASD 3 to 6 years old	Single-blind, randomised, controlled	6 months	No significant benefit in managing ASD symptoms
González-Domenech et al., 2019 (26)	Spain	28 ASD 3 to 16 years old	Crossover, randomised, Single-blind	6 months	No significant changes in behavioural symptoms
González-Domenech et al., 2020 (25)	Spain	37 ASD 2 to 18 years old	Crossover, randomised, Single-blind	12 months	No significant behavioural changes

GFCF- Gluten-Free and Casein-Free; ASD - Autism spectrum disorder

and the control group. This research features a lower subject number (n=12) and follow up period of four weeks. [28]

In 2016, Hyman *et al*. conducted placebo-controlled research and discovered no appreciable impact of GFCF diet on physiological and behavioural issues or characteristics of autism. This research had several advantages notwithstanding its small number of participants (n=14), including the research design, the exclusion of any pharmaceutical therapy during the investigation, and for the prior four months.[29] The study conducted by Hyman *et al*. in 2016 had several advantages despite its small number of participants (n=14) and a short follow-up duration of four months. Firstly, the research design was a placebo-controlled study, which is considered a robust design for evaluating the effectiveness of interventions. This design allows for the comparison of the intervention (GFCF diet in this case) with a placebo, enabling researchers to assess the true impact of the intervention on the outcomes of interest. Additionally, the exclusion of any pharmaceutical therapy during the investigation is advantageous as it eliminates potential confounding effects of concurrent medications on the observed outcomes. This ensures that any changes in physiological and behavioural issues can be more confidently attributed to the GFCF diet rather than being influenced by other treatments. Furthermore, the duration of the study, although relatively short, provides valuable insights into the short-term effects of the GFCF diet, which can be important for understanding its immediate impact on individuals with ASD.[30] In 2019, a study conducted by Piwowarczyk *et al*.[31] revealed no difference in maladaptive behaviours, intellectual abilities and autistic behaviour between the children who were on gluten free diet and gluten diet.

A noteworthy feature was how each participant served as both a treatment and a control. The patient enrolment for this study is challenging, this approach lowered inter-subject heterogeneity and enables researchers to conduct studies with lower sample numbers which would be useful.[32,33] A lower sample size is advantageous in research due to increased cost-efficiency, time savings, and enhanced feasibility, especially in cases of logistical challenges or budget constraints. Smaller samples align with ethical considerations and can suffice for focused investigations, offering meaningful insights without sacrificing efficiency. Researchers must carefully balance these advantages with considerations of generalisability and statistical power based on their specific study requirements. Trials that have shown no conclusive results are all double-blind. The main limitations for conducting studies to associate GFCF diet in children with ASD are the limited sample size and relatively short time of follow-up, which are both brought on by the massive costs and methodological challenges.[34]

CONCLUSION

Currently, scientific and clinical evidence are still insufficient to offer GFCF diet as a therapy to all ASD patients. Therefore, further studies consisting of randomised, double blinded trials with placebo and extended follow up periods are necessary.

Take Home Message

- GFCF diet shows mixed results: 30% of studies showed benefit, while 70% showed no significant improvement in ASD symptoms.
- The opioid theory remains controversial: although introduced as a foundational hypothesis, the opioid-excess theory remains controversial and lacks conclusive proof.
- Clinical trials often have limitations: many studies suffer from small sample sizes, short follow-up periods, and lack placebo controls, which reduces scientific reliability.
- Some subgroups may benefit more: the ScanBrit study and others found that children aged 7–9 responded more positively, and those with GI symptoms were also more likely to benefit.
- More high-quality research is needed specifically large, placebo-controlled, double-blind trials.

Abbreviations

ASD	Autism spectrum disorder
PDD	Pervasive developmental disorder
SPCD	Social pragmatic communication disorder
CDC	Centre for Disease Control
GFCF	Gluten-free, casein-free
GI	Gastrointestinal
TNF-α	Tumour necrosis factor-α

Declarations

Conflict of interests

The authors declare no conflict of interests.

Ethical consideration

Not required for review article.

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