Effect of Gluten Free Casein Free Diet on Maladaptive Behavior in Autistic Children: Meta Analysis

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ABSTRACT

Background: Children with Autism tend to experience gastrointestinal disorders more often. Interactions between the gut and brain microbiome influence mood and behavior in health and disease. Diet therapy, specifically the gluten and casein-free diet (GFCF) is an elimination diet that involves removing certain proteins from the normal diet, such as gluten and casein. The right intervention can change behavior for the better and through the right handling. This study aims to estimate the effect of a casein-free gluten-free diet on maladaptive behavior in autistic children, through a meta-analysis of primary studies conducted by previous authors.

Subjects and Method: This study is a systematic review and meta-analysis with the following PICO, population: Autism children, intervention: gluten-free casein-free diet, comparison: no casein-free gluten-free diet, outcome: maladaptive behavior. The articles used in this study were obtained from three databases, namely PubMed, Google Scholar, and Science Direct, using the search key "Diet Gluten Free Casein Free" OR "Non-Diet Gluten Free Casein Free" OR "GFCF" AND "Autism" OR "Autism" AND "Maladaptive" AND "Randomize Controlled Trial" OR "RCT". The included article is a full-text Randomized Controlled Trial study design from 2002 to 2022 and reports the mean and SD in multivariate analysis. The selection of articles is done using PRISMA flow diagrams. Articles were analyzed using the Review Manager 5.4 application.

Results: A total of 7 randomized controlled trial studies conducted in the Americas, Europe, and Asia were selected for meta-analysis. Autistic children who received casein free gluten free had an average score of -0.11 units of maladaptive behavior lower than those who were not given a diet (SMD= -0.11 95% CI= -.035 to 0.13; p= 0.360).

Conclusion: The casein free gluten free diet has an effect on reducing the risk of maladaptive behavior in children with autism.

Keywords: casein free gluten free diet, gfcf, maladaptive behavior, autism

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BACKGROUND

Autism is a complex developmental disorder including delays and difficulties in social interaction, language, and cognitive, emotional, motor, and sensory abilities (Green & Wieder, 2006 in Suprajitno, 2017). Autism Spectrum Disorder (ASD) is one of the most common neurodevelopmental disorders worldwide, affecting around 1 in 54 children, with a male to female ratio of 4:1 (Maenner et al., 2020). Studies over the last decade have shown that children with ASD tend to

have more frequent gastrointestinal disorders. Interactions between the gut and brain microbiomes influence mood and behavior in health and disease (Madra et al., 2020). Autism-associated gene mutations known to alter synapse function in the CNS (central nervous system) also influence inflammatory responses and modify the enteric nervous system resulting in abnormal gastrointestinal motility and structure.

Autistic children suffer from general GI symptoms 4.4 times more often than children with other neurological disorders (McElhanon et al., 2014). GI (gastrointensial) symptoms tend to be strongly associated with autism severity and with increased irritability, anxiety, and social withdrawal (Gorrindo et al., 2012).

The results of research conducted by O'Banion D., et al (1978) in Millward (2008), show that foods such as wheat, corn, tomatoes, sugar, mushrooms and dairy products play an important role in producing behavioral problems in these children. Movement of microbes across the brain-gut is known to be responsible for many pathophysiological disorders of the brain such as autism, as well as other gastrointestinal diseases, including intestinal disorders.

Imbalances in gut microbial composition, frequently observed in individuals with ASD. Therefore this imbalance is believed to be the cause of gastrointestinal symptoms. The function of the digestive tract and blood-brain barrier (BBB) in children with ASD is affected. Peptides, toxins and pro-inflammatory cytokines that are not digested properly then enter the blood circulation and are carried up to the central nervous system so that they can affect brain function.

The resulting incompletely digested peptides act as opioid agonists reducing pain sensitivity and increasing the severity of

autism-specific behaviors. However, it is not known exactly how opioid peptides trigger ASD symptoms once they reach the brain (Gorrindo, et al 2012).

Diet therapy, specifically the elimination diet, is considered an alternative treatment for preventing this condition. The gluten-free casein-free diet (GFCF) is an elimination diet that involves eliminating certain proteins from the normal diet, such as gluten and casein (Baspinar et al., 2020).

Several studies have shown that behavior improves by eliminating nutrients, and that behavior worsens when exposed to the same nutrients again (Evangeliou, 2003).

Appropriate intervention can change behavior for the better and through appropriate, early, intensive and optimal treatment, people with autism can carry out activities like children in general so that later they can develop and be independent in society. However, the possibility of improving behavior depends on the severity of the existing disorder (Hasdianah, 2013).

This study aims to estimate the effect of a casein-free gluten-free diet on maladaptive behavior in autistic children, through a meta-analysis of primary studies conducted by previous authors.

SUBJECTS AND METHOD

1. Study Design

This study was conducted using a systematic review and meta-analysis. Search for articles using 3 databases, namely: Pubmed, Google Scholar and Science Direct which were published in 2010-2022. This is done using the following keywords: "Diet Gluten Free Casein Free" OR "Non Diet Gluten Free Casein Free" OR "GFCF" AND "Autism" OR "Autism" AND "Maladaptive" AND "Randomize Controlled Trial" OR " RCTs".

2. Steps of Meta-Analysis

Meta analysis was carried out in 5 steps as follows:

1) Formulate research questions in the PICO format (Population, Intervention, Comparison, Outcome).
2) Search for primary study articles from various electronic databases including Pubmed, Google Scholar and Science Direct.
3) Conduct screening and critical assessment (Critical Appraisal) and primary research articles.
4) Extracting data and synthesizing effects into Revman 5.4.
5) Interpret and conclude results.

3. Inclusion Criteria

Inclusion criteria in this study included articles with a cross-sectional study design, full text available articles, results of the analysis used a randomized controlled trial (RCT), the year of the article used the range of 2010-2022 and the research outcome was maladaptive behavior.

4. Exclusion Criteria

Exclusion criteria in this study included not full text, articles published before 2002 and the results of the analysis were not using RCTs.

5. Operational Definition of Variables

The Gluten Free Casein Free Diet is a diet that is carried out by eliminating/-eliminating food or drink sources that contain casein and gluten.

Maladaptive behavior is the behavior of children who are unable to adjust or adapt to their surroundings naturally, and are unable to adapt according to their age developmental stages.

6. Study Instruments

Article selection was carried out using the PRISMA flow diagram and critical assessment from a randomized controlled trial study sourced from the Center for Evidence Based Management (CEBMa) (2014).

7. Data Analysis

The articles in this study were collected according to the PRISMA flowchart and analyzed using the Review Manager 5.4 application. The analysis was carried out by calculating the effect size and heterogeneity consistency value (I^2) of the selected research results.

RESULTS

Search for articles in this study through databases that include PubMed, Google Scholar, and Science Direct. The process of reviewing related articles can be seen in the PRISMA flow diagram in Figure 1. In the initial search process it yielded 1,630 articles, after the process of deleting published articles, 1,107 articles were found with 660 of them meeting the requirements for further full-text review. A total of 7 articles that met the quality assessment were included in the quantitative synthesis using meta-analysis. The selected research articles came from three continents, namely 2 articles from the Americas, 1 article from the Asian continent and 4 articles from the European continent, can be seen in Figure 2.

Table 1, researchers conducted an assessment of the quality of the study. The seven articles that have been obtained from several databases need to be assessed to determine the quality of the articles before being used as material for meta-analysis. This research was conducted using a checklist for critical assessment from a randomized controlled trial study sourced from the Center for evidence-based management (CEBMa) (2014). This assessment criteria consists of 12 question criteria with each given a score of 1 = if the answer is yes, and 0 = if no answer, then added up. The results of the summation of the scores that have been carried out, it was found that 1 article received a total score of 12 answers, namely Elder et al. (2006). In addition, there are 3 articles with a total score of 10, namely Anna et al. (2019), Whiteley et al. (2010) and Knivsberg et al. (2003), two articles with a total score of 11 namely Johnson et al. (2011)

and Nani et al. (2017), as well as one article with a score of 9, namely Gonzales et al. (2019).

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┌─────────────────────────────────────┐
│ Articles identified through database │
│        search (n=1,630)              │
└─────────────────────────────────────┘
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│ Articles after duplicates were removed│
│            (n =523)                   │
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┌─────────────────────────────────────┐        ┌──────────────────────────────────┐
│    Filtered articles (n=1,107)       │──────▶ │ Issued articles (n= 445)         │
└─────────────────────────────────────┘        │ Irrelevant title = 386           │
                  │                             │ Not full-text= 35                │
                  │                             │ Not RCT = 18                     │
                  │                             │ No English = 6                   │
                  ▼                             └──────────────────────────────────┘
┌─────────────────────────────────────┐        ┌──────────────────────────────────┐
│ Full Text articles that are considered│──────▶│ Complete articles issued with reasons│
│         eligible(n=660)              │        │ (n= 647)                         │
└─────────────────────────────────────┘        │ Article does not include Mean= 473│
                  │                             │ Gluten casein free intervention = 73│
                  ▼                             │ Maladaptive behavior outcome = 90 │
┌─────────────────────────────────────┐        └──────────────────────────────────┘
│ Articles included in the meta-analysis│
│          synthesis (n= 7)            │
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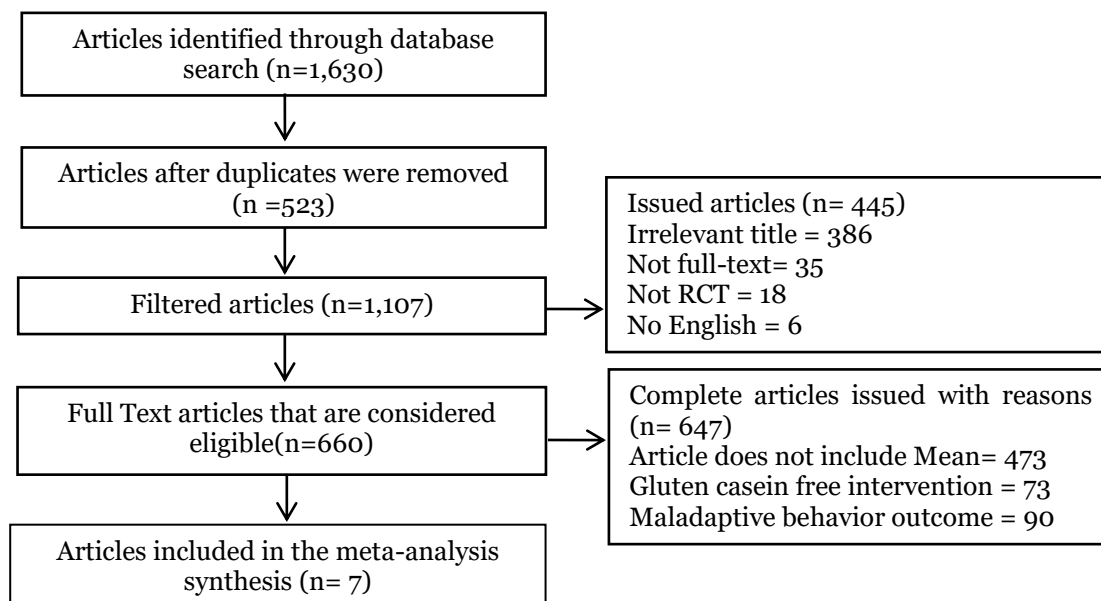
Figure 1. PRISMA flowchart diagram of the effect of a gluten-free, casein-free diet on maladaptive behavior in autistic children

Figure 2. Map of the research area on the effect of a gluten free diet casein free on autistic children's maladaptive behavior

Table 1. Critical appraisal checklist for randomized control trial (RCT) studies in meta-analysis

Author (Year)	1	2	3	4	5	6	7	8	9	10	11	12	Total
Anna et al. (2019)	1	1	1	1	1	0	1	1	1	1	0	1	10
Elder et al. (2006)	1	1	1	1	1	0	1	1	1	1	1	1	12
Johnson et al. (2011)	1	1	1	1	1	0	1	1	1	1	1	1	11
Gonzales et al. (2019)	1	1	1	0	1	0	1	1	1	1	0	1	9
Knivsberg et al. (2003)	1	1	1	1	1	0	1	1	1	1	0	1	10
Nazni et al. (2008)	1	1	1	1	1	0	1	1	1	1	1	1	11
Whiteley et al. (2010)	1	1	1	1	1	0	1	1	1	1	0	1	10

(Note: Header row shows "Criteria of Questions" spanning columns 1–12.)

Description of the question criteria:

1 = Does the research address a clear research focus?
2 = Is the Randomized Controlled Trial research method suitable for answering research questions?
3 = Are there enough subjects in the study to establish that the findings did not occur by chance?
4 = Were subjects randomly allocated to the experimental and control groups? If not, could this introduce bias?
5 = Were inclusion/exclusion criteria used?
6 = Were the two groups comparable at study entry?
7 = Were objective, unbiased outcome criteria used?
8 = Are objective and validated measurement methods used to measure the results? If not, were the results assessed by someone who was not aware of the group assignment (i.e. was the assessment blinded)?
9 = Is the effect size practically relevant?
10 = How precise is the estimated effect? Are there confidence intervals?
11 = Could there be confounding factors that have not been taken into account?
12 = Can the results be applied to your research?

Description of the answer score:

0 = No
1 = Yes

Table 2. Summary of primary study articles included in the meta-analysis

Author (Year)	Country	Sample	P	I	C	O
Nazni et al. (2008)	India	C=50 I=30	Autism 3-11 years	2 month GFCF diet	Regular Diets	Maladaptive Behavior
Anna et al. (2019)	Polandia	C=33 I=33	Autism 36-69 months	6 month GFCF diet	Regular Diets	Maladaptive Behavior
Gonzales et al. (2012)	Spanyol	C=15 I=16	Autism 3-15 years	2 month GFCF diet	Regular Diets	Maladaptive Behavior
Knivsberg et al. (2003)	Norwegia	C=10 I=10	Autism 3-18 years	12 month GFCF diet	Regular Diets	Maladaptive Behavior
Whitely et al. (2014)	Denmark	C=29 I=26	Autism 4-10 years	12 month GFCF diet	Regular Diets	Maladaptive Behavior
Elder et al. (2018)	USA	C=7 I=7	Autism 2-16 years	6 week GFCF diet	Regular Diets	Maladaptive Behavior
Johnson et al. (2014)	USA	C=8 I-14	Autism 3-5 years	3 month GFCF diet	Healthy diet without sugar	Maladaptive Behavior

Table 2 shows that 7 articles from primary studies as evidence of the linkage of the effect of a gluten-free, casein-free diet on maladaptive behavior in autistic children. The primary studies that met the criteria totaled 7 randomized control trial research articles, with 2 articles from the Americas (Texas, Florida), 4 articles from the European continent (Poland, Spain, Norway, Denmark) and 1 article from India so that a total sample of 288 were involved. with an age range of 36 months - 18 years.

Table 3. Data on standard mean deviation (SMD) and 95% confidence interval (95% CI) on the effect of a gluten-free, casein-free diet on maladaptive behavior in autistic children

Author	SMD	CI 95%	
		Lower Limit	Upper Limit
Nazni et al. (2008)	-0.26	-0.71	0.19
Anna et al. (2019)	0.14	-0.34	0.63
Gonzales et al. (2012)	-0.12	-0.82	0.59
Knivsberg et al. (2003)	-1.37	-2.36	-0.37
Whitely et al. (2010)	-0.01	-0.54	0.52
Elder et al. (2018)	0.26	-0.79	1.31
Johnson et al. (2014)	0.06	-0.81	0.93

Table 3 displays the Std.Mean different for each selected article. It is necessary to pay attention to the collection of articles by selecting articles that have controlled for confounding factors, which can be seen from the study inclusion requirements, namely the adjusted odds ratio (aOR). According to Murti (2018), confounding factor is mixing the estimation of the relationship between exposure and the disease being studied, by other factors that are related, both to disease and exposure.

1. Forest Plot

Study or Subgroup	GFCF Mean	SD	Total	non GFCF Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Anna et al 2019	18.5	6.11	33	17.57	6.57	33	23.9%	0.14 [-0.34, 0.63]
Elder et al 2006	33.6	8.6	7	31.2	8.7	7	5.0%	0.26 [-0.79, 1.31]
Gonzales et al 2019	59.9	20.9	15	62.9	28.9	16	11.2%	-0.12 [-0.82, 0.59]
Johnson et al 2011	40.1	9.3	14	39.5	8.7	8	7.4%	0.06 [-0.81, 0.93]
Knivsberg et al 2003	5.6	2.4	10	11.2	5	10	5.6%	-1.37 [-2.36, -0.37]
Nazni et al 2008	1.34	0.43	50	1.46	0.5	30	27.0%	-0.26 [-0.71, 0.19]
Whiteley et al 2010	0.0168	0.5221	26	0.0252	0.6572	29	19.9%	-0.01 [-0.54, 0.52]
Total (95% CI)			155			133	100.0%	-0.11 [-0.35, 0.13]

Heterogeneity: Chi² = 8.36, df = 6 (P = 0.21); I² = 28%
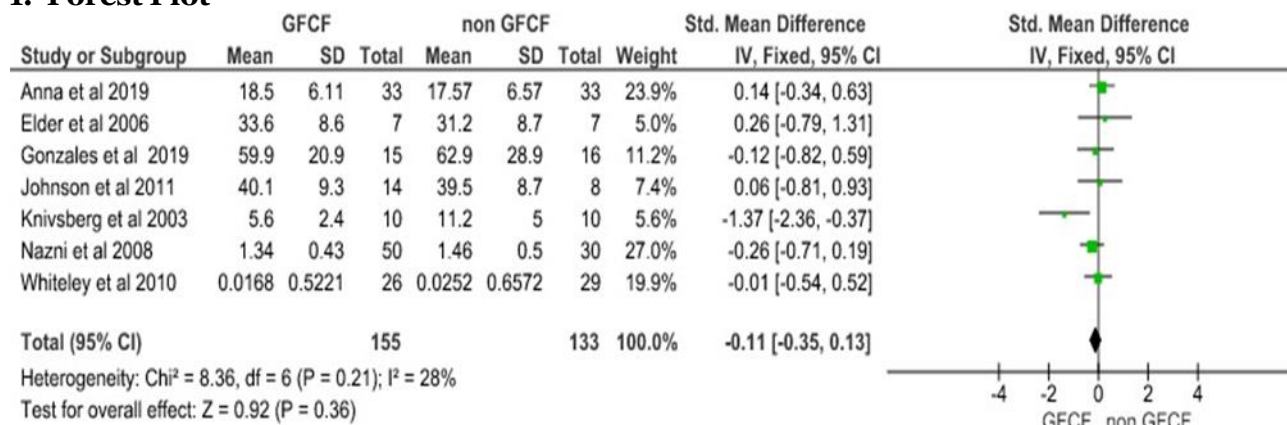Test for overall effect: Z = 0.92 (P = 0.36)

Figure 3. Forest plot of the effect of a gluten-free, casein-free diet on maladaptive behavior in autistic children

The forest plot in Figure 3 shows that there is an effect of a gluten-free, casein-free diet on maladaptive behavior in autistic children. but statistically not significant. Autistic children who received casein free gluten free had an average score of -0.11 maladaptive behavior lower than those who were not given a diet (SMD= -0.11 95% CI= -.035 to 0.13., p=

0.360). The Forest Plot in Figure 4.3 also shows a small variation in the effect estimates between studies (I2= 28%., p= 0.210) so that

the calculation of the average estimate is done by means of a fixed model.
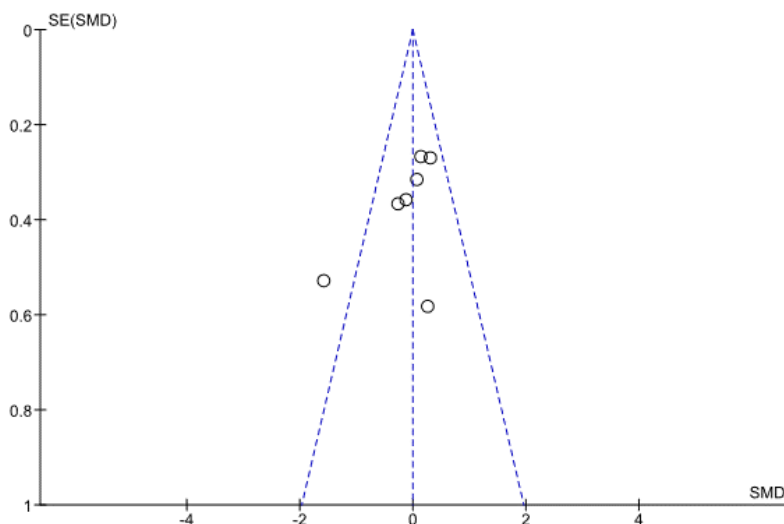
2. Funnel Plot

Figure 4. Funnel plot of the effect of a gluten-free, casein-free diet on maladaptive behavior in autistic children

The funnel plot in Figure 4 shows a more or less balanced distribution of effect estimates between studies to the right and left of the vertical mean of the estimate, so this meta-analysis indicates no publication bias.

<div style="background:maroon;color:white;text-align:center;">DISCUSSION</div>

Systematic review and meta-analysis in this study were carried out with the aim of increasing the generalization of the findings and obtaining convincing conclusions from the results of various similar studies regarding the effect of a gluten-free, casein-free diet on maladaptive behavior in children with autism. The selection of primary articles for meta-analysis processing uses a predetermined PICO reference. The selection of articles also uses research that has controlled for confounding factors, which can be seen from the research inclusion requirements, namely RCTs. Primary studies that met the criteria totaled 7 research articles as sources originating from three continents, namely 2 articles from America,

1 from Asia, and 4 articles from Europe, involving a total sample of 288.

The heterogeneity that appears in the articles that have been collected is formed due to the diversity of the population which is not taken from just one region, and the instruments used in each article vary. The population in this study were children and adolescents, but when searching for articles, several articles were found with autistic children as research subjects who were only female. Heterogeneity in the instrument part of the articles collected, the tools used by the researchers. The results of a meta-analysis study showed that a casein-free gluten-free diet could reduce maladaptive behavior in children with autism by -0.11 times (95% CI -0.35 to 0.13 p = 0.36). However, the effect of casein-free gluten-free on reducing the

risk of maladaptive behavior in autistic children, statistically not significant. Autistic children who received casein-free gluten free had an average score of -0.11µ lower maladaptive behavior than those who were not given a diet (SMD= -0.11 95% CI= -.035 to 0.13., p= 0.360. This study is in line with research by Bagaskorowati et al., (2022) which states that some foods that children with autism and ADHD cannot consume, such as consumption ingredients that contain gluten (protein contained in wheat and its processed products) and casein (protein in milk and animal and various processed products) consumption needs to be reduced because it causes hyperactivity in children.

Other research according to Djati et al. (2017) stated that there was an effect of a casein-free gluten-free diet on the maladaptive behavior of autistic children where there was a relationship between gluten consumption patterns and adherence to the GFCF diet with the behavior of autistic children with a result of p<0.05. This research is strengthened by another study by Dewanti and Machfud (2014) regarding the development of autistic children where autistic children who do a gluten free casein free diet have good development compared to autistic children without a gluten free casein free diet and have a significant effect.

The advantage of this study is that the results are more representative because they are taken from various articles. However, the researcher realizes the limitations of this research. The limitation of this study is the lack of homogeneity. on the instrument used for each article, search bias due to only searching through three databases, and publication bias shown in the funnel plot results.

AUTHORS CONTRIBUTION

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

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