Gluten and casein supplementation does not increase symptoms in children with autism spectrum disorder

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ABSTRACT

Aim: A gluten- and casein-free diet is often given to children with autism spectrum disorder (ASD). We aimed to determine the effect of gluten and casein supplementation on maladaptive behaviour, gastrointestinal symptom severity and intestinal fatty acids binding protein (I-FABP) excretion in children with ASD.

Methods: A randomised, controlled, double-blind trial was performed on 74 children with ASD with severe maladaptive behaviour and increased urinary I-FABP. Subjects were randomised to receive gluten–casein or a placebo for seven days. We evaluated maladaptive behaviour before and after supplementation, using I-FABP excretion, the approach withdrawal problem composite subtest of the Pervasive Developmental Disorder Behavior Inventory and the Gastrointestinal Symptom Severity Index.

Results: The mean approach withdrawal problem composite score was significantly higher before supplementation than after, both in the placebo and in the gluten–casein group. However, the mean difference was not significant and may have been caused by additional therapy. There was no significant difference in gastrointestinal symptoms and urinary I-FABP excretion.

Conclusion: Administering gluten–casein to children with ASD for one week did not increase maladaptive behaviour, gastrointestinal symptom severity or urinary I-FABP excretion. The effect of prolonged administration or other mechanisms of enterocyte damage in ASD should be explored.

INTRODUCTION

The prevalence of autism spectrum disorder (ASD) has risen over the years. Its global prevalence in 2012 was 11.3 per 1000 children (1), suggesting that both nongenetic and genetic factors (2) contribute to its pathomechanism (3,4).

The hallmarks of ASD, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revision, are abnormal or impaired development in social interaction and communication and a markedly restricted, repetitive and monotonous (stereotypical) repertoire of activities and interests (5). One of the most crippling aspects of ASD is the maladaptive behaviours of sensory perception impairment, ritualism and resistance to change, social pragmatic impairment, semantic pragmatic impairment, impairment in regulation of awareness to surroundings, specific phobias and aggressiveness (6).

Several studies have linked maladaptive behaviours in ASD with gastrointestinal dysfunction, intestinal bacteria, intestinal inflammation and colitis (7,8). It has been suggested that impaired intestinal permeability leads to abnormal intestinal absorption of gluten and casein in the form of opioid peptides, which cause aggravation of maladaptive behaviour due to their morphine-like effect on the brain (9,10). However, a number of studies have found no association between gastrointestinal symptoms and/or disorders with ASD (11,12).

Key notes

- We explored the effect of gluten and casein supplementation on maladaptive behaviour, gastrointestinal symptom severity and intestinal fatty acids binding protein (I-FABP) excretion in children with ASD.
- A randomised, controlled, double-blind trial was performed on 74 children with ASD, who were randomised to receive gluten–casein or a placebo for seven days.
- Administering gluten–casein to children with ASD did not increase maladaptive behaviour, gastrointestinal symptom severity or urinary I-FABP excretion.
Although the evidence remains controversial, professionals and parents often impose a gluten-free, casein-free (GFCF) diet unselectively to ASD children without clear guidelines on its indications or outcome measurement parameters. Results of studies on the effect of such diets are conflicting; trials are small, nonrandomised or open-label, precluding the generalisation of study results on this topic (13–16). Outcomes that rely on parental observations are particularly susceptible to bias in the absence of a solid blinding method. Only three small, randomised controlled trials on the GFCF diet have been published to date (14–16). Furthermore, these studies have investigated the effect of eliminating gluten and casein from the diet, whereas the effect of provocation with gluten and casein has not been studied using a randomised controlled trial design. Without carefully deliberated guidelines, imposing food restrictions to children may adversely affect the child’s growth (17).

In a previous study, we found a significant association between urinary intestinal fatty acids binding protein (I-FABP) excretion, a marker of enterocyte damage, and maladaptive behaviour in 66 healthy controls, 111 children with mild maladaptive ASD and 48 children with severe maladaptive ASD subjects. All the subjects were between two years old and 10 years old (Pusponegoro et al., unpublished data). According to our findings, maladaptive behaviour in children with ASD was not associated with gastrointestinal symptom severity, intestinal inflammation, intestinal bacteria and bacterial products and intestinal permeability. There was no evidence of urinary opioid peptide excretion in the children with and without ASD. The children with ASD and severe maladaptive behaviour had significantly more enterocyte damage than the children with ASD and mild maladaptive behaviour and the control children. Our previous results suggest that enterocyte damage may have mediated the deleterious effect of dietary gluten and casein on the children’s behaviour and that the subgroup of children with ASD with elevated urinary I-FABP may have been more likely to respond to dietary intervention.

This study aimed to determine the effect of dietary gluten and casein supplementation on maladaptive behaviour, gastrointestinal symptom severity and urinary I-FABP excretion in ASD children with severe maladaptive behaviour.

**METHODS**

This was a randomised, double-blind, placebo-controlled trial to determine the effect of gluten–casein supplementation on maladaptive behaviour, gastrointestinal symptom severity and urinary I-FABP excretion. We recruited subjects from a previous study that measured the association of maladaptive behaviour with gastrointestinal dysfunction parameters (Pusponegoro et al., unpublished data). Detailed information on the inclusion and exclusion criteria can be found online (Appendix S1). Subjects were children with ASD who had severe maladaptive behaviour and a urinary I-FABP value of more than 96.97 pg/mg creatinine. We used the urinary I-FABP cut-off point established in the aforementioned study (Pusponegoro et al., unpublished data). Detailed information can be found online (Appendix S2). Written parental informed consent was obtained prior to starting the intervention.

Subjects were randomised in two arms, an experimental arm that received a standardised amount of gluten–casein supplementation and a control arm that received a placebo. Block randomisation was carried out by a third party not involved in the study, and the randomisation list was concealed from the investigator.

Subjects in the experimental arm were supplemented with a total of 11 g of gluten and 12 g of casein in the form of six biscuits. Control subjects were asked to consume placebo biscuits that were identical in number, size, shape and taste to the intervention biscuits but contained a total of 30 g of rice meal. The biscuits were manufactured by PT Kalbe Nutritionals (Jakarta, Indonesia). Each subject had to consume six biscuits daily for seven consecutive days. Subjects, parents and investigators blinded to the type of intervention given. Subjects were allowed to discontinue the intervention at any time if the child showed markedly increased maladaptive behaviour or gastrointestinal symptoms. Parents were asked to report such occurrences to the investigator. The degree of maladaptive behaviour, gastrointestinal symptom severity index (GSSI) and urinary I-FABP was measured before and after the intervention. Comparisons between pre-intervention and post-intervention values were carried out using the Student’s *t*-test, when data distribution was normal, and the Wilcoxon signed ranks test for abnormally distributed data.

The degree of maladaptive behaviour was assessed using the approach withdrawal problems composite (AWPC) subtest of the Pervasive Developmental Disorder Behavior Inventory (PDDBI) (Hogrefe Ltd, Oxford, UK) (18). The AWPC subtest questionnaire measures seven categories of maladaptive behaviour, comprising sensory/perceptual approach behaviours, ritualism/resistance to change, social/pragmatic problems, semantic/pragmatic problems, arousal/regulation problems, specific fears and aggressiveness. Each category consists of a number of subcategories containing four questions regarding certain behaviours. Administration of the questionnaire was guided by one of two trained developmental psychologists. Parents were asked to answer each question on a scale of zero to three with zero being does not show the behaviour and three being usually and typically shows the behaviour. A raw score was computed according to the instructions in the manual, from which a *t*-score was generated. Maladaptive behaviour was considered severe when the *t*-score was more than or equal to 61 and mild when the *t*-score was less than or equal to 60 (6).

The GSSI was measured using a questionnaire which looked at symptoms such as borborygmi, flatulence, abdominal bloating, nausea and/or vomiting, abdominal pain, diarrhoea and constipation, based on the Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders.
Using this questionnaire, the severity of gastrointestinal symptoms was expressed as a score ranging from zero to 28, with higher scores corresponding to higher severity levels (19,20).

For the determination of urinary I-FABP, we used a Human I-FABP ELISA Kit (Hycult Biotech, Uden, The Netherlands). Urinary I-FABP values obtained were corrected for urinary creatinine levels. The assay was carried out at the Prodia Laboratory Research Division.

This study protocol was approved by the Standing Committee for Medical Research Ethics of the Medical School, Universitas Indonesia.

RESULTS

We randomised 74 subjects, 38 into the experimental group and 36 into the control group. Because 12 subjects dropped out of each of group, 24 subjects remained in the experimental group and 26 subjects in the control group. Reasons for dropping out included parental concern about the potential adverse effects, inability or refusal to consume the prescribed amount of biscuits for the required amount of time and relocation to another city (Fig. 1). Baseline subject characteristics were comparable between the two groups (Table 1).

In both groups, the mean AWPC t-score decreased significantly after supplementation: in the intervention group, it was 56.95 before and 39.38 after supplementation ($p < 0.001$), and in the control group, it was 57.08 before and 39.63 after ($p < 0.001$). The change in the degree of maladaptive behaviour was not significantly different between the two groups ($p = 0.971$) (Table 2).

At baseline, all subjects had a GSSI of zero. After intervention, the median (range) GSSI was zero (zero to seven) in the control group and three (zero to nine) in the intervention group (Table 2). In the control group, there was no significant difference before and after supplementation ($p = 0.102$), whereas in the intervention group this difference was significant ($p = 0.027$). However, the difference between the two groups was not significant ($p = 0.067$) and remained so after Monte Carlo adjustment ($p = 0.070; 99\%\ CI$ of $p 0.063–0.077$) (Table 3).

Analysis of urinary I-FABP levels was carried out after the data were cleaned from six outliers with values of more than 700 (745.84; 838.57; 849.90; 880.84; 930.38 and 1454.98). Median urinary I-FABP did not change significantly before and after supplementation ($p = 0.123$), both in the control group (155.32 versus 211.49) and in the intervention group (148.95 versus 135.39, $p = 0.689$). However, I-FABP showed an increasing trend in the control group, while it showed a decreasing trend in the

![Figure 1](#) Recruitment of study subjects.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Rice meal group</th>
<th>Gluten-casein group</th>
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<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Sex</td>
<td>Male/female</td>
<td>Male/female</td>
</tr>
<tr>
<td></td>
<td>23/13</td>
<td>21/3</td>
</tr>
<tr>
<td>Median age in years</td>
<td>5.1 (4.3–6.1)</td>
<td>5.4 (4.8–6.7)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDD-NOS</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Autism</td>
<td>18</td>
<td>17</td>
</tr>
</tbody>
</table>

PDD-NOS = Pervasive Development Disorder Not Otherwise Specified.
intervention group. There was no significant difference in urinary I-FABP changes between the two groups (p = 0.416), even after adjustment (p = 0.427; 99% CI of p 0.414–0.440) (Table 3).

**DISCUSSION**

We performed a randomised, double-blind, controlled trial on the effect of a week-long gluten–casein challenge on the degree of maladaptive behaviour, gastrointestinal symptom severity and urinary I-FABP excretion of children with ASD showing severe maladaptive behaviour. Previous randomised trials on this topic have studied the effect of gluten–casein elimination on behavioural changes in ASD (14–16). To our knowledge, our study is the first randomised controlled trial to study the behavioural effects of adding gluten and casein to the diets of children with ASD who were already on a GFCF diet. Our hypothesis was that a supplementation with gluten–casein may worsen maladaptive behaviour. But mean AWPC t-score postsupplementation was not significantly different between groups and was significantly reduced compared with presupplementation scores. This suggests the absence of a derogatory effect of provocation with gluten and casein on behaviour in our study population. However, this finding may be influenced by the short duration of the intervention, which was only seven days, and by the high dropout rate of 30%. Previous randomised, controlled studies that have suggested an association between gluten and casein with autism traits have used an elimination diet as the intervention with observation periods ranging from 12 weeks to 24 months. Knivsberg et al. (14) found that autistic traits, including ‘behaviour that is strange or difficult for others to understand’, were significantly reduced in a small group of 10 children fed a GFCF diet for one year, while in the control group behaviour remained unchanged. In a preliminary randomised trial in 15 children with ASD, Elder (13) reported no significant difference in autism symptoms between the diet and control groups, save for a number of anecdotal reports from parents to teachers. A randomised, controlled, single-blind trial in 72 children with ASD reported that the ameliorative effect of GFCF diet on communication, repetitive behaviour, attention and socialisation was time dependent and observable after 12 months of intervention (16). The length of observation in our study after the start of gluten–casein supplementation was only seven days. In a previous study by Lucarelli et al. (21), children with autism given a one-time oral challenge with cows’ milk protein after eight weeks of a cows’ milk elimination diet showed worsening of behavioural symptoms within two weeks.

The reduction of maladaptive behaviour in the present study may, in part, result from therapeutic interventions administered to subjects during the course of the study, such as behavioural intervention, sensory integration therapy and other therapeutic modalities that were deemed necessary by the treating clinician. The first, pre-intervention, measurement of maladaptive behaviour was carried out at enrolment of each subject. The first, pre-intervention, measurement of I-FABP was carried out after all the subjects had been recruited, which took about three months. The time gap between pre-intervention and postintervention maladaptive behaviour measurement was longer in subjects recruited earlier than in subject recruited
later. During this time gap, subjects had received other therapies such as sensory integration and speech and occupational therapy. According to a meta-analysis by Virues-Ortega et al., behavioural intervention for a period as brief as nine weeks, 12 and 18 weeks is long enough to result in a measurable improvement in maladaptive behaviour (22).

Presupplementation testing for maladaptive behaviour was carried out before, or at the time of, determining urinary I-FABP testing, whereas enrolment into the study and gluten–casein supplementation was carried out after urinary I-FABP results were known. This means that the time gap between pre-intervention and postintervention assessment of maladaptive behaviour extended for more than the seven-day intervention period. Even brief, targeted intervention may result in changes in autistic features, including behaviour (23,24). In comparing pre-intervention and postintervention scores, we did not control for therapeutic modalities received or time elapsed between pre- and post-testing.

Gastrointestinal symptoms showed a modest but statistically significant increase after the gluten–casein challenge, but not after the placebo supplementation, although the difference between the gluten–casein group and the placebo group after the challenge was not statistically significant different. Our results suggest that gluten–casein supplementation may cause an increase of gastrointestinal symptoms in children with ASD, but these did not affect maladaptive behaviour during the one-week study period. Some guidelines have stated that children with ASD who report gastrointestinal symptoms should be treated as any child with such symptoms (25,26).

Although this is one of the few studies to report on a prospective, double-blind intervention with casein and gluten supplementation in children with ASD, the study does have many weaknesses. It is not clear whether the taste of the casein/gluten-free biscuits was really identical to the taste of those containing casein and gluten. The chosen amounts supplemented was arbitrary. The time gap between inclusion in the study and the introduction of the dietary intervention varied and this may interfere with the interpretation of the results. The high dropout rate (30%) and the short intervention of seven days are other weaknesses of this study. As the intake of gluten and casein was recorded prior to the study period, previous intake of gluten and casein may have caused chronic enterocyte damage undetectable by I-FABP examination. The short duration of the study may also have precluded the detection of an observable effect on urinary peptides.

In conclusion, a short gluten–casein challenge was not associated with increased maladaptive behaviour or urinary I-FABP excretion in children with ASD with severe maladaptive behaviour, although it may cause increased gastrointestinal symptoms. A time–treatment interaction may exist in the effect of provocation with gluten and casein in children with ASD. Future studies should incorporate a longer observation period and should take into consideration external factors such as the effect of therapies received by the child. As GI symptoms did increase, it may be that if these children had been followed for a longer period of time, then the maladaptive behaviour would have increased as well. The pathogenesis of gastrointestinal symptoms and enterocyte damage in children with ASD still needs to be explored.

CONFLICT OF INTEREST
The authors report no conflict of interest.

FUNDING
No funding.

References


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

*Appendix S1* Inclusion and exclusion criteria.