The Gluten-Casein-Free Diet in Children with Autism: A Clinical Results of the Ophthalmic and Behavioral Manifestations*

Öznur Özge ÖZCAN**, İbrahim ŞAHBAZ***, Emre TAŞDÖNDÜREN****, Mesut KARAHAN*****

Abstract

Aim: To evaluate the ophthalmic and behavioral effects of a gluten-free casein-free diet (GCFD) in autism spectrum disorder (ASD).

Method: The study was conducted from November 2020 to January 2023. We designed an 8-week clinical trial of GCFD on corneal reflex, pupil size, body mass index (BMI), weight, Autistic Disorder Index, Communication, Social interaction, and Stereotype Behavior.

Results: A total of thirty participants completed the trial. GCFD reduced BMI [%95 CI -4.8895 to -2.1305 (P < 0.0001)], weight [%95 CI -8.5153 to -0.0647 (P = 0.0467)], Autistic Disorder Index [%95 CI -28.0359 to -16.2841 (P < 0.0001)], Communication [%95 CI -3.3325 to -1.6675 (P < 0.0001)], Social Interaction [%95 CI -4.3190 to -2.4010 (P < 0.0001)], and Stereotype Behavior [%95 CI -2.3939 to -0.2461 (P =0.01)] significantly. GCFD also reduced left [%95 CI -2.2421 to -1.6779 (P <0.0001)] and right pupil size [%95 CI -2.2999 to -1.7201 (P <0.0001)] significantly. Conversely, corneal reflex was significantly increased [95% CI 0.2159 to 2.1241 (P = 0.01)]. No differences were observed for IPD.

Conclusion: This study suggested that the GCFD can effectively control ASD behaviors as well as regulate autonomic functions related to ophthalmic markers such as pupil size and corneal reflex, but more research is needed.

Keywords: Autism spectrum disorders, cognitive functions, corneal reflex, gluten-free casein-free diet, plusoptix a09, pupil size

Özgün Araştırma Makalesi (Original Research Article)
Geliş / Received: 20.10.2023 & **Kabul / Accepted:** 11.12.2023
DOI: https://doi.org/10.38079/igusabder.1378810
* This article was produced from a master's thesis. Title of the Thesis: "A Prospective Cohort Study of the Effects of Gluten-Free Casein-Free Diet on Body Mass Index, Autism Grade, and Behavior in Children with Autism" Author: Emre TAŞDÖNDÜREN. Thesis advisor: Prof. Dr. Mesut KARAHAN. Üsküdar University Institute of Health Sciences, 2021.
** Assist. Prof., Üsküdar University, Vocational School of Health Sciences, Department of Electroneurophysiology, Istanbul, Türkiye. E-mail: oznurozge.ozcan@uskudar.edu.tr ORCID https://orcid.org/0000-0001-8992-0556
*** Assist. Prof. Dr., Şinasi Can Hospital, Ophthalmology, Istanbul, Türkiye. E-mail: ibrahim.sahbaz@uskudar.edu.tr ORCID https://orcid.org/0000-0002-5934-5367
**** MSc, Üsküdar University, Institute of Health Sciences, Department of Neuroscience, Istanbul, Türkiye. E-mail: emre.tsdndrn@hotmail.com ORCID https://orcid.org/0000-0002-0728-788X
***** Prof. Dr., Üsküdar University, Vocational School of Health Sciences, Department of Biomedical Device Technology, Istanbul, Türkiye. E-mail: mesut.karahan@uskudar.edu.tr ORCID https://orcid.org/0000-0002-8971-678X

ETHICAL STATEMENT: Üsküdar University The Human Ethics Committee approved the protocol (number: 61351342/2021-526, date: 26.11.2020). Informed consent was obtained from all participants and their parents.

Otizmli Çocuklarda Glutensiz Kazeinsiz Diyet: Oftalmik ve Davranışsal Belirtilerin Klinik Sonuçları

Öz

Amaç: Otizm spektrum bozukluğunda (ASD) glutensiz kazeinsiz diyetin (GCFD) oftalmik ve davranışsal etkilerini değerlendirmektir.

Yöntem: Çalışma Kasım 2020'den Ocak 2023'e kadar gerçekleştirildi. Kornea refleksi, gözbebeği büyüklüğü, vücut kitle indeksi (BMI), ağırlık, Otistik Bozukluk İndeksi, İletişim, Sosyal Etkileşim ve Stereotip Davranış üzerinde GCFD'nin 8 haftalık bir klinik çalışması yapıldı.

Bulgular: Çalışmaya toplamda 30 çocuk dahil edildi. GCFD, BKİ'yi [%95 CI 4,8895 ile -2,1305 (P < 0,0001)], ağırlığı [%95 CI -8,5153 ila -0,0647 (P = 0,0467)], Otistik Bozukluk İndeksini [%95 CI -28.0359 to -16.2841 (P < 0.0001)], İletişim [%95 CI 3,3325 ila -1,6675 (P < 0,0001)], Sosyal etkileşim [%95 CI -4,3190 ila -2,4010 (P < 0,0001)] ve Stereotip Davranışı [%95 CI -2,3939 ila -0,2461 (P =0,01)] önemli ölçüde azalttı. GCFD ayrıca soldaki [%95 CI -2,2421 ila -1,6779 (P <0,0001)] ve sağ gözbebeği boyutunu [%95 CI -2,2999 ila -1,7201 (P <0,0001)] önemli ölçüde azalttı. Tersine, kornea refleksi önemli ölçüde arttı (%95 CI 0,2159 - 2,1241 (P = 0,01)). IPD için hiçbir fark gözlenmedi.

Sonuç: Bu çalışma, GCFD'nin OSB davranışlarını etkili bir şekilde kontrol edebileceğini ve ayrıca gözbebeği boyutu ve kornea refleksi gibi oftalmik belirteçlerle ilgili otonomik işlevleri düzenleyebileceğini göstermiştir ancak daha fazla araştırmaya ihtiyaç vardır.

Anahtar Sözcükler: Otizm spektrum bozuklukları, bilişsel fonksiyonlar, kornea refleksi, glutensiz kazeinsiz diyet, plusoptix a09, pupil çapı

Introduction

Autism spectrum disorder (ASD) is a disease with a multifactorial infrastructure by early-onset differences in interaction, communication, sensory atypicality, and stereotyped behaviors[1]. Nutrition may play a role in the autonomic nervous system activity through the brain-gut axis due to the increased epithelial permeability observed in ASD[2]. When examining the causes of increased obesity and high body mass index (BMI) in children with autism compared to typically developing (TD) children, impaired eating behavior, gastrointestinal symptoms (GI), multiple drug use, and gut microbiota diversity were significantly reduced[3-6]. The opioid peptides mediated by casein and gluten metabolism reach the central nervous system by crossing the brain-intestinal barrier. Neuroinflammation in ASD is associated with opioid peptides, and the casein and gluten mechanism theory is critical[7]. With the hydrolysis of cereal and milk proteins, gliadorphine and casomorphine appears, which have a morphine-like effect on the brain, and high opioid peptide levels may negatively affect brain function resulting in isolation and unusual behavior problems in children with ASD[8-12]. In only four of the nine clinical trials (CTs) with 521 participants, a gluten-free casein-free diet (GCFD) has a positive contribution on language problems, stereotype behaviours, aggressive, hyperactive and attention deficit in ASD[13]. Conversely, Piwowarczyk et al.

reported that GCFD had no significant effects on ASD symptoms, as measured by standardized scales (Six CTs with 214 participants)[14]. The etiology of ASD has been still uncertain. Therefore, elucidating the underlying pathology may assist in risk assessment and facilitate process management. Non-harmful and existing evidence for the diet's benefits in ASD must be elucidated. This study aimed to obtain more data on the behavioral symptoms of GCFD on ASD.

The pupil size could be a marker to understand a number of psychopathological or physiological variables, including cognition, attention, communication and learning. Changes in light trigger the pupil response, which adjusts the amount of brightness falling on the retina by instinctively constricting or dilating the pupil[15,16]. According to the strength of the environmental stimulus and the age of the individual, the amplitude and rapidity of pupil size change might vary[17-20]. These alterations might also reflect cognitive function[21,22]. Furthermore, the pupil size could be changed by the neurotransmitter mechanism, particularly norepinephrine ehich can be affected from gliadorphine and casomorphine[23]. Daluwatte et al. found a significant correlation between pupillary light reflex (PLR) constriction amplitude and a range of sensory behaviors in the ASD group compared to normally developing children[24]. An atypical pupillary reflex has been reported in the emerging literature with potential clinical biomarker for the behavioral and cognitional status of ASD[25,26]. In our previous study, ASD children had larger pupil size and interpupillary distance (IPD) compared to the control group (normally developing children)[27], corneal reflex was not significant, however, wider pupils were detected under light compared to controls. However, one study reported no difference in pupil size between autistic and healthy children[28].

To the best of our knowledge, this is the first study to evaluate the efficacy of 8-weeks of GCFD in children with ASD on ophthalmic findings such as corneal reflex, IPD and pupil size.

Material and Methods

Ethics Approval

Üsküdar University The Human Ethics Committee approved the protocol (number: 61351342/2021-526, date: 26.11.2020). Informed consent was obtained from all participants and their parents.

Participants and Sample Size

This study was conducted with thirty children ages 2-11 years and from November 2020 to January 2023. Sample size can be estimated from clinical studies reporting the effects of GCFD on ASD included in current systematic reviews and meta-analyses[14,28,29].

Inclusion Criteria:

Children, male or female, 2 to 11 years old (inclusive)

Confirmed diagnosis of ASD according to the DSM-IV Symptom Checklist[30]

Ability to maintain a gluten- and casein-free diet during the study

Exclusion Criteria:

Cardiovascular, psychiatric, autoimmune, cancer, etc. children with secondary different disease

Children with any drug use

Children currently receiving treatment with any alternative medical methods (eg.hyperbaric).

Children with celiac disease

Children who have received any dietary treatment before

Study Design

To fulfil the research objectives, the authors planned a prospective clinical trial. The children were prospectively enrolled. GCFD education was given to the parents of the children by trained dietician. During the diet, a 24-hour diet recall was collected from the parents. The trial was registered at www.clinicaltrials.gov with the identifier NCT05848336. This study was conducted within the framework of the World Medical Association ethical rules[31].

GCFD Procedure

The content of the nutrition program is as follows:

Foods containing gluten (pasta, bread, etc.), casein (unfermented dairy products), and their disguised sources have been eliminated from the nutrition program. Also, packaged foods (chocolate, crackers, etc.) containing additives such as artificial preservatives, food coloring, and sweeteners that create a tendency to consume were avoided from the nutrition program. Goat milk contains type A2 casein, and most of the casein is digested in fermented dairy products. Therefore these have been allowed to be consumed in small amounts during the diet. In order to increase calcium intake, the consumption of some foods (dill, kale, spinach, chard, arugula, broccoli, parsley, legumes, nuts, tahini, etc.) has increased. Elimination was done gradually, considering nervousness, anxiety, etc., due to the effects of opioid mechanisms of action and gastrointestinal system symptoms[32-34]. The parents were also given a brochure with a list of foods that contain gluten and casein, as well as instructions for making Turkish-inspired gluten- and casein-free meals.

Gilliam Autism Rating Scale 2 (GARS-2)

Based on DSM-IV autism diagnostic criteria, GARS-2 is a behavior checklist for children and teenagers aged 3 to 22. The three subscales of the scale are communication, social interaction, and stereotypical behavior. Each subscale contains 14 items that are scored on a four-point scale (0: Never observed, 1: Rarely observed, 2: Sometimes observed, 3: Often observed). The Turkey Assessment Tools Index (TOAD) collaborated in the initial review of the Turkish version for linguistic clarity and cultural appropriateness[35]. The total score obtained from the scale is

converted into standard scores, and an OBI score is obtained. An OBI score of 85 and above indicates a high probability of having ASD, a score between 70-84 indicates a medium probability, and a score of 69 and below indicates a low probability—the internal consistency coefficients of the Turkish version of the scale range from 0.77 to 0.85. Confirmatory factor analysis shows that the model fit of the scale's factor structure is at a reasonable level ($\chi 2$=1730.08, sd=813, $\chi 2$/sd=2.13, CFI=0.89, RMSA=0.071)[36].

Anthromopetric Measurements

The height and weight of all the participants were recorded using standard anthropometric measure techniques with the Jadever-Türkter brand NLD-W model scale in order to develop recommendations. WHO growth standards were used to calculate and classify anthropometric data[37].

Plusoptix® A09

Ophthalmic measurements were made with a binocular photorefractometer (Plusoptix® A09, GmbH, Nuremberg, Germany)[38,39]. Procedure was performed according to previously published study[27].

Statistical Analysis

For statistical analysis, SPSS version 22.00 (Statistical Package for Social Science) was used (IBM Corp. Released. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The quantitative data were shown as mean, standard deviations, and ranges for the parametric data, while the qualitative data were shown as numbers and percentages. The normality of variables was examined using the Kolmogorov-Smirnov test. An Independent t-test was used to compare two independent groups (baseline and intervention groups) using numerical data and a parametric distribution. The equality of proportions was compared using a two-tailed test with a 5% alpha level, and $p< 0.05$ was statistically significant.

Results

Cohort Description

The study was carried out between November 2020 and January 2023. Thirty participants with a chronological age of 2-11 years were potentially eligible for inclusion. Table 1 shows the Baseline characteristics of the participants. A total of thirty participants (13 female and 17 male) entered the study. All participants completed the study with no dropouts. The mean age of the participants was 4.49±2.5.

Table 1. Baseline characteristics of the participants

	Children with Austism (n=30)
Age, months (MD±SD)	4.49 ± 2.5
Male	17
Female	13
BMI (MD±SD)	20.86 ± 3.15 kg/m²
Use of psychiatric drugs	NA

Abbreviations Body mass index (BMI); mean difference (MD); not applicable (NA); standart deviation (SD)

Anthropometric Measurements

Weight and BMI were significantly decreased in within group after the GCFD intervention (PRE VS. POST, P<0.05). The mean weight of children with ASD was 21.11±8.46 kg and changed to 16.82 ± 7.88 kg after the GCFD (p=0.04). The mean BMI (kg/m²) values measured before GCFD was 20.86±3.15 and changed to 17.35±2.08 after GCFD intervention (P<0.0001). GCFD is effective in the management of weight and BMI of children with an obese profile in this sample group aged between 2 and 11 years.

Behavioral Effects

Table 2 presents PRE VS. POST results related to autistic and behavioral symptoms, including descriptive statistics of the analysis of the variables. Generally, there were significant differences on the GARS-2 results between at baseline and the 8-week follow-up (P<0.05, Paired Samples Test).

Table 2. Behavioral results at baseline and follow-up

	GARS-2		
Variables	**Baseline**	**8weeks-GCFD** 79,05 ± 12,15	**p**
Autism Index (N=30)	101,21 ± 10,53		.000*
Stereotyped Behaviors (N=30)	10,37 ± 1,89	9,05 ± 2,25	0.01*
Communication (N=30)	10,81 ± 1,47	8,31 ± 1,74	.000*
Social Interaction (N=30)	9,68 ± 1,60	6,32 ± 2,08	.000*

*Abbreviations: Gilliam Autism Rating Scale-2 (GARS-2); wk (Week), *p<0,05*

Opthalmic Measurements

We had complete opthalmic measurements in n=30 subjects. All participants had abnormal corneal reflex and large pupil size. Left pupil size decreased significantly and changed by -

1.96±0.04 mm after 8 weeks-GCFD intervention. Similarly, right pupil size changed by -2.01±0.03 and significantly reduced (PRE VS. POST, in table 3) as distinct from corneal reflex changed by 1.17±1.21 and significantly increased and also IPD but not significantly.

Table 3. Changes in ophthalmic measurements

	Ophthalmic measurements		
Variables Pupil Size (mm)	**Baseline**	**8 weeks-GCFD**	**p**
Left eye	7.03 ± 0.46	5.07 ± 0.62	.000*
Right eye	7.03 ± 0.44	5.02 ± 0.66	.000*
IPD	53.89 ± 3.67	54.53 ± 2.97	0.209
Corneal Reflex	3.49 ± 2.21	4.66 ± 1.39	0.01*

*Abbreviations: Interpupillary Distance (IPD), millimeter (mm), wk (Week), *p<0,05*

Discussion

Few studies have investigated the behavioral effects of GCFD on children with ASD, but its effect on ophthalmic findings is unknown. Recognizing the limitations of the difficulties of such an intervention (GCFD) in ASD with behaviorally heterogeneous features. In this study, we investigated a significant difference in opthalmic and behavioral changes with the 8- weeks GCFD in children with ASD. The majority of participants were male, and the rate of medication use was typical of the ASD population as a whole[40-42]. A systematic comparative review by Jobski et al. found that 17% of children and adolescents with ASD and 42% of those without the disorder used antipsychotic medications[43]. Comorbidities are to blame for the high rate of medication use in the general ASD population[41,42,44]. It was congruent with the literature, although the presence of comorbidity in our study was an exclusion criterion. The results align with studies that assume that pupil size can be used as an autism indicator[15,16,45]. Few studies have investigated the intervention and differences in baseline pupillary response are atypical in ASD[25-28,46]. Anderson and Colombo stated that the pupil size of the ASD group was larger compared to control[47], results were similar in our previous study[27]. Another study has found that children with ASD differed in pupil size and pupillary constriction compared to other groups, according to visual scanning evaluation[48]. Upper opioid peptide levels play a role in the emergence of symptoms such as loss of eye contact, learning disorder, hyperactivity, and self-harm[49]. Studies on experimental animals have shown the importance of the opioid system in maintaining ASD-like social behaviors. Pellissier et al. reported increased levels of opioid peptides in blood serum, CSF, and urine samples from individuals with ASD[50]. However, none of them investigate the effects of a dietary intervention on ASD on pupillary and corneal responses. Ophthalmic evaluation of a recent ASD case report had absent corneal reflex, in the right eye compared to intense corneal opacity with dryness in the left eye[51]. Another 10-year-old case of ASD reported loss of vision due to punctate

epithelial erosions, corneal and conjunctival keratinization[52]. Gutierrez et al. reported that amblyopia, strabismus, optic neuropathy, refractive errors and ocular motility disorders are common in patients with ASD especially in pediatric population[53] but the ophthalmic examination of ASD has still major deficiencies in the literature. In particular, pupil and corneal studies can provide important information about ASD symptoms and biomarkers. As demonstrated in recent publications by Singman regarding the development of new technologies to facilitate ophthalmological examination, instruments such as the PlusoptiX photorefractometer are important for screening for risk factors in patients with ASD[54]. Also, atypical ophthalmic findings in children with ASD may be thought to be related to casomorphine and gliadomorphin, because Hafid et al. detected high levels of casomorphine and gliadomorphine in urinalysis in 20 of 30 children prior to GCFD intervention[55]. Our study found that 8 weeks-GCFD significantly reduced pupil size and increased corneal reflex in ASD. The research has the advantages of being one of the few studies to examine the behavioral and ophthalmic effects of a GCFD in children with ASD.

GARS-2 evaluation of behavioral autistic symptoms was the study's preliminary outcome. The research discovered that the GARS-2 scores significantly differed between the baseline and the 8-week follow-up. These results imply that the GCFD may improve behavioral autistic traits in children. Harris and Card reported improved behavior patterns with the GCFD[56]. Especially some studies found that a GCFD was effective in improving acute ASD behaviors, physiological symptoms, and social behaviors in children who have GI, allergy, and food sensitivity abnormalities[57,58]. Ghalichi et al. found significant decreases in stereotyped behaviors, communication, and social interaction but no correlation for communication in a subgroup of children with ASD[59]. Mulloy et al. shown that the GCFD intervention was ineffective in treating the behavioral pattern of ASD[58]. Similarly, Seung et al. reported no significant difference in communication between 6 weeks-GCFD and regular diet[60]. Marí-Bauset et al. recommend that GCFD should be only used after the diagnosis of an intolerance or allergy to foods containing the allergens but helpful to regulate BMI in ASD[8]. Clinical studies also have insufficient evidence for BMI and weight control of GCFD.

Clinical studies have shown that six or eight weeks GCFD is helpful in improving gastrointestinal symptoms associated with ASD (NCT01116388), but no effective in terms of post-intervention autistic symptoms, non-adaptive behaviors or cognitive abilities, and also no signifcant diferences in BMI between groups were found (NCT02280746)[61]. In contrast, we found that GCFD had a significant reduction in weight and BMI in children with ASD. A systematic review reported inconsistent evidence for 6 CTs to examine the efficacy of a GCFD on ASD symptoms[14]. González-Domenech et al. demonstrated 6-months consuming GCFD did not effective on urinary beta-casomorphin concentrations, language, sociability, sensory speech communication, cognitive awareness, autistic isolation and impairment of physical health in ASD[62]. Similarly, Navarro et al. reported 6 weeks GCFD did not changed hyperactivity, irritability and cognitive functions in

twenty children with ASD[7], Elder et al.'s results were similar after 12 weeks of GCFD[63]. Conversely, Ghalichi et al.[59] and our study found GCFD intervention significantly reduced behavioral problems. These studies often included a control diet and also GCFD applied at different lengths of time. Generally, all these clinical studies have discussed cases such as sample size, age, duration of intervention, blinding, lack of control or more precise dietary analysis. GCFD intervention ranged from seven days to twelve months. The duration of the intervention and sample size (vary from 7 to 100) does not change the inconsistency in the results obtained. The effects of the intervention were evaluated by various scales with validity and reliability in ASD. Despite the variety of scales and diet duration, the results of clinical studies on ASD-related behaviors are still inconsistent. Comparing diets with different ingredients and randomization of ASD individuals with different characteristics may limit the precise investigation of the effects of GCFD. This may be the main reason why the results are so inconsistent. Observing the effects of GCFD on a single sample may be more clinically effective because patients with ASD can vary widely in terms of genetic, morphological, and environmental factors[64,65]. Nutritional interventions need to be prospectively evaluated individually in the management of ASD[66].

Limitations

The research has some limitations, such as the small sample size and the use of parent-reported data for scales and also further investigation is urgently necessary to verify these findings.

Conclusion

Consequently, in our study, GCFD was beneficial for measures in the Autistic Disorder Index, Communication, Social interaction, and Stereotype Behavior subscale of the GARS-2 scale and significantly effective on ocular differences in ASD such as corneal reflex and pupil size. Further investigations are needed to confirm these results and explore the possible molecular mechanisms of optic nerve and brain connections that cause this effect. Studies with longer intervention and larger samples are required. Our results suggests to clinicians and researchers that autistic children with different sensory patterns may be more likely to experience social, adaptive, and/or attention/behavioral difficulties and associated corneal and pupil values can be balanced with nutritional strategies.

Author Contributions: Concept/Design: MK, İŞ, ET, ÖÖÖ; Data acquisition: ET, İŞ; Data analysis and interpretation: ÖÖÖ, MK, ET; Drafting manuscript: MK, ÖÖÖ; Final approval and accountability: MK, İŞ;

Financial Disclosure: Thanks to Üsküdar University and Dünya Bursa Göz Hospital

REFERENCES

1. Lai MC, Lombardo MV, Baron-Cohen S. Autism. *The Lancet*. 2014;383:896-910. doi: 10.1016/S0140-6736(13)61539-1.
2. Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: Comorbidity or causative mechanisms? *Bioessays*. 2014;36(10):933–939. doi: 10.1002/bies.201400075.
3. Dhaliwal KK, Orsso CE, Richard C, Haqq AM, Zwaigenbaum L. Risk factors for unhealthy weight gain and obesity among children with autism spectrum disorder. *Int J Mol Sci*. 2019;20(13):3285. doi: 10.3390/ijms20133285.
4. Bandini LG, Curtin C, Phillips S, Anderson SE, Maslin M, Must A. Changes in food selectivity in children with autism spectrum disorder. *J Autism Dev Disord*. 2017;47(2):439–446. doi: 10.1007/s10803-016-2963-6.
5. Maneeton N, Maneeton B, Putthisri S, Woottiluk P, Narkpongphun A, Srisurapanont M. Risperidone for children and adolescents with autism spectrum disorder: A systematic review. *Neuropsychiatr Dis Treat*. 2018;14:1811–1820. doi: 10.2147/NDT.S151802.
6. Kang DW, Park JG, Ilhan ZE, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PloS one*. 2013;8(7):e68322. doi: 10.1371/journal.pone.0068322.
7. Navarro F, Pearson DA, Fatheree N, Mansour R, Hashmi SS, Rhoads JM. Are 'leaky gut' and behavior associated with gluten and dairy containing diet in children with autism spectrum disorders? *Nutr Neurosci.* 2015;18(4):177–185. doi: 10.1179/1476830514Y.0000000110.
8. Marí-Bauset S, Zazpe I, Mari-Sanchis A, Llopis-González A, Morales-Suárez-Varela M. Evidence of the gluten-free and casein-free diet in autism spectrum disorders: A systematic review. *J Child Neurol.* 2014;29(12):1718–1727. doi: 10.1177/0883073814531330.
9. Priyatna A. Amazing Autisme. Jakarta: Elex Media Kompetindo. 2010. https://opac.perpusnas.go.id/DetailOpac.aspx?id=141789. Accessed September 29, 2023.
10. Bandini LG, Anderson SE, Curtin C, et al. Food selectivity in children with autism spectrum disorders and typically developing children. *J Pediatr.* 2010;157(2):259–264. doi: 10.1016/j.jpeds.2010.02.013.
11. Dinan TG, Cryan JF. Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. *Psychoneuroendocrinology*. 2012;37(9):1369–1378. doi: 10.1016/j.psyneuen.2012.03.007.
12. Ciéslińska A, Kostyra E, Savelkoul HFJ. Treating autism spectrum disorder with gluten-free and casein-free diet: The underlying microbiota-gutbrain axis mechanisms. *HSOA J Clin Immunol Immunotherapy*. 2017;3(1):9-19.
13. Alamri ES. Efficacy of gluten- and casein-free diets on autism spectrum disorders in children. *Saudi Med J.* 2020;41(10):1041-1046. doi: 10.15537/smj.2020.10.25308.

14. Piwowarczyk A, Horvath A, Łukasik J, Pisula E, Szajewska H. Gluten- and casein-free diet and autism spectrum disorders in children: A systematic review. *Eur J Nutr*. 2018;57(2):433-440. doi: 10.1007/s00394-017-1483-2.

15. Alpern M, Mccready DW, Jr Barr. The dependence of the photopupil response on flash duration and intensity. *J Gen Physiol*. 1963;47(2):265–278. doi: 10.1085/jgp.47.2.265.

16. Lowenstein O, Loewenfeld IE. The pupil: Anatomy, Physiology, and Clinical Applications. Detroit: Iowa State University Press, Ames and Wayne State University Press.1993.https://www.scirp.org/(S(351jmbntvnsjt1aadkozje))/reference/referencespapers.aspx?referenceid=2071826. Accessed September 29, 2023.

17. Sahraie A, Barbur JL. Pupil response triggered by the onset of coherent motion. *Graefes Arch Clin Exp Ophthalmol*. 1997;235(8):494–500. doi: 10.1007/BF00947006.

18. Ellis CJ. The pupillary light reflex in normal subjects. *Br J Ophthalmol*. 1981;65(11):754–759. doi: 10.1136/bjo.65.11.754.

19. Lobato-Rincón LL, Cabanillas-Campos Mdel C, Bonnin-Arias C, Chamorro-Gutiérrez E, Murciano-Cespedosa A, Sánchez-Ramos Roda C. Pupillary behavior in relation to wavelength and age. *Front Hum Neurosci*. 2014;8:221. doi: 10.3389/fnhum.2014.00221.

20. Ly V, Bottelier M, Hoekstra PJ, Arias Vasquez A, Buitelaar JK, Rommelse NN. Elimination diets' efficacy and mechanisms in attention deficit hyperactivity disorder and autism spectrum disorder. *Eur Child Adolesc Psychiatry*. 2017;26(9):1067–1079. doi: 10.1007/s00787-017-0959-1.

21. Laeng B, Ørbo M, Holmlund T, Miozzo M. Pupillary stroop effects. *Cogn Process*. 2011;12(1):13–21. doi: 10.1007/s10339-010-0370-z.

22. Nassar MR, Rumsey KM, Wilson RC, Parikh K, Heasly B, Gold JI. Rational regulation of learning dynamics by pupil-linked arousal systems. *Nat. Neurosci*. 2012;15(7):1040–1046. doi: 10.1038/nn.3130.

23. DiCriscio AS, Troiani V. Pupil adaptation corresponds to quantitative measures of autism traits in children. *Sci Rep*. 2017;7:6476 doi: 10.1038/s41598-017-06829-1.

24. Daluwatte C, Miles JH, Sun J, Yao G. Association between pupillary light reflex and sensory behaviors in children with autism spectrum disorders. *Res Dev Disabil*. 2015;37:209–215. doi: 10.1016/j.ridd.2014.11.019.

25. Lynch G. Using pupillometry to assess the atypical pupillary light reflex and LC-NE system in ASD. *Behav Sci*. 2018;2(11):108. doi: 10.3390/bs8110108.

26. Nyström P, Gliga T, Nilsson Jobs E, et al. Enhanced pupillary light reflex in infancy is associated with autism diagnosis in toddlerhood. *Nat Commun*. 2018;9:1678. doi: 10.1038/s41467-018-03985-4.

27. Şahbaz İ, Taşdöndüren E, Özcan ÖÖ, et al. Comparison of interpupillary distance, pupillary diameter and corneal reflex measured with Plusoptix A09 in normally developing children

and autism. *İstanbul Gelişim Üniversitesi Sağlık Bilimleri Dergisi*. 2022;18:668-677. doi: 10.38079/igusabder.1088174.

28. Nuske HJ, Vivanti G, Dissanayake C. Brief report: evidence for normative resting-state physiology in autism. *J Autism Dev Disord*. 2014;44(8):2057-63. doi: 10.1007/s10803-014-2068-z.

29. Keller A, Rimestad ML, Friis Rohde J, et. al. The effect of a combined gluten- and casein-free diet on children and adolescents with autism spectrum disorders: A systematic review and meta-analysis. *Nutrients*. 2021;13(2):470. doi: 10.3390/nu13020470.

30. APA. Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM 5). (2013). Washington, DC: American Psychiatric Association. https://psycnet.apa.org/record/2013-14907-000. Accessed September 29, 2023.

31. Declaration of Helsinki. The World Medical Association (WMA). (1998). The WMA declaration of Helsinki 1960 with recommendations on biomedical research on human subjects (modified in 1975, 1980 and 1989). *Chirurgia (Bucur),* 93(2), 138–140. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2008.pdf. Accessed September 29, 2023.

32. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. *BMC Gastroenterol*. 2011;11:22. doi: 10.1186/1471-230X-11-22.

33. Feingold BF. Hyperkinesis and learning disabilities linked to artificial food flavors and colors. *Am J Nurs*. 2011;75(5):797–803.

34. Mazurek MO, Vasa RA, Kalb LG, et. al. Anxiety, sensory over-responsivity, and gastrointestinal problems in children with autism spectrum disorders. *J Abnorm Child Psychol*. 2013;41(1):165-176. doi: 10.1007/s10802-012-9668-x.

35. Diken İH, Ardıç A, Diken Ö, Gilliam JE. Exploring the validity and reliability of Turkish version of gilliam autism rating scale-2: turkish standardization study. *Eğitim ve Bilim*. 2012;37(166):318-327.

36. Akçamuş MÇ, Bakkaloğlu H, Demir Ş, Bahap-Kudret Z. The validity and reliability study of the Repetitive Behavior Scale-Revised- Turkish Version in autism spectrum disorder. *Alpha Psychiatry*. 2019;1(20):65-72. doi: 10.5455/apd.42649.

37. WHO. (2006). WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development. Switzerland: World Health Organization. https://www.who.int/publications/i/item/924154693X. Accessed September 29, 2023.

38. Payerols A, Eliaou C, Trezeguet V, Villain M, Daien V. Accuracy of PlusOptix A09 distance refraction in pediatric myopia and hyperopia. *BMC Ophthalmol*. 2016;16:72. doi: 10.1186/s12886-016-0247-8.

39. Yan XR, Jiao WZ, Li ZW, Xu WW, Li FJ, Wang LH. Performance of the Plusoptix A09 photoscreener in detecting amblyopia risk factors in Chinese children attending an eye clinic. *PloS one*. 2015;10(6):e0126052. doi: 10.1371/journal.pone.0126052.

40. Chiarotti F, Venerosi A. Epidemiology of autism spectrum disorders: A review of worldwide prevalence estimates since 2014. *Brain Sci.* 2020;10(5):274. doi: 10.3390/brainsci10050274.

41. Coury DL, Anagnostou E, Manning-Courtney P, et al. Use of psychotropic medication in children and adolescents with autism spectrum disorders. *Pediatrics*. 2012;130(Suppl 2):S69–S76. doi: 10.1542/peds.2012-0900D.

42. Frazier TW, Shattuck PT, Narendorf SC, Cooper BP, Wagner M, Spitznagel EL. Prevalence and correlates of psychotropic medication use in adolescents with an autism spectrum disorder with and without caregiver-reported attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol.* 2011;21(6):571–579. doi: 10.1089/cap.2011.0057.

43. Jobski K, Höfer J, Hoffmann F, Bachmann C. Use of psychotropic drugs in patients with autism spectrum disorders: A systematic review. *Acta Psychiatr Scand.* 2017;135(1):8–28. doi: 10.1111/acps.12644.

44. Houghton R, Ong RC, Bolognani F. Psychiatric comorbidities and use of psychotropic medications in people with autism spectrum disorder in the United States. *Autism Res*. 2017;10(12):2037–2047. doi: 10.1002/aur.1848.

45. Cohen N, Moyal N, Henik A. Executive control suppresses pupillary responses to aversive stimuli. *Biol Psychol.* 2015;112:1–11. doi: 10.1016/j.biopsycho.2015.09.006.

46. Nuske HJ, Vivanti G, Dissanayake C. No evidence of emotional dysregulation or aversion to mutual gaze in preschoolers with autism spectrum disorder: An eye-tracking pupillometry study. *J Autism Dev Disord.* 2015;45(11):3433-45. doi: 10.1007/s10803-015-2479-5.

47. Anderson CJ, Colombo J. Larger tonic pupil size in young children with autism spectrum disorder. *Dev Psychobiol.* 2009;51(2):207–211. doi: 10.1002/dev.20352.

48. Anderson CJ, Colombo J, Jill Shaddy D. Visual scanning and pupillary responses in young children with Autism Spectrum Disorder. *J Clin Exp Neuropsychol.* 2006;28(7):1238–1256. doi: 10.1080/13803390500376790.

49. Knivsberg AM, Reichelt KL, Nødland M. Reports on dietary intervention in autistic disorders. *Nutr Neurosci.* 2001;4(1):25–37. doi: 10.1080/1028415x.2001.11747348.

50. Pellissier LP, Gandía J, Laboute T, Becker JAJ, Le Merrer J. μ opioid receptor, social behaviour and autism spectrum disorder: Reward matters. *Br J Pharmacol.* 2018;75(14):2750-2769. doi: 10.1111/bph.13808.

51. Sheth F, Shah J, Patel K. et al. A novel case of two siblings harbouring homozygous variant in the NEUROG1 gene with autism as an additional phenotype: A case report. *BMC Neurol.* 2023;23:20. doi: 10.1186/s12883-023-03065-1.

52. Kinlin LM, Vresk L, Friedman JN. Vision loss in a child with autism spectrum disorder. *Paediatr Child Health*. 2019;24(3):148-150. doi: 10.1093/pch/pxy058.

53. Gutiérrez C, Santoni JLM, Merino P, de Liaño PG. Ophthalmologic manifestations in autism spectrum disorder. *Turk J Ophthalmol*. 2022;52(4):246-251. doi: 10.4274/tjo.galenos.2021.46588.

54. Singman E, Matta N, Fairward A, Silbert D. Evaluation of plusoptiX photoscreening during examinations of children with autism. *Strabismus*. 2013;21(2):103-5. doi: 10.3109/09273972.2013.786736.

55. Hafid A, Ahami AOT. The efficacy of the gluten-free casein-fren diet for Morroccan autistic children. *Curr Res Nutr Food Sci Jour*. 2018;6(3):734-741. doi: 10.12944/CRNFSJ.6.3.15.

56. Harris C, Card B. A pilot study to evaluate nutritional influences on gastrointestinal symptoms and behavior patterns in children with Autism Spectrum Disorder. *Complement Ther Med*. 2012;20:437-440. doi: 10.1016/j.ctim.2012.08.004.

57. Pennesi CM, Klein LC. Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum disorder: Based on parental report. *Nutritional Neuroscience*. 2012;15(2):85–91. doi: 10.1179/1476830512Y.0000000003.

58. Mulloy A, Lang R, O'Reilly M, Sigafoos J, Lancioni G, Rispoli M. Gluten-free and casein-free diets in the treatment of autism spectrum disorders: A systematic review. *Res Autism Spect Dis*. 2009;217:2-12. doi: 10.1016/j.rasd.2009.10.008.

59. Ghalichi F, Ghaemmaghami J, Malek A, Ostadrahimi A. Effect of gluten free diet on gastrointestinal and behavioral indices for children with autism spectrum disorders: A randomized clinical trial. *World J Pediatr*. 2016;12(4):436–442. doi: 10.1007/s12519-016-0040-z.

60. Seung H, Rogalski Y, Shankar M, Elder J. The Gluten-and Casein-free diet and Autism: Communication outcomes from a preliminary double-blind clinical trial. *J Med Speech Lang Pathol*. 2007;15:337-345.

61. Piwowarczyk A, Horvath A, Pisula E, Kawa R, Szajewska H. Gluten-free diet in children with autism spectrum disorders: A randomized, controlled, single-blinded trial. *J Autism Dev Disord*. 2020;50(2):482-490. doi: 10.1007/s10803-019-04266-9.

62. González-Domenech PJ, Díaz Atienza F, García Pablos C, Fernández Soto ML, Martínez-Ortega JM, Gutiérrez-Rojas L. Influence of a combined gluten-free and casein-free diet on behavior disorders in children and adolescents diagnosed with autism spectrum disorder: a 12-month follow-up clinical trial. *J Autism Dev Disord*. 2020;50(3):935-948. doi: 10.1007/s10803-019-04333-1.

63. Elder JH, Shankar M, Shuster J, Theriaque D, Burns S, Sherrill L. The gluten-free, casein-free diet in autism: Results of a preliminary double blind clinical trial. *J Autism Dev Disord*. 2006*;*36(3):413-20. doi: 10.1007/s10803-006-0079-0.

64. Santos JX, Rasga C, Marques AR, et. al. A role for gene-environment interactions in autism spectrum disorder is supported by variants in genes regulating the effects of exposure to xenobiotics. *Front Neurosci*. 2022;19(16):862315. doi: 10.3389/fnins.2022.862315.

65. Tordjman S, Somogyi E, Coulon N. et al. Gene × Environment interactions in autism spectrum disorders: Role of epigenetic mechanisms. *Front Psychiatry*. 2014;4(5):53. doi: 10.3389/fpsyt.2014.00053.

66. Fan X, Miles JH, Takahashi N, Yao G. Abnormal transient pupillary light reflex in individuals with autism spectrum disorders. *J Autism Dev Disord*, 2009;39(11):1499–1508. doi: 10.1007/s10803-009-0767-7.