Original Article

Increased intake of health-promoting foods as a benefit of the exclusion of gluten and casein from the diet of ASD patients

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Abstract. *Background:* The high intake of nuts, seeds, berries and cruciferous vegetables has beneficial effects on the immune system, as well as communication skills and behaviour. Gluten- and/or casein-free diets are the most common interventions in ASD patients; however, scientific evidence for their use is poor. *Objective:* The aim is to explore the relationship between the implementation of a gluten-free diet or a casein- and gluten-free diet in ASD children and the frequency of consumption of nuts, seeds, berries and cruciferous vegetables. *Methods:* A sample of 88 ASD patients and their mothers was followed for 12 months after making their free choice of a gluten-free diet, a gluten- and casein-free diet or a regular diet. The Food Frequency Questionnaire (FFQ) was used to gather information about the frequency of consuming assortment groups of products. *Results:* Children with ASD on a gluten- and casein-free diet have significantly higher intake of seeds, berry fruits and cruciferous vegetables than controls, while patients on a gluten-free diet consumed more frequently seeds and cruciferous vegetables. In both groups, after dietetic intervention, the frequency of nut consumption tended to be higher than in patients on a regular diet. *Conclusions:* Unflagging interest and frequently reported subjective feelings of improvement in parents of autistic children on elimination diets may be due to the healthy balance of modified diets and their multidirectional impact.

Key words: Autism, gluten-free diet, casein-free diet, nuts, berry fruits

Autism manifests itself as a syndrome of profound behavioural disorders in three main areas: difficulties in establishing and sustaining social contacts, specific speech disorders, and deficits of imagination (1,2). Prevalence ranged from 1.09/10,000 to 436/10,000, with a median prevalence of 100/10,000 (1). Unfortunately, there have been no official epidemiological data on the prevalence of ASD in Polish population, but it seems to be rising rapidly (3). Inappropriate eating patterns, narrow preferences for foods and refusal of new foods are common among children diagnosed with ASD. It is estimated that up to 72–83% of children with ASD may be affected by those problems, while this phenomenon affects 10–50% of healthy children (4,5). Patients who exhibit this type of nutritional behaviour tend to prefer a monotonous diet (1,4,6). They are likely to choose food products with high energy density and low nutritional value, e.g. rich in refined carbohydrates, crispy snacks or sweets, and at the same time reject vegetables and fruit (7,8).

The literature has reported various factors responsible for ASD (1,2,6,9). Results from several in-depth reviews showed a plethora of immunological abnormalities as possible risk factors for autism (9-11). Moreover, evidence from several observational studies and clinical trials suggests that high intake of nuts, seeds, berries and cruciferous vegetables has beneficial effects on the immune system, as well as communication skills and behaviour in the general population (12-16). Recently, nuclear peroxisome proliferator-activated receptors (PPARs) and regulatory T cells (Tregs) were identified as promising targets for pharmacologic treatment in ASD, alongside nutritional therapy with nuts and berries (17-21). Diets vary across countries

and cultures, and comparisons between them can be compromised due to different backgrounds. Overall, scientific evidence for the elimination of casein and gluten from the diet of autistic children is poor (6,22,23), which contrasts with the unflagging interest of parents in these interventions, for which information is being sought from friends, support groups and media sources more so than from medical professionals (24). Parental descriptions make claims for high rates of success from gluten- and/or casein-free diets as an intervention in ASD, but at the same time it is not possible to identify what specific behavioural outcomes caregivers saw as changed (22,24). Research exploring the relationship between the introduction of a gluten-free diet (GFD) and a casein- and gluten-free diet (CGFD) in autistic children, and the frequency of consumption of nuts, seeds, berries and cruciferous vegetables has never been undertaken in monoethnic populations. Thus, the aims of this study were to investigate the changes in consumption of selected health-promoting foods after the exclusion of gluten and casein from the diet of Polish ASD patients.

Materials and Methods

Ethical Approval

The study was approved by the local Bioethics Committee. Written informed consent was obtained from the parents of recruited autistic children.

Sample

Patients with autism (ICD 10: F84.0) attending the Pediatric Clinic and outpatient services in the Mother and Child Institute in Warsaw in the years 2014–2016 were recruited for the study. The final sample included 88 children aged 3–7 years old, who have never before been treated with elimination diets, and their mothers. All participants were Caucasian of Polish origin recruited from the same geographical region. Mothers were offered a free choice of elimination diet (GFD or CGFD) or no diet for their children, and follow-up with a dietitian for at least one year. Exclusion criteria were food allergies, coeliac disease, as well as small credibility of the interview according to comorbidities. Mothers were asked to fill in a questionnaire upon qualifying for the study and after 12 months of using the elimination or regular diets.

Research tool

The Food Frequency Questionnaire (FFQ), developed by Wądołowska (25-27), was used to gather information about the frequency of consuming 62 assortment groups of products, representing eight main groups of food. Respondents had a choice of six categories of food intake frequency: (1) never or almost never, (2) once a month or less frequently, (3) several times a month, (4) several times a week, (5) daily, (6) several times a day. The questionnaire was validated and characterised by a high correlation between double measurements. The correlation coefficient for the frequency of consumption of product groups was on average 0.78 (95% average confidence: 95% CI = 0.73–0.83) (25).

Statistical analysis

Categorical variables were presented as a sample percentage (%) and continuous variables as means and standard deviation (SD). In order to compare data comprehensively, the Shapiro-Wilk test, the chi-squared test and the Wilcoxon signed rank test for dependent variables were used. P-values <0.05 were considered as significant. All statistical analyses were carried out in the STATISTICA PL software (StatSoft, Krakow, Poland).

Results

The sample of patients comprised mostly of boys (66; 75%) (Table 1). Of all participating mothers, 40.9% decided not to introduce an elimination diet. During the one-year follow-up, the intake of nuts, seeds, berries and cruciferous vegetables remained unchanged in ASD children on a regular diet (Table 2). The intake frequency of nuts, seeds and cruciferous vegetables increased in a statistically significant manner in patients on GFD (Table 3). A statistically significant increase in the consumption of berries and cruciferous vegetables was observed among users of

Table 1. General baseline characteristics of the groups

Variable	Gluten-free diet (GFD) N=25	Gluten- and casein-free diet (CGFD) N=27	Control group (regular diet) N=36	p
Age (months, X ±SD)	48.6 ±16.1	48.1 ±15.1	48.7 ±14.5	0.90
Gender (male %)	72	85	69	0.33
Cesarean section (%)	44	44	42	0.94
Length of breast-feeding > 3 months (%)	68	70	69	0.39

Table 2. Comparison of investigated food eating models of autistic children without dietetic treatment (Control group)

Food groups	At the beginning of observation (N=36) Subgroups of food intake frequency (n, %)						After 12 months of observation (N= 36) Subgroups of food intake frequency (n, %)						p
	Never or almost never	Once a month or less often	Several times a month	Several times a week	Every day	Several times a day	Never or almost never	Once a month or less often	Several times a month	Several times a week	Every day	Several times a day	
Nuts	16 (44.4)	8 (22.2)	8 (22.2)	3 (8.3)	1 (2.8)	0	18 (50)	8 (22.2)	4 (11.1)	6 (16.7)	0	0	0.548
Seeds	16 (44.4)	8 (22.2)	8 (22.2)	3 (8.3)	1 (2.8)	0	20 (55.6)	8 (22.2)	3 (8.3)	4 (11.1)	1 (2.8)	0	0.132
Berry fruits	14 (38.9)	6 (16.7)	11 (30.6)	3 (8.3)	2 (5.6)	0	15 (41.7)	6 (16.7)	11 (30.6)	2 (5.6)	1 (2.8)	1 (2.8)	0.732
Cruciferous vegetables	12 (33.3)	5 (13.9)	15 (41.7)	1 (2.8)	2 (5.6)	1 (2.8)	12 (33.3)	5 (13.9)	14 (38.9)	2 (5.6)	2 (5.6)	2 (5.6)	0.346

Table 3. Comparison of investigated food eating models of autistic children on gluten-free diet (GFD)

Food groups	Before dietetic treatment (N=25) Subgroups of food intake frequency (n, %)						After 12 months on the diet (N=25) Subgroups of food intake frequency (n, %)						p
	Never or almost never	Once a month or less often	Several times a month	Several times a week	Every day	Several times a day	Never or almost never	Once a month or less often	Several times a month	Several times a week	Every day	Several times a day	
Nuts	12 (48)	6 (24)	2 (8)	2 (8)	3 (12)	0	9 (36)	0	6 (24)	10 (40)	0	0	**0.012**
Seeds	10 (40)	5 (20)	3 (12)	4 (16)	2 (8)	1 (4)	5 (20)	2 (8)	6 (24)	8 (32)	3 (12)	1 (4)	**0.035**
Berry fruits	8 (32)	4 (16)	6 (24)	4 (16)	3 (12)	1 (4)	5 (20)	2 (8)	10 (40)	5 (20)	3 (12)	0	0.369
Cruciferous vegetables	3 (12)	4 (16)	13 (52)	5 (20)	0	0	2 (8)	1 (4)	9 (36)	10 (40)	3 (12)	0	**0.003**

Significant results are highlighted in bold font

CGFD (Table 4). The implementation of GFD resulted in more frequent consumption of seeds (p=0.003) and cruciferous vegetables (p=0.004) in comparison to patients without diet modification (Table 5). Children on CGFD exhibit a higher frequency of intake of seeds (p=0.018), berry fruits (p=0.003) and cruciferous vegetables (p=0.001) than controls (Table 5). Seeds were never/almost never eaten by 55.6% of children without dietetic treatment, 20% of patients on GFD and 29.6% of patients on CGFD. In both groups with dietetic intervention, the frequency of nut consumption tended to be higher than in patients on a regular diet (Table 5). Nuts were eaten at least several times a week and almost never by 16.6% and 50% of patients without dietetic treatment, 29.6% and 26% of patients on CGFD, and 40% and 36% of patients on GFD respectively.

Table 4. Comparison of investigated food eating models of autistic children on casein- and gluten-free diet (CGFD)

Food groups	Before dietetic treatment (N=27) Subgroups of food intake frequency (n, %)						After 12 months on the diet (N= 27) Subgroups of food intake frequency (n, %)						p
	Never or almost never	Once a month or less often	Several times a month	Several times a week	Every day	Several times a day	Never or almost never	Once a month or less often	Several times a month	Several times a week	Every day	Several times a day	
Nuts	10 (37)	6 (22.2)	5 (18.5)	4 (14.8)	2 (7.4)	0	7 (25.9)	3 (11.1)	9 (33.3)	6 (22.2)	2 (7.4)	0	0.226
Seeds	9 (33.3)	7 (25.9)	4 (14.8)	5 (18.5)	2 (7.4)	0	8 (29.6)	3 (11.1)	5 (18.5)	7 (25.9)	4 (14.8)	0	0.158
Berry fruits	10 (37)	2 (7.4)	6 (22.2)	6 (22.2)	3 (11.1)	0	4 (14.8)	3 (11.1)	7 (25.9)	7 (25.9)	4 (14.8)	2 (7.4)	0.062
Cruciferous vegetables	4 (14.8)	4 (14.8)	7 (25.9)	10 (37)	2 (7.4)	0	2 (7.4)	1 (3.7)	7 (25.9)	10 (37)	7 (25.9)	0	**0.033**

Significant result is highlighted in bold font

Table 5. Differences in investigated food eating models between autistic children after 12 month of dietetic treatment (GFD or GCFD) and after observation without dietetic treatment (control group)

Food groups	Group of patients with higher frequency of consumption	Group of patients with lower frequency of consumption	p
Nuts	GFD	Control group	0.080
	CGFD	Control group	0.088
Seeds	GFD	Control group	**0.003**
	CGFD	Control group	**0.018**
Berry fruits	CGFD	Control group	**0.003**
	GFD	Control group	0.092
Cruciferous vegetables	CGFD	Control group	**0.001**
	GFD	Control group	**0.004**

Significant results are highlighted in bold font

Discussion

Research on dietary patterns in children with ASD is often marred by lack of comparison groups; therefore, definitive conclusions cannot be drawn from the literature (23,24). In the presented study, two groups of patients with ASD under dietetic intervention were compared to control patients on a regular diet. The population under investigation was ethnically homogeneous. Our study showed that implementation of GFD/CGFD in ASD patients may result in higher frequency of consumption of nuts, seeds, berries and cruciferous vegetables, which has been shown to be linked with mental health (12-16).

There is growing acceptance that the incidence of autism has dramatically increased over the past half century (1-3). During this time, in Poland a large part of the population has moved to cities. Extensive home orchards and formerly typical gardens with black-, white- and red-currants, and gooseberries were liquidated, while the iconic cruciferous vegetable – the white cabbage (sauerkraut in winter) – is no longer the most important vegetable (28). The habit of buying ready-made bunches of vegetables, obligatorily containing a slice of a head of Savoy cabbage, imitating the tronchuda cabbage, for cooking soup, presumably associated with the influx of influential immigrants to Poland in the eighteenth century, has disappeared. Convenient food has largely replaced the relatively expensive shelled almonds, hazelnuts and walnuts as a snack. Knowledge of various recipes using berries (e.g. natural cranberry lemonade, bilberry/cranberry kissel, baked gooseberry turnovers, cranberry soup, raw redcurrant jelly) has decreased. The habit of harvest-

ing wild plants, including gluten-free grains of manna grass used specifically in children's diets, has been abandoned (29,30). Interestingly, Portugal with a high consumption of vegetables and traditional dishes with tronchuda cabbage is a country with relatively low/medium incidence of ASD (2,31). There is growing evidence of impairment of dynamic sulfur compounds homeostasis in ASD (13,32). Nuts, seeds and berries have been part of the human diet since prehistoric times. Interestingly, of the plant foods foraged by modern-day hunter-gatherers, an average 41% is made up of fruit rich in antioxidants but not in sugar, in comparison to the currently selected cultivars, of which 26% comes from tree nuts and unrefined whole seeds, but not from small grass seeds as in the past, 24% from underground storage structures and 9% from leaves, flowers, gums and miscellaneous parts of plants (33). Various historical sources reveal the association of nuts as food and popular drugs in several civilisations: hazelnuts have been cultivated in China for more than 5,000 years, pharaonic Egyptians valued pine nuts, and walnuts and pistachios are mentioned in the Bible, while Hippocrates, the Greek "father of medicine" and physicians from Muslim Iberia (Al-Andalus) regarded almonds as especially nutritious (34–36). Recently, a plethora of protective and therapeutic mechanisms of nut and seed constituents against neurological disorders are reported (12,37).

In our study, we have focused selectively on diet changes but not behavioural outcomes. Chan et al. (38), however, reported that according to the rules of the Shaolin temple a diet with a high intake of nuts positively influenced behavioural symptoms in autistic children. Recently, Lynch et al. (39) showed that anti-inflammatory phytochemical sulforaphone isolated from sprouts of broccoli, belonging to the *Brassicaceae* family, may reduce symptoms of autism. In mice, the polyphenolic compound resveratrol, which is mainly detected in berries, may ameliorate immunological dysregulation (40). Bearing the above observations in mind, the unflagging interest and subjective feelings of improvement in parents of autistic children on GFD/CGFD may not be linked solely to the direct results of gluten and/or casein elimination, but rather to the balance of the modified diet, including the interrelatedness of certain foods and its multidirectional impact (41). However, diet modifications may be among the factors strongly associated with the likelihood of financial problems in families that have a child with autism (42). Careful examination of exposure to different diets combined with broad analytical methods are necessary to understand fully the relationship between diet and autism and its underlying causal mechanisms, which may result in optimisation of diet composition. Further research should also include an evaluation of the recipes used and the methods of processing food products (43-45).

Conversely, the results of our study do not support the hypothesis that the popularity of GFD/CGFD diets among parents of ASD children is only due to a psychological mechanism, whereby parents are guided by feelings of regret for not using the diet, in fear that they had not tried all available interventions (46). Conscious selection of foods in the diet gains new faces, including aspects of the functioning of the family. Very recently, Harris et al. (47) showed that interventions intended to optimize nutrition in children with elevated autistic traits may integrate behavioural strategies to support parents' responding to their child's food selectivity.

In conclusion, despite doubts about the advisability of excluding gluten and casein *per se* from the ASD patients' diet (23), dietary interventions in autism should still form a particular research focus.

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