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Omega-3 and omega-6 polyunsaturated fatty acid intake and aberrant behaviors in Jordanian children with autism spectrum disorders (ASD): A pilot study

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ABSTRACT

Background: This study aimed to investigate the relationship between the daily intake of omega-3 polyunsaturated fatty acids (PUFA) and omega-6 PUFA, and aberrant symptoms in Jordanian children and adolescents.
Method: A sample of 37 children and adolescents aged between, 3–18 y, (76% males) diagnosed with autism spectrum disorders (ASD) were included in this study. Behavior and mental development were assessed using the Aberrant Behavior Checklist (ABC) questionnaire. Food frequency questionnaires were used to assess omega-3 PUFA and omega-6 PUFA intake. Food items were analyzed using gas chromatography to measure their omega fatty acid content. A Chi-square test was conducted to determine the difference between autism rating scale items and omega-3 PUFA, omega-6 PUFA, and the ratio of omega-3 PUFA: omega-6 PUFA intake.
Results: Omega-3 PUFA and omega-6 PUFA mean intakes were 0.31 ± 0.29 g/day and 5.15 ± 2.91 g/day, respectively. The results indicate that there is no significant difference in most autism scale items with omega-3 PUFA intake. Omega-6 PUFA was found to have a significant association with several scale items (p-value < 0.05). Moreover, we found that foods high in omega-3 PUFA (g/100 g) were walnuts (9.20), tuna (0.92), and sardine (0.90). Foods high in omega-6 PUFA were sunflower oil (63.3), corn oil (56.0), and soybean oil (53.0).
Conclusion: The results indicated that omega-3 PUFA and omega-6 PUFA mean intake were found to be lower than the recommended values in children with autism. This information will be useful for healthcare providers to consider omega-3 PUFA and omega-6 PUFA when planning dietary meals for individuals with autism.

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1. Introduction

Autism spectrum disorder (ASD) is a developmental disability that can result in a variety of difficulties with social interaction, communication, and behavior (Belmonte et al., 2004). The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines ASD as the presence of restricted, repetitive patterns of behaviors, interests, or activities (Belmonte et al., 2004). Over the past few decades, there has been an increase in the prevalence of ASD in several countries (Matson & Kozlowski, 2011). The Autism and Developmental Disabilities Monitoring (ADDM) Network sites estimated the prevalence of ASD grew from 6.7 (one in 150) per 1000 children aged 8 years in the surveillance years 2000 and 2002 to 27.6 (one in 36) in the surveillance year 2020 (Ghanizadeh, 2008). However, due to the lack of studies and information, tracking the prevalence of ASD in Jordan is difficult. According to estimates, Jordan reported 8000 cases of ASD in 2018 (Masri et al., 2013). Recently, there is growing evidence that nutritional therapy can make a significant difference for children with autism (Doreswamy, Dec 22 et al., 2020). Most children with autism suffer from severe digestive problems, and for that reason restoring balance in the gut is a key focus of nutritional therapy (Al-Hadid & Ahmad, 2008). A study conducted on autism participants in Jordan found several in-correct dietary practices and distinct negative dietary patterns are apparently such as the consumption of soft drinks, sweets and foods containing additives, which were the most problematic food groups with a reported negative effect on autism (Sadock & Sadock, 2007). Moreover, they found that intake of certain macro and micro- nutrients such as vitamins D and B12, thiamin, riboflavin, niacin, folic acid, calcium, selenium, magnesium, and iodine were clearly insufficient (Sadock & Sadock, 2007).

Polyunsaturated fatty acids (PUFAs) have been a topic of interest for many psychiatric diseases. PUFAs play an important role in brain functioning due to their anti-inflammatory properties and ability to keep healthy function of the membrane and myelin sheath of brain cells (Chez et al., 2007). Studies have implicated these fatty acids in various cognitive processes, including neurotransmission, synaptic plasticity, and neuroinflammation (Chez et al., 2007). Since the human body is unable to synthesize PUFAs de novo, it relies on the conversion of precursor fatty acids(Burdge,2018) However, the efficiency of this conversion process may vary among individuals due to genetic differences, a factor of particular interest when investigating PUFA intakes and their potential impact on neurological outcomes Several researchers have hypothesized that dietary behavior changes that led to an imbalance in PUFA consumption may be to blame for the recent rise in the prevalence of ASD (Chez et al., 2007). One of the most well-known PUFAs are the omega-3 and omega-6 PUFAs. Animal and vegetable oils are the sources of the latter, whilst the former is primarily derived from seafood (Miral et al., 2008). The Council on Food and Nutrition of the American Medical Association has established recommended daily intake ranges for omega-3 and omega-6 PUFAs based on age in children (Institute of Medicine, 2002). Specifically, the suggested intake is 0.7–1.2 g per day for omega-3 PUFA and 7–12 g per day for omega-6 PUFA (Institute of Medicine, 2002). According to several studies, the ideal intake of omega-6 PUFA to omega-3 PUFA should be between 1:1 and 4:1 (Miral et al., 2008). Omega-3 PUFA supplementation resulted in a slight reduction in emotional reactivity and oppositional behaviors in attention-deficit/hyperactivity disorder patients (ADHA), according to a recent meta-analysis (Doaei et al., 2018). Although social dysfunction is the primary symptom of ASD, many patients also experience other emotional or behavioral issues, such as hyperactivity and poor impulsivity (Wainwright, 2002; Berger et al., 2002).

Consequently, omega-3 PUFA could benefit children with ASD. For the brain's and the auditory and visual processing system to develop and function normally, omega-3 PUFA, particularly docosahexaenoic acid (DHA), are essential (Richardson, 2004). Evidence indicates that children with ASD have low blood concentrations of omega-3 PUFA and an increased omega-6 PUFA to omega-3 PUFA ratio, which may be caused by a poor diet or a variation in how fatty acids are metabolized and incorporated into cellular membranes in children with autism (American Psychiatric Association, 1994; Esmaillzadeh et al., 2007). In children, it also found clinically significant improvements in ASD symptoms with supplements in-take of omega-3–6-9, but effects were confined to one subscale (Keim et al., 2018).

According to research by Amminger et al. on 13 children with ASD. The Aberrant Behavior Checklist (ABC-H) hyperactivity score was reduced by 4.0 points in the treatment group compared to an increase of 3.0 points in the placebo group ($p = 0.098$), a non-significant finding with an effect size of 0.71. Moreover, Bent et al. (2011). randomly assigned 27 ASD children to receive omega-3 PUFA for 12 weeks versus a placebo, and they discovered that the omega-3 PUFA group's hyperactivity decreased by 2.7 points on the ABC-H compared to the placebo group's 0.3 points ($p = 0.4$), a difference that was statistically in-significant (effect size = 0.38). However, both earlier investigations were small and lacked the statistical power to prove efficacy, they actually demonstrate patterns that favored the omega-3 PUFA group.

To the best of our knowledge, few studies have focused on the effect of food intake of omega-3 PUFA and omega-6 PUFA on autism. Most studies in this field have focused on the effect of omega-3 PUFA supplementation alone on autism (Amminger et al., 2007; Bent et al., 2011). The main objectives of this study are: 1- To determine Omega-3 and omega-6 PUFAs concentrations in various food items. 2- To assess the association between omega-3 and omega-6 PUFAs intake from various food groups and the severity of autism symptoms in children and adolescents.'.

2. Methods

2.1. Subjects

Out of 45 participants, a total of 37 Jordanian children and adolescents aged between 3 to 18 years old and diagnosed with autism spectrum disorder. The participants included in our study were exclusively recruited from centers for autistic children, namely the Al-Aooj center, Al-Banafsaj center, and the Cerebral Palsy Foundation, located in the northern region of Jordan (Irbid) during the period of April-July 2015. Each participant had already been diagnosed with autism spectrum disorder by qualified and experienced clinicians specializing in developmental disorders. Participants who did not provide information on their age, sex, height, weight and food frequency questionnaire (FFQ) were excluded. Moreover, subjects who had any acute infection lasting longer than a week prior to the inclusion process were also excluded. The Human Research Review Committees of the participating centers gave their approval to the study. In addition, questionnaires were distributed to participants through parents' personnel interviews in which the purpose of the study was fully explained. Parents of participants gave their written consent.

2.2. Assessment of dietary omega-3 and omega-6 PUFas intake

A questionnaire (see Appendix A) was constructed according to (Sublette et al., (Lyall et al., 2013)) which was originally validated to assess omega-3 and omega-6 PUFAs. This questionnaire was developed using the National Cancer Institute's Diet History Questionnaire as a model (El-Ansary et al., 2011). The FFQ, which took participants approximately 5 min to complete, included an extensive list of 10 specific food groups that are commonly consumed by Jordanian society and were classified as in rich in omega-3 and omega-6 PUFAs content in previous studies (Lachman, 2014; Bloch & Qawasmi, 2011). The included food groups were: seafood (e.g. tuna, sardine), nuts (e.g. almond, walnut, pistachio), seeds (e.g. flax seed, sunflower seed, pumpkin seed), oils (e.g. corn oil, sunflower oil, olive oil, soybean oil), vegetable (e.g. turnip, cabbage, cauliflower, spinach), fruits (e.g. strawberry, kiwi, mango, guava), meat poultry and egg, milk product (e.g. cheese, sour cream), cereal and legumes (e.g. oat, bean, maize), and herbs (e.g. thyme, mint). The FFQ provided a range of food portion sizes. For instance, the terms small (2 ounces or less), medium (2–7 ounces), and large were used to describe the portion sizes (more than 7 ounces). The Diet History Questionnaire (DHQ) Nutrient Database's parallel items were used to calculate the sex-specific portion sizes (small, medium, and large) in grams per serving (Richardson et al., 2012). For each item, participants also were asked about frequency of intake over the last 6 months, with categories ranging from never to number of times each month, each week, or each day. Moreover, personal and medical questions modification were added to meet the objectives of this research such as monthly income, presence of other children diagnosed with autism, allergies, and drug use.

Daily dietary omega-3 and omega-6 PUFAs intake were determined by multiplying the portion size of a single serving of each food by its reported frequency of intake, then multiplying the total amount consumed by the nutrient content of the food and summing the nutrient contributions of all food items using the US Department of Agriculture Food Composition data (Amminger et al., 2010).

2.3. Evaluation of behavior and mental development

The child's behavior and mental development were evaluated by a questionnaire (see Appendix B) which is used in autism centers to measure the child's improvement. This questionnaire was adapted from the Aberrant Behavior Checklist (ABC) questionnaire (Freeman et al., 2006). The ABC questionnaire is an informant rating instrument that was empirically derived by principal component analysis. It has 58 components, which resolve to five sub-scales. The following are the subscales and their corresponding item counts: (15 items) irritability; (16 items) lethargy; (16 items) social disengagement; (7 items) stereotypic behavior; (16 items) hyperactivity; and (18 items) inappropriate speech (4 items). Any adult who is well acquainted with the client can complete the ABC form. This might be a parent, teacher, workshop leader, caseworker, or informant in yet another position. Completion times vary depending on reading ability; however, most raters can finish the ABC in 10–15 min. The validity and reliability of the aberrant behavior checklist have been tested (Richardson & Montgomery, 2005).

2.4. Anthropometric measurements

In all centers measurements were made three times by qualified researcher. To the nearest 0.05 kg, weight was measured and recorded using an advanced Tanita scale MC-780 U. To the nearest 0.1 cm, the height was measured and recorded from the top of the head to the sole of the feet. All measurements were made while barefoot and in under-pants. Body weight (kg) divided by height (m) squared (kg/m2) was used to compute BMI and compared to the weight-for-stature percentiles of the USA growth chart from the Centers for Disease Control and Prevention.

Table 1
Food Items.

Food group	Food items
Seafood	tuna, sardine canned in oil
Nuts	almond, walnut, pistachio
Seeds	flax, sunflower, pumpkin seed
Oils	corn, olive, sunflower, soybean oil
Vegetable	turnip, cabbage, spinach, cauliflower
Fruit	strawberry, kiwi, mango, guava
Meat and poultry	egg, chicken, meat(beef)
Milk product	Labaneh, cheese
Cereals and legume	oat, beans, maize
Herbs	mint, thyme

2.5. Polyunsaturated fatty acids analysis

Food items were analyzed to determine the amount (g/100 g of food) of omega-3 and omega-6 content using gas chromatography according to the procedure described by Chritopherson and Glass, (1969) method (Yehuda et al., 2002).

2.5.1. Reagents

All reagents are of analytical grade and were obtained from Sigma Aldrich chemicals. Analytical grade reagents hexane, potassium hydroxide, anhydrous methanol, and acetic acid, were purchased from Sigma –Aldrich Chemicals.

2.5.2. Preparation of fatty acid methyl ester

Fatty acid methyl esters (FAMEs) of oil were extracted from food samples (Table 1) and prepared according to Christopherson and Glass, (1969) method (Yehuda et al., 2002). Briefly; 50 mg of extracted oil was weighed, dissolved in 1 ml hexane (GC grade), and mixed by vortex for 1 min 0.2 ml of 2 M-potassium hydroxide, prepared in anhydrous methanol, was added and mixed for 30 s until the solution becomes clear, and then 0.2 ml of acetic acid was added and mixed for 30 s

2.5.3. Gas-liquid chromatography analysis

The prepared methyl esters were analyzed using a capillary GLC column (Restek, Rtx-225, USA, cross bond 50%-phenyl methyl-polysiloxane, 60 m, 0.25 mm/D, 0.25 μmdf) immediately after esterification by injection 1 μl of the hexane layer through the injection port of the GLC (model GC-2010, Shimadzu. Inc., Koyoto, Japan). The FAMEs were inject-ed after adjusting the GLC conditions; column oven temperature was 180° C for 10 min, increased to 200° C 5° C/min and kept at 200° C for 5 min, then increased to 210° C 3° C/min and kept at 210° C for 20 min. The injector temperature was 250° C, the flame ionization detector temperature was 260° C, the flow rate was 1.2 ml/min N2, and the split ratio used was 70. The fatty acids methyl esters (FAMEs) were identified using a chromatogram of fatty acids standard. Two replicates were used to calculate the mean value.

Table 2
General characteristics of Jordanian children with autism 3–18 years, where the results represented as a number and % value.

Variables	N (%)$
Sex	
Male	28(75.7)
Female	9(24.3)
Age	
Less than 7	21(56.8)
7-10	10(27.0)
Greater than 10	6(16.2)
Family Income (JD)	
Less than 200	5(13.5)
200 – 500	22(59.5)
Greater than 500	10(27.0)
Member in the family diagnosed with autism	
Yes	5(13.5)
No	32(86.5)
Number of meals	
Less than 3 meals	11 (29.7)
Three meals	24 (64.9)
More than 3 meals	2 (5.4)
Omega-3 daily intake (g)	
Lower than 0.21	12(32.4)
0.21-0.28	13(35.1)
higher than 0.28	12(32.4)
Omega-6 daily intake (g)	
lower than 3.61	12(32.4)
3.61-5.76	13(35.1)
higher than 5.76	12(32.4)
Body Mass Index (BMI)*	
Underweight	5(13.5)
Normal	21(56.8)
Overweight	11(29.7)
Using Drugs	
Yes	5(13.4)
No	32(86.5)

$Data are presented as percentage. *Body mass index (BMI) was calculated and compared to CDC growth chart for children.

3. Statistical analysis

The statistical package for social sciences software (SPSS, version 19, Chicago. Inc) was used for data processing and data analysis. Characteristics of subjects' variables were described using frequency distribution of categorical variables and mean and standard deviation for continuous variables. The chi-square was used to test the distribution of categorical variables on different omega-3 and omega-6 PUFAs categories.

One-way analysis of variance (ANOVA) was used to examine differences between numbers of meals and snacks categories and intake of omega-3 and omega-6 PUFAs. Moreover, the differences among age categories and omega-3 and omega-6 PUFAs intake were evaluated us-ing ANOVA. Finally, a chi-square test was conducted to determine the difference between autism rating scale items and omega-3, omega-6 and the ratio of omega-3: omega-6 PUFAs intake. p-value of < 0.05 was considered the cut-off level for statistical significance.

4. Results

4.1. General participants characteristics

Table 2 represents the general characteristics of a total of 37 Jordanian children with autism, who participated in this study. The age of participants ranged from 3 to 18 years old. The majority of sample was males with 75.7%, and only 24.3% females' participants.

The results indicated that (59.5%) of participants have a family income between 200–500 JD (middle SES), 13.5% of the participants have less than 200 JD family income (low SES) and only 27% of the participants exceeded the 500 JD monthly income (high SES). Moreover, the results shown that 13.5% of the participants have one member of the family diagnosed with autism, while the rest of the participants indicated that they are the only one in the family who has autism. Regarding BMI, (56.8%) participants were classified as normal weight and (29.7%) were classified as overweight. The number of daily meals consumed by children with autism, indicates that most of the participants (64.9%) consume three meals a day.

4.2. Relationship of Dietary Habits on omega-3 and omega-6 PUFAs Intake

The association between participants' dietary habits and omega-3 and omega-6 PUFAs intake was presented in supplementary (Table S1). The results revealed that the number of meals consumed by the targeted groups was 2, 3, 4, and 5 meals, where the mean value of omega-3 PUFA intake was, 0.35 ± 0.41, 0.31 ± 0.23, 0.22 ± 0.11, and 0.20 ± 0.09, respectively. Regarding omega-6 PUFA the mean intake were 5.47 ± 3.30 and 4.80 ± 2.52 and 3.23 ± 1.76 and 6.57 ± 4.32 g, respectively. These results indicated that no significant correlation was found between the number of meals and omega-3 or omega-6 PUFAs intake. The consumption of omega-3 PUFA was the high (0.35 g) in the two meals group and the values were decreased when the participants ate more frequently till it reached the lowest (0.2 g) in the five meals group. The consumption of omega-6 PUFA has the same tendency except for the five meals group shows the highest value of omega-6 PUFA. On the other hand, results indicated that the number of snacks consumed was 1 and 2 a day, with a mean value of omega-3 PUFA intake was 0.32 ± 0.29, and 0.21 ± 0.29, and omega-6 were 5.06 ± 2.59 and 4.11 ± 5.81 g. Upon these results, the number of snacks does not have any significant effect on omega-3 and omega-6 PUFAs intake. Moreover, there was no significant difference in omega-3 and omega-6 intake among the meal number.

4.3. Relationship between age and omega-3 and omega-6 PUFAs daily intake

The results showed a significant relationship between age and omega-3 and omega-6 PUFAs intake (Table 3), where it was higher in age > 10 years than in age < 7. The results also showed that participants aged between 7 and 10 years have the lowest omega-3 consumption values, compared to the younger or older participants.

4.4. General characteristics of the autism rating scale

Supplementary Table S2 shows the rating scale items for autism derived from the global system called the ABC checklist. This scale is composed of 15 items that assess a child's behavioral and mental development.

Table 3
The relationship between age and omega-3 and omega-6 PUFAs intake.

	Age categories			
	Less than 7	7-10	> 10	p-value
Omega-3 daily intake (g)	0.60 ± 0.24^{a}	0.21 ± 0.099^{b}	0.60 ± 0.48^{a}	0.016
Omega-6 daily intake (g)	4.39 ± 2.33^{b}	4.78 ± 3.01^{b}	7.53 ± 2.36^{a}	0.037

All values are means±SD. Differences among age categories and omega-3 and omega-6 PUFAs intake were evaluated using ANOVA. Values within the same column with similar superscript letters are not different. Boldface values indicate significance. P-value of < 0.05 was considered the cut-off level for statistical significance.

4.5. Relation of omega-3 PUFA and the autism rating scale

Table 4 shows the association between omega-3 PUFA and autism rating scale items. The result indicates that there is no significant difference in most items', except for one scale (put the rings in the column), which has a p-value of 0.025.

4.6. The relation of omega-6 PUFA and the autism rating scale

Table 5 shows the association between omega-6 PUFA daily intake and autism rating scale. The result showed a significant difference in some ratings (p-value is <0.05). These items are putting the ring in the column, visual eye contact, and the ability to distinguish sound.

4.7. The relation of the omega-6 to omega-3 PUFAs ratio and the autism rating scale

Table 6 shows the association between the ratio of omega-6 to omega-3 PUFAs on autism rating scale items. The result showed a

Table 4
The association between omega-3 PUFA daily intake (g) and autism rating scale items.

Scale items	Omega-3 Intake	Responds		P-value
		Yes	No	
		N (%)[$]	N (%)	
Visual contact	Lower 0.21	2(16.7)	10 (Ross et al., 2007)	0.289
	0.21-0.28	6 (Institute, 2009)	7 (Richardson & Montgomery, 2005)	
	Higher 0.28	4(33.3)	8 (Hamazaki et al., 2005)	
Ability to distinguish sounds	Lower 0.21	1(33.3)	11(32.4)	0.998
	0.21-0.28	1(33.3)	12(35.3)	
	Higher 0.28	1(33.3)	11(32.4)	
Says yes when calling his name	Lower 0.21	5(35.7)	7(30.4)	0.362
	0.21-0.28	3(21.4)	10(43.5)	
	Higher 0.28	6(42.9)	6(26.1)	
Social communication	Lower 0.21	3(42.9)	9 (Zanarini & Frankenburg, 2003)	0.805
	0.21-0.28	2(28.6)	11(36.7)	
	Higher 0.28	2(28.6)	10(33.3)	
Discrimination geometric shapes	Lower 0.21	3(23.1)	9(37.5)	0.215
	0.21-0.28	7(53.8)	6 (Richardson et al., 2012)	
	Higher 0.28	3(23.1)	9(37.5)	
Discrimination color	Lower 0.21	5 (Richardson et al., 2012)	7(41.2)	0.203
	0.21-0.28	6 (Zanarini & Frankenburg, 2003)	7(41.2)	
	Higher 0.28	9 (Vancassel, 2001)	3(17.6)	
Discrimination animals name	Lower 0.21	1(16.7)	11(35.5)	0.147
	0.21-0.28	1(16.7)	12(38.7)	
	Higher 0.28	4(66.7)	8(25.8)	
Discrimination fruits name	Lower 0.21	2(28.6)	10(33.3)	0.805
	0.21-0.28	2(28.6)	11(36.7)	
	Higher 0.28	3(42.9)	9 (Zanarini & Frankenburg, 2003)	
Independence	Lower 0.21	2(14.3)	10(43.5)	0.109
	0.21-0.28	5(35.7)	8(34.8)	
	Higher 0.28	7 (Institute, 2009)	5(21.7)	
Put the rings in the column	Lower 0.21	1(6.7)	11 (Institute, 2009)	0.021
	0.21-0.28	7(46.7)	6(27.3)	
	Higher 0.28	7(46.7)	5(22.7)	
Distinguishes the big parts of the body	Lower 0.21	4(30.8)	8(33.3)	0.839
	0.21-0.28	4(30.8)	9(37.5)	
	Higher 0.28	5(38.5)	7(29.2)	
Distinguishes danger and avoid it	Lower 0.21	0(0)	12(36.4)	0.129
	0.21-0.28	1 (Richardson et al., 2012)	12(36.4)	
	Higher 0.28	3[75]	9(27.3)	
Execution of the order	Lower 0.21	3(17.6)	9 (Vancassel, 2001)	0.176
	0.21-0.28	8(47.1)	5 (Richardson et al., 2012)	
	Higher 0.28	6(35.3)	6 (Zanarini & Frankenburg, 2003)	
Aggressive to other people	Lower 0.21	0(0)	12(35.3)	0.326
	0.21-0.28	1(33.3)	12(35.3)	
	Higher 0.28	2(66.7)	10(29.4)	
Hyperactivity	Lower 0.21	1 (Richardson et al., 2012)	11(33.3)	0.805
	0.21-0.28	2 (Institute, 2009)	11(33.3)	
	Higher 0.28	1 (Richardson et al., 2012)	11(33.3)	

[$] Data are presented as number and percentages, n (%). Boldface values indicate significance. P-value of < 0.05 was considered the cut-off level for statistical significance.

Table 5
The association between omega-6 PUFA daily intake (g) and autism rating scale.

Scale items	Omega-6 intake	Responds		P-value
		Yes	No	
		N (%)$	N (%)	
Put rings in the column	Lower 3.61	1(6.7)	11 (Institute, 2009)	0.008
	3.61-5.76	9[60]	4(18.2)	
	Higher 5.76	5(33.3)	7(31.8)	
Visual eye contact	Lower 3.61	1(8.3)	11 (Agostoni et al., 2017)	0.014
	3.61-5.76	8(66.7)	5 (Bent et al., 2011)	
	Higher 5.76	3 (Richardson et al., 2012)	9 (Sathe et al., 2017)	
Ability to distinguish sounds	Lower 3.61	0(0)	12(35.3)	0.049
	3.61-5.76	3(100)	10(29.4)	
	Higher 5.76	0(0)	12(35.3)	

$ Data are presented as number and percentages (%). Boldface values indicate significance. P-value of < 0.05 was considered the cut-off level for statistical significance.

Table 6
The ratio between omega-6 to omega-3 PUFAs on autism rating scale items.

Scale items	Omega-6: omega-3 daily intake (g)	Responds		p-value
		Yes N (%)$	No N (%)	
Independence	< 14.88	7 (Institute, 2009)	5(22.7)	0.026
	14.89-19.71	1(7.1)	11 (Institute, 2009)	
	> 19.72	6(42.9)	6(27.3)	
Execution of the order	< 14.88	4(100)	8 (Richardson et al., 2012)	0.011
	14.89-19.71	0	12(37.5)	
	> 19.72	0	12(37.5)	
Aggressive to other people	< 14.88	3(100)	9(27.3)	0.038
	14.89-19.71	0	12(36.4)	
	> 19.72	0	12(36.4)	

$ Data are presented as number and percentages (%). Boldface values indicate significance. P-value of < 0.05 was considered the cut-off level for statistical significance

significant difference in 3 rating scales (independence, execution of the order, and aggression towards other people). Also, the results indicated that the omega-6 to omega-3 PUFAs ratio exceeds the optimum ratio.

4.8. Omega-3 and omega-6 PUFAs amounts in food

Omega-3 and omega-6 PUFAs concentrations in various food items (seafood, nuts, seeds, oils, fruits, vegetables, dairy products, meat, and herbs) are shown in Table 7. The results showed that the highest omega-3 PUFA amount was in walnuts (9.16 g/100 g) followed by soy-bean oil, flaxseed, tuna, and sardine with a concentration of 6.38, 5.46, 0.917, and 0.898 g/100 g, respectively. A moderate amount of omega-3 PUFA was found in corn oil, olive oil, guava, thyme, and almond 0.85, 0.74, 0.53, 0.45, and 0.36, respectively. The lowest amounts of omega-3 PUFA were found in thyme, almond, kiwi, oat, turnip, sunflower oil, mint, strawberry, maize, and cabbage ranging from 0.451 to 0.015 g/100 g. Also, the results (Table 7) showed that the highest omega-6 PUFA content was in sunflower oil (63.3 g/100 g), followed by corn oil, soybean oil, walnut, and sunflower seed, 55.98, 52.8, 37.13, 31.984 and 19.95 g/100 g, respectively. A moderate amount of omega-6 PUFA was found in pistachio, almond, olive oil, tuna, chicken, and sardine 13.7, 11.323, 9.5, 4.68, 3.211, and 2.748 g/100 g, respectively. The lowest amounts of omega-6 PUFA were found in kiwi, strawberry, guava, spinach, mango, turnip, and cabbage with concentrations ranging from 1.0 to 0.002 g/100 g.

5. Discussion

The main results suggest that no correlation was found between omega-3 and omega-6 PUFAs intakes and number of meals consumed per day or body mass index. Meanwhile, the significant determinant of omega-3 and omega-6 PUFAs intake in this study was age. Moreover, we found that there are correlations and trends in omega-3 and omega-6 PUFAs intake with items on the Aberrant Behavior Checklist.

In accordance with this work, Amminger et al (Zanarini & Frankenburg, 2003). examined the effects of omega-3 PUFA supplementation in children with autism and found that omega-3 PUFA treatment reduced hyperactivity and stereotyped behavior. Another study found that giving children with ASD an omega-3 PUFA supplement for 12 weeks reduced their hyperactivity (Hibbeln et al., 1998). Moreover, it has been found that omega-3 and omega-6 PUFAs supplementation for 3 months improved language development in children at risk for ASD (Hamazaki et al., 2005). On other hand, a systematic review identified that omega-3 PUFA supplementation

Table 7
Average omega-3 and omega-6 PUFAs concentration (g/100 g of food) in various food items collected from different local markets in Jordan.

Food groups	Food items	Fat content g/100 g of food	Omega-3 Mean	Omega-6 Mean
Seafood	Tuna canned in oil	8.08	0.917	4.68
	Sardine canned in oil	11.45	0.898	2.748
Nuts and seeds	Walnut	65.21	9.16	37.13
	Almond	52.83	0.355	11.323
	Pistachio	45.97	0.189	13.7
	Flax seed	36.29	5.64	19.7
	Pumpkin seed	49.05	0.155	19.95
	Sunflower seed	49.80	0.053	31.984
Oils	Soybean oil	100	6.38	52.8
	Corn oil	100	0.85	55.98
	Olive oil	100	0.74	9.5
	Sunflower oil	100	0.08	63.3
Vegetables	Cauliflower	0.45	0.109	0.052
	Turnip	0.30	0.084	0.015
	Spinach	0.26	0.04	0.057
	Cabbage	0.17	0.015	0.002
Fruits	Guava	0.95	0.532	0.062
	Kiwi	0.52	0.264	0.076
	Mango	0.27	0.051	0.022
	Strawberry	0.30	0.037	0.066
Meat and milk products	Chicken	12.40	0.25	3.211
	Meat (beef)	3.40	0.046	0.201
	Labaneh	2.32	0.17	1.52
	Egg	10.01	0.115	1.053
	Cheese	31.25	0.021	0.628
Cereals and legume	Oat	4.64	0.092	1.779
	Beans	0.32	0.059	0.1
	Maize	1.41	0.019	0.49
Herbs	Thyme	7.43	0.451	1.004
	Mint	0.73	0.038	0.393

improved attention deficiency in children with autism (Lauritzen et al., 2016). Another study reported that omega-3 PUFA treatment improved reading skills in children with lowest initial reading skills (Mischoulon & Freeman, 2013). There is evidence to suggest that omega-3 and omega-6 PUFAs play an important role in reducing autism symptoms such as aggression and impulsivity.

Reviews of research involving children aged two to eight years old found no evidence supporting the benefits of omega-3 PUFA supplementation for enhancing behaviors such as internalizing, adaptive functioning, and hyperactivity in those with ASD (Karhu et al., 2020; Sathe et al., 2017; Williamson et al.,2017). However, specific trials targeting children within different age brackets revealed noteworthy outcomes. In a study with participants aged three to ten years, a three-month dietary supplementation with DHA (247 mg) demonstrated enhancements in a broad spectrum of learning and language skills (Patrick and Salik., 2005). Another trial focusing on children aged three to 11 years, supplementing with DHA (240 mg), EPA (52 mg), gamma-linolenic acid (48 mg), and omega-6 (20 mg) for three months, reported a statistically significant reduction in autism severity for 67% of the participants (Ooi et al., 2015). A third trial, including children aged seven to 18 years and supplementing with DHA (840 mg), EPA (192 mg), gamma-linolenic acid (144 mg), and omega-6 (66 mg) for three months, documented statistically significant improvements in various domains of social responsiveness and attention (Ross et al., 2007). However, the nonrandomized design of these trials presents challenges in attributing effects solely to the treatment, and the broad age ranges of participants hinder a nuanced understanding of potential impacts during early childhood, when interventions may be most influential. Noteworthy, a key difference between previous trials of young children (i.e., age 8 years and younger) is the combination of long-chain polyunsaturated fatty acids used.

Although the underlying mechanism of how omega-3 PUFA affects the brain and symptoms of ASD is not fully understood, the possible explanation of these results is that it may be linked to the modulation of serotonergic and dopaminergic systems. The noradrenergic system may be related to omega-3 PUFAs including DHA, eicosapentaenoic acid (EPA), and arachidonic acid (Hamazaki et al., 2005; Lauritzen et al., 2016). Even at the low dose of 762 mg of EPA plus DHA per day, supplementation from both fatty acids reduced plasma norepinephrine concentrations in healthy participants. The central noradrenergic system, which is important for regulating impulsive behaviors linked to these neurotransmitters like aggression, may also be altered by DHA (Hamazaki et al., 2005; Lauritzen et al., 2016). DHA and EPA also play important roles in the synthesis of membrane phospholipids, particularly in the central nervous system (Mischoulon & Freeman, 2013). Omega-3 PUFA, which is one of the macronutrients, are thought to play a neuro-protective role in the production of synaptic maintenance (Hallahan & Garland, 2005), modulation of brain cell signaling, regulation of monoamines production, and receptor signal transduction pathway (Hallahan & Garland, 2005; Doaei et al., 2019; Galli et al., 1972), which may help to explain the role of omega-3 PUFA in psychiatric disorders like autism (Galli et al., 1972; Agostoni et al., 2017). Omega-3 PUFA and their metabolic products provide a solid foundation because they play a role in ASD through their role in brain structure and brain function, neurotransmission, cell membrane structure, and microbial domain organization (Vancassel, 2001). DHA has a significant role in neuronal cell signaling as evidenced by the fact that it is substantially abundant in neural and synaptic membranes. It is sterically incompatible with cholesterol and results in either DHA or cholesterol-rich lipid rafts. It is preferentially integrated into phosphatidylethanolamine and phosphatidylserine in the inner layer of synaptic membranes. The fluidity of the fatty acid chains in membranes, ion permeability, flexibility, protein activity, phase behavior, and fusion are also affected by DHA Regarding omega-6 PUFA, lower intake was associated with lower respond to some autism rating scales. Small studies have shown that children with autism have increased levels of omega-6 PUFA metabolites, prostaglandin E2, increases the risk of neuroinflammation, which can lead to excessive production of reactive oxygen species causing DNA damage, proteolysis, and lipid damage, affecting the growth, development, and migration of neurons (Hallahan & Garland, 2005; Doaei et al., 2019; Galli et al., 1972). However, no studies evaluated the relation between omega-6 PUFA only and autism.

In our study we found that the ratio between omega-6 to omega-3 PUFAs significantly associated with (independence, execution of the order, and aggression towards other people) autism rating scale. A similar finding was reported on neurotypical people, which found that omega-3 PUFA deficit or imbalanced ratio between omega-3 and omega-6 PUFAs have been linked to various neurodevelopment disorders such as attention deficiency, hyperactivity disorder, dyslexia, and dyspraxia (Johnson, 2011; Freeman,2000). In the same vein, these results agree with Johnson et al., study which found an imbalance ratio between omega-3 to omega-6 PUFAs in the blood sample of patients with autism (Anonymous, 2013). To the best of our knowledge, there are no studies that examine the relation of omega-3 and omega-6 PUFAs from dietary sources, most of studies focus on the supplantation effect.

Regarding our results of analysis omega-3 and omega-6 PUFAs in various food items. The supplementary table, S3, shows a comparison between the average omega-3 and omega-6 PUFAs amount (g/100 g food item) in some Jordanian food items, which were tested with corresponding items listed in other countries including Finland, France, Denmark, and Canada. The results showed that most of the omega-3 and omega-6 PUFAs contents of the selected food items were similar to four of the mentioned countries. However, the only difference in omega-3 PUFA content were found in tuna (0.917 g/100 g), which was higher than the values of Finland tuna which was 0.4 g/100 g (ANSES, 2012), France tuna with a content of 0.293 g/100 g (Institute, 2009), and Denmark tuna with a content of 0.71 g/100 g (US Department, 2015). The possible explanation of this difference could be depending on some factors including the diet of each fish species, the season and location of the catch, the age and physiological status of the individual organism, and reproductive cycles [52].

This study has several strength points. First, this study can be generalized as a national Jordanian study, since it was carried out on children with autism from different centers in Irbid/Jordan. The omega-3 and 6 PUFAs intake were precisely determined on food items locally produced or imported. Moreover, this study examines the relation be-tween omega-3 and omega-6 PUFAs intake and autism symptoms. However, this study had some limitations. First, although the sample size was calculated with the power accepted for the study, the results should be confirmed so further studies with larger population samples and longitudinal observation are needed. Moreover, this study's nature does not allow us to assess the behavior over a period of time and did not provide information in determining the cause-and-effect association. The FFQ were used for collecting the food consumption data, and therefore, a social bias should be considered. Finally, this study did not take into account total energy intake, physical activity, and paternal education. Despite the limitation, these results may suggest useful information to promote proper selection of healthy foods for reduce the severity of autism in children. Further studies are needed to increase our understanding on the effect of omega-3 and omega- 6 PUFAs on social and behavioral disorders of children with autism.

6. Conclusion

The main findings of this study indicate a potential correlation between omega-3 and omega-6 PUFAs intake and the amelioration of autism symptoms, accounting for age and sex variations. Notably, the ratio of omega-6 to omega-3 PUFAs demonstrated a significant association with the autism rating scale, particularly in aspects such as independence, execution of orders, and aggression towards others. Furthermore, the results indicated that omega-3 and omega-6 PUFAs mean intake were found to be lower than recommended value in children with autism, emphasizing the importance of considering these fatty acids in dietary planning. This study highlights specific food items rich in omega-3 and omega-6 PUFAs, such as walnuts, soybean oil, flaxseed, tuna, and sardines for omega-3 PUFA, and sunflower oil, corn oil, soybean oil, walnuts, and sunflower seeds for omega-6 PUFA. This information is valuable for healthcare providers in planning dietary interventions for individuals with autism. Caregivers and autism centers are encouraged to incorporate good sources of omega polyunsaturated fatty acids into the diets of children with autism. Further longitudinal studies are needed to increase our understanding on the effect of omega-3 and omega- 6 PUFAs on social and behavioral disorders of children with autism.

Ethics approval and consent to participate

This study was approved by Human Ethical Committee approval at Jordan University of Science and Technology.

CRediT authorship contribution statement

Sondos Flieh: Writing – review & editing, Writing – original draft, Formal analysis. **Taha Rababah:** Supervision. **Ali Almajwal:** Software. **Numan Al-Rayyan:** Writing – review & editing. **Ghazi Magableh:** Visualization. **Tha'er Ajouly:** Software. **Muhammad Al-u'datt:** Writing – review & editing. **Sana Gammoh:** Writing – review & editing.

Declaration of Competing Interest

We declare no conflict of interest.

Data Availability

The authors do not have permission to share data.

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Author contributions

Flieh.SM analyzed the data and wrote the manuscript. Rababah.T, and the rest of co-author revised the manuscript and provided their essential comments. All authors have read and agreed to the published version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rasd.2024.102386.

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