RESEARCH ARTICLE

Omega-3 Fatty Acid Supplementation Improves Attention Deficit-Hyperactivity Disorder Symptoms in Children

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Abstract: ***Background***: Attention Deficit-Hyperactivity Disorder (ADHD) is one of the most common neuropsychiatric disorders in children. Increasing evidence suggests an association between omega-3 fatty acid and ADHD. We aimed to investigate the effects of 6-month omega-3 fatty acid supplementation on the fatty-acid profile of erythrocytes and on the clinical severity of ADHD symptoms in children.

Methods: Recruitment included 32 children aged 6-14 years diagnosed with ADHD at the Child and Adolescent Psychiatry Department at Ziv Medical Center, Israel. Intervention included refrigerated omega-3 fatty acid supplementation normalized to body weight. Blood samples were taken at baseline, 3 and 6 months after intervention for quantification of fatty acids from erythrocyte membranes. ADHD symptoms were assessed by parents and teachers according to the ADHD Rating-Scale and the Strengths and Difficulties Questionnaire. ADHD severity was additionally assessed by a psychiatrist according to the Clinical Global Impressions Scale.

Results: Supplementation of omega-3 fatty acid in children with ADHD raised their omega-3 index statistically significantly from an average of 4.4% omega-3 index at baseline to 11.6% after 6 months and had beneficial effects on ADHD symptoms, as measured by validated questionnaires and in accordance with a pediatric psychiatrist examination.

Conclusion: Our pilot study showed that dietary supplementation of omega-3 fatty acid increased the blood omega-3 index levels and improved ADHD symptoms even at the midpoint of 3 months.

Keywords: ADHD, fatty acid, omega-3 fatty acid, supplementation, CGI-S, ADHD-RS

1. BACKGROUND

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders in children [1]. ADHD is defined as a persistent and pervasive pattern of inattention and/or hyperactivity-impulsivity that interferes with function and results in a negative impact on psychological, social and academic/occupational ability. The conventional treatment is based on the combination of psychological therapy and pharmacotherapy. Structural and functional neuroimaging studies have recognized a possible link between ADHD status and the central nervous system (CNS) network and function [2]. Yet, the cause of ADHD is not precisely known, and its etiology is multifactorial, involving both neurobiological and environmental influences. Optimal brain function requires a stable metabolic environment, including the supply of all necessary nu-

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trients. Omega-3 fatty acid is one of the nutrient factors possibly involved in ADHD pathogenesis [3].

The CNS is the organ system with the second largest concentration of lipid content. Out of their dry weight, nerve tissues contain about 60% lipids, and approximately 40% of these lipids are polyunsaturated fatty acids (PUFAs) [4]. Long-chain PUFAs are necessary for normal brain development and function. PUFAs are subcategorized according to the position from the methyl end of the acyl chain of their first double bond. They are, therefore, subcategorized as omega-3 [alpha-linolenic acid (ALA, 18:3n3), eicosapentaenoic acid (EPA, 20:5n3), docosapentaenoic acid (22:5n3), docosahexaenoic acid (DHA, 22:6n3)], omega-6 [linoleic acid (LA, 18:2n6), dihomo-gamma-linolenic acid (DGLA, 20:3n6), arachidonic acid (20:4n6)], and omega-9 families. Dietary consumption of the essential fatty acids LA and ALA is necessary for human growth and development since mammals lack the ability to synthesize them. There are various mechanisms by which PUFAs could affect cell functions [5]. In general, omega-3 fatty-acids have anti-inflammatory [6, 7], anti-arrhythmic [8], and anti-thrombotic [9] effects, while most omega-6 fatty acids tend to cause inflammation and thrombus formation [10]. The main omega-3 PUFA in the CNS is DHA, which has critical roles in complex structural and functional processes [11]. The high concentration of DHA in brain gray matter suggests that these fatty acids may play roles in neural function. DHA is transferred across the placenta [12], is present in human milk, and normally accumulates in the brain during fetal and infant development. Dietary intake of DHA has been shown to directly increase DHA levels in brain membranes, therefore basing the relevance of fatty acids in the diet on those in the brain [13]. While DHA is synthesized in the human liver, it is unclear if this biosynthesis is sufficient to support optimal brain development and function [14]. The omega-3 index, calculated as the sum of EPA and DHA, is expressed as a percentage of total fatty acids in red blood cell (RBC) membranes and is based on a standardized analytical method [15]. This index reflects the dietary fatty-acid intake mean completed within the RBC lifespan (up to 120 days). A target range for the omega-3 index of 8–11% has been associated with the lowest mortality risk in coronary heart disease and incidence of mental health diseases [16].

Some studies have shown that physical ADHD symptoms are similar to the symptoms observed in the deficiency of essential fatty acids in humans [17]. Animal studies have shown that the depletion of DHA from the brain results in learning deficits [18]. Other studies suggest an association between nutritional low levels of PUFAs, especially omega-3, and increased incidence of ADHD [19]. Additional observations showed reduced levels of omega-3 fatty acid in the blood of children with versus without ADHD [20-22]. The effect of omega-3 fatty acid supplementation has been widely studied, however, the methodological heterogeneity across studies, including variations in study duration, type and dosage of supplementation, makes it difficult to compare the findings and draw strong conclusions about the efficiency [23, 24]. Our study aimed to evaluate the effects of 6-months dietary supplementation of omega-3 fatty acid on clinical severity of ADHD symptoms as well as on the fatty-acid profile in children's RBC membranes.

2. METHODS

2.1. Study Design and Ethics

The study was authorized by the Ziv Medical Center Helsinki Committee, Israel Ministry of Health, and registered at clinicaltrials.gov under number NCT02391428. Written informed consent was obtained from parents, and verbal agreement was established with children before entering the study.

2.2. Participants

A total of 32 Israeli children aged between 6 and 14 years (mean age=10.2 years, SD=2.0) were involved in the study. They were recruited through the Department of Child and Adolescent Psychiatry at the Ziv Medical Center in Safed in the north of Israel. Children's diagnosis of ADHD was confirmed by a child psychiatrist according to the criteria of Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) [25]. Exclusion criteria included a history of other psychiatric disorders, allergy to fish, severe chronic or autoimmune disorders, or previous supplementation of omega-3 fatty acid.

2.3. Blood Samples

(3 ml) were taken by venipuncture at three time-points: recruitment (before intervention), 3-

months, and 6-months. RBCs were isolated immediately after collection by centrifugation at 2,500g, for 10 minutes at room temperature and then stored at -80°C for subsequent analyses. Researchers were blinded for the status of samples when performing analysis.

2.4. Fatty-acid composition

Fatty-acid composition was analyzed in separated and thawed RBCs by the omega-3 index methodology using gas chromatography. Fatty-acid methyl esters were generated from RBCs by acid transesterification and analyzed using gas chromatography (GC2010, Shimadzu, Duisburg, Germany), using hydrogen as carrier gas. A total of 26 fatty acids were quantified and validated by a standard mixture of fatty acids. Results were calculated as a percentage of total identified fatty acids after response factor correction.

2.5. Intervention

Children with ADHD were given omega-3 capsules (fish oil containing 400 mg EPA and 200 mg DHA) and vitamin E capsules (400 IU alpha tocopherol per 3000 mg omega-3) with clear instructions to store all capsules in the refrigerator. Effective absorption of omega-3 capsules requires fat digestion, so children were instructed to take capsules with routine fat in a meal. The dose was set at 100 mg EPA+DHA per kg body weight per day, based on numerous safety and efficacy studies with similar and even higher dosages of omega-3 fatty acid supplementation in children with ADHD [20, 26]. Participants were required to take capsules daily for 6 months and were instructed to maintain their usual level of physical activity and diet during the intervention and to report any symptoms, medications, or changes in habits.

2.6. ADHD Symptoms Evaluation

At the beginning of the study and after 3 and 6 months, all children were clinically evaluated for ADHD symptoms by a child psychiatric examination, including Clinical Global Impression-Severity scale (CGI-S) assessment [27]. The CGI-S is a 7-point scale that measures the overall clinical status of a subject, with scores ranging from 1 (not severe) to 7 (extremely severe). Additionally, symptom severity was assessed using questionnaires filled out by a teacher and parents of every child, after giving explanations at the initiation of the study, and 3 and 6 months later. The questionnaires used were:

1. **ADHD Rating Scale IV (ADHD-RS)** [28] is composed of 18 symptoms of ADHD as defined in the DSM-5 which are scored by a parent or a teacher. The ADHD-RS uses a four-point response scale that ranges from 0 to 3 (0=never or rarely, 1=sometimes, 2=often, and 3=very often). The test includes 9 items reflecting symptoms of inattentiveness and 9 items reflecting symptoms of hyperactivity/impulsivity. Higher scores indicate worse attention and impulse inhibition abilities. If the child's total score ranged below 9 or between 10-14, the child was classified as having mild or moderate ADHD, respectively. Children who scored 15 or above in the ADHD-RS were considered to have severe ADHD.
2. **The strengths and difficulties questionnaire (SDQ)** [29] is a behavioral screening questionnaire which included 25 items on psychological attributes offering information about “emotional symptoms”, “conduct problems”, “hyperactivity/inattention”, “peer relationship problems”, and “prosocial behavior” [30]. Items were scored by a 3-point scale: not true, somewhat true or certainly true, with higher scores indicating greater difficulties (range 0-40). SDQ scores were categorized as accepted in previous studies to 'normal', 'borderline' or 'abnormal'.

Finally, the average scores of the questionnaires were calculated and considered as criteria for clinical response.

2.7. Statistical Analysis

For categorical variables, summary tables are provided, giving sample size and absolute frequencies. For continuous variables, summary tables are provided giving arithmetic mean (M) and standard deviation (SD). The independent-sample t-test or Mann-Whitney non-parametric tests were applied for testing differences between control and ADHD groups. The Friedman Multiple Comparisons non-parametric tests were applied for testing quantitative parameters between the 3 times of measurements (0, 3 and 6 months). P-value of 5% or less was considered statistically significant. All data were analyzed using SPSS version 24 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

The study included 32 Israeli children with ADHD. After parental signing of informed consent, participants with ADHD were given a supply of refrigerated omega-3 capsules in the form of fish oil concentrate capsules containing 400 mg EPA and 200 mg DHA. Dosage was calculated according to body weight (100 mg/kg of omega-3 daily), the average pill supplementation of omega-3 fatty acid was 6.3±1.9 capsules per day. Additionally, vitamin E capsules were supplemented to eliminate fatty acid oxidation during the study. Our results showed that omega-3 fatty acid was a safe supplement and did not induce any serious adverse effects and was generally well tolerated. These findings are in agreement with other studies assessing the safety of dosage and long-term administration. Compliance was measured according to consumed pill count, as well as self-reports. High compliance was defined when over 80% of prescribed pills were taken. Out of the 32 ADHD children- 24 children (75%) completed 3 months of the trial, and 15 children (47%) completed the 6-months intervention with high compliance (Table **1**). The majority of dropouts occurred due to swallowing problems (n=9), family issues (n=5), and feeling a lack of influence (n=3).

3.1. Omega-3 supplement effectively alters the fatty-acid profile in ADHD children

Dietary supplementation of omega-3 fatty acid in children with ADHD altered the fatty-acid composition of RBC membranes already after 3 months, as seen in the data summarized in Table **2**. As expected, when comparing the omega-3 fatty acid levels at baseline to levels after 3 months of intervention, there was a rise in the omega-3 index (11.35% vs. 4.4% averages, $p<0.001$, Fig. **1** and Table **2**). In children who completed the 6-month trial, the average omega-3 levels were maintained and even slightly raised, as compared to 3-months of intervention (Fig. **1** and Table **2**). The supplementation of omega 3 fatty acid statistically significantly altered relative levels of most fatty-acids screened (n=21/26 measures), including raising levels of DHA by almost 2 folds and EPA by almost 10 folds, while lowering relative levels of omega-6. Thus, the omega-6/omega-3 ratio was also lower in ADHD children after 3 and 6-months of intervention, as compared to baseline (Table **2**). These results show that the omega-3 fatty acid supplementation had an effect on the fatty-acid profiles in RBC membranes.

Evaluation of omega-3 index and Clinical Global Impression-Severity scale (CGI-S) were performed at the beginning of the study and after 3 and 6 months of intervention (n=15). Mean omega-3 fatty acid (in blue ◆), represents index as calculated by the sum of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) ±statistical error of the mean . CGI-S (in red ▲), presented mean ±statistical error of the mean as clinically examination for ADHD symptoms.

3.2. Omega-3 Fatty Acid Supplementation Improves Symptoms in Children with ADHD

We tested whether the omega-3 fatty acid intervention also has an effect on ADHD symptoms. We first compared the results of the CGI-S before vs. after the intervention as assessed by a child psychiatrist. At baseline, the average CGI-S score of participants was markedly ill=4.9 (range 4-6) (Fig. **1**). Following the intervention, we observed

Table 1. Comparison of supplementation compliance at 3 and 6 months.

Compliance of Omega-3		Body weight (Kg)		80%+		50-80%		<50%		p
Intervention (months)	**Pills (n)**	**Mean**	**SD**	**n**	**%**	**n**	**%**	**n**	**%**	
3	4-5	25.9	2.6	12	50.0	1	50.0	1	14.3	0.018
	6-7	36.6	4.1	3	12.5	1	50.0	5	71.4	
	8-10	51.1	7.1	9	37.5	0	0	1	14.3	
6	4-5	27.6	3.2	10	66.7	1	20.0	2	33.3	0.100
	6-7	40.4	6.9	2	13.3	0	0	2	33.3	
	8-10	58.2	9.5	3	20.0	4	80.0	2	33.3	

Compliance was measured according to pills count and self-reports throughout the intervention. Pills dosage was calculated according to body weight.

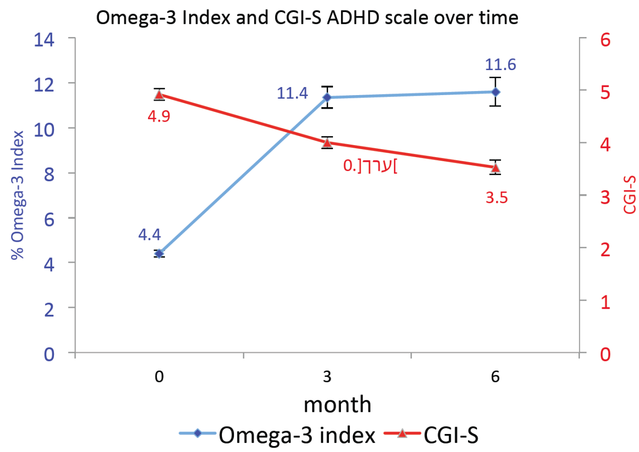

Fig. (1). Omega-3 index and CGI-S ADHD scale at 0, 3, and 6-months. Evaluation of omega-3 index and Clinical Global Impression-Severity scale (CGI-S) were performed at the beginning of the study and after 3 and 6-months of intervention (n=15). Mean omega-3 fatty acid (in blue ◆), represents index as calculated by the sum of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) ±statistical error of the mean. CGI-S (in red ▲), presented mean ±statistical error of the mean as clinically examination for ADHD symptoms. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

an improvement as seen by lowering CGI scores to 4.0±0.6 after 3 months, and further lowering to 3.5±0.5 after 6 months in children with ADHD (p<0.001, Fig. **1**). Scoring after 6 months included 7 participants with CGI-S=3, and 8 with CGI-S=4. Additionally, at baseline, the ADHD-RS average score, as reported by parents and teachers was higher than 15 indicating worse attention and impulse inhibition abilities (Fig. **2** and Table **3**). At starting position also, SDQ scores were categorized as 'abnormal', indicating greater ADHD difficulties (Table **4**). There appeared to be an improvement in the ADHD symptoms reported by both parents and teachers, as seen by the results of the ADHD-RS (Fig. **2**) and SDQ questionnaires (Table **4**).

ADHD Rating Scale-IV (ADHD-RS) questionnaires filled by parents (A) and teachers (B) were used to evaluate clinical severity of attention-deficit hyperactivity disorder (ADHD) symptoms at the beginning of the study and after 3-months of intervention. Averages were calculated for compliant participants with complete questionnaires at both times (n=24). Values are reported as mean ±standard deviation. *Indicate significant differences between groups.

According to the ADHD-RS questionnaire, statistically significant improvement was found in the mean total scores of the questionnaires obtained from both the parents and teachers (Fig. **2** and Table **3**). Comparing answers between baseline and after 3 months of omega-3 fatty acid supplementation showed statistically significant lower scores in both "inattentive" and "hyperactive" parameters as reported by the parents following intervention (Fig. **2**). While this was also the trend in the teacher responses, differences did not appear as statistically significant. The inattention and hyperactivity parameters remained improved after 6 months of omega-3 fatty acid supplementation, as compared to the baseline (Table **3**). According to the SDQ questionnaires, ADHD symptoms also improved after 3-months of intervention, as seen by the statistically significantly lower score of total difficulties, filled by both parents and teachers (Table **4**). Statistically significant improvement was additionally shown in the subcategories “emotional symptoms”, “hyperactivity” and “prosocial behaviors”, according to the parents. Teacher SDQ questionnaires revealed that the “conduct problems” category decreased statistical significantly after 3-months of omega-3 fatty acid supplementation (Table **4**).

4. DISCUSSION

The goal of the present study was to explore the effects of 6-month supplementation of omega-3 fatty acid in children diagnosed with ADHD. Dietary supplementation of omega-3 fatty acid in children with ADHD raises omega-3 and improves symptoms. Omega-3 fatty acid levels were measured from RBCs as they reflect the dietary fat intake and potentially reflect the fatty acid composition in the CNS as well [31]. RBC fatty-acid

Table 2. Variation of fatty-acid profiles from RBC membranes of children with ADHD comparing composition at 0, 3, and 6 months.

Fatty-acid			Time (months)			p
			0	3	6	
Saturated		Myristic C14_0	0.24a	0.21ab	0.20b	0.030
		Palmitic acid C16_0	21.33	21.63	21.57	0.431
		Stearic C18_0	17.55	17.84	17.89	0.076
		Eicosanoic C20_0	0.16	0.16	0.15	0.404
		Docosanoic C22_0	0.22a	0.20b	0.22a	<0.001
		Lignoceric C24_0	0.58a	0.42b	0.42b	0.001
Mono unsaturated		Palmitoleic C16_1n7	0.21a	0.14b	0.14b	<0.001
		Oleic C18_1n9	14.51	14.20	14.39	0.229
		Eicosenoic C20_1n9	0.28	0.30	0.28	0.753
		Nervonic C24_1n9	0.57a	0.46b	0.47b	0.011
Polyunsaturated	Omega-6	Linoleic C18_2n6	12.65a	11.09b	11.07b	0.001
		gamma Linolenic C18_3n6	0.09a	0.06b	0.05c	<0.001
		Eicosadienoic C20_2n6	0.26a	0.18b	0.17b	<0.001
		DGLA C20_3n6	1.71a	1.15b	1.23b	<0.001
		Arachidonic C20_4n6	17.30a	13.54b	13.44b	<0.001
		Docosatetraenoic C22_4n6	3.87a	1.87b	1.66b	<0.001
		Docosapentaenoic C22_5n6	1.12a	0.40b	0.34b	<0.001
		Total onega-6	36.78a	28.87b	29.34b	<0.001
	Omega-3	**ALA C18_3n3 ↓**	0.16a	0.12b	0.11b	**<0.001**
		EPA C20_5n3	0.40b	4.09a	3.98a	<0.001
		Docosahexaenic C22_5n3	2.11b	4.17a	4.22a	<0.001
		DHA C22_6n3	4.00b	7.27a	7.63a	<0.001
		Total onega-3	6.60b	14.72a	14.4a	<0.001
		Omega-3 Index	4.40b	11.35a	11.60a	**<0.001**
Ration O6/O3			5.68a	2.10b	2.34b	<0.001
Trans		C16_1n7t trans	0.07a	0.06ab	0.05b	0.021
		C18_1t	0.42a	0.37ab	0.29b	0.003
		C18_2n6tt	0.010	0.006	0.005	0.326
		C18_2n6ct	0.016a	0.007b	0.005b	<0.001
		C18_2n6tc	0.10a	0.08b	0.07b	0.002
		Total Trans-fatty acids	0.64a	0.52b	0.40c	<0.001

Analysis of fatty acid from red blood cells (RBC) membranes was performed *via* gas chromatography. Fatty-acid values were expressed as percentage of total fatty-acids and reported as mean. Averages were calculated for compliant participants throughout the 6-month intervention (n=15). Values with different superscript letters in the same row present significant differences between groups. The letters indicate significantly higher (a), lower (b) or the lowest (c). Dihomo-gamma-linolenic acid (DGLA); Total omega–6 include 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, and 22:5. Total omega–3 includes 18:3, 20:5, 22:5, and 22:6. Alpha Linolenic (ALA); Omega-3 Index was calculated as the sum of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Total trans-fatty acids include: C16:1n7t, C18:1t, C18:2n6tt, C18:2n6ct, and C18:2n6tc. p-value ≤0.05 is represented in bold and shows a significant difference.

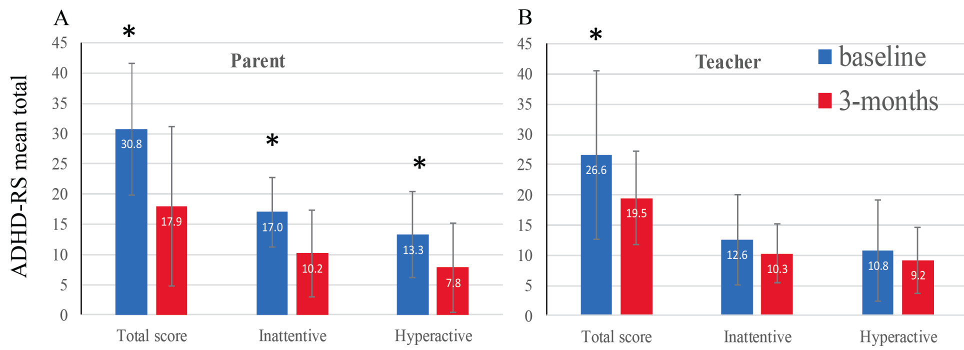

Fig. (2). ADHD-RS questionnaire obtained from parents and teachers at 0, and 3-months. ADHD Rating Scale-IV (ADHD-RS) questionnaires filled by parents (**A**) and teachers (**B**) were used to evaluate clinical severity of attention-deficit hyperactivity disorder (ADHD) symptoms at the beginning of the study and after 3-months of intervention. Averages were calculated for compliant participants with complete questionnaires at both times (n=24). Values are reported as mean ±standard deviation. *Indicate significant differences between groups. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 3. ADHD-RS questionnaire obtained from parents and teachers at 0 and 6 months

ADHD-RS Questionnaire	Months		p
	0	6	
Parent-sum	31.50±10.6	22.54±11.2	0.013
Inattentive	16.89±4.5	13.66±4.8	0.034
Hyperactive	14.18±8.4	9.86±6.4	0.026
Teacher-sum	26.60±11.6	23.75±13.3	0.386
Inattentive	16.18±5.6	13.50±6.7	0.066
Hyperactive	11.00±7.7	10.36±7.4	0.929

ADHD Rating Scale-IV (ADHD-RS) questionnaire was used to evaluate clinical severity of attention-deficit hyperactivity disorder (ADHD) symptoms in the beginning of the study and after 6 months of intervention filled by parents and teachers. Averages were calculated for compliant participants with complete questionnaires at both times (n=15). Values are reported as mean ±standard deviation. p-value ≤0.05 shown in bold, points to a significant difference.

Table 4. SDQ scores obtained from parents and teachers at 0 and 3 months.

SDQ questionnaires Scores		Intervention (Months)		p	Banding of SDQ Score		
		0	3		Normal	Borderline	Abnormal
Parent	Total Difficulties score	21.74±7.9	16.29±6.2	**0.005**	0-13	14-16	17-40
	Emotional symptoms	4.66±2.9	3.16±2.6	**0.008**	0-3	4	5-10
	Conduct Problems	3.33±2.1	2.69±2.1	0.205	0-2	3	4-10
	Hyperactivity	7.31±2.3	5.43±1.6	**0.003**	0-5	6	7-10
	Peer Problems	3.13±2.1	2.70±1.8	0.147	0-2	3	4-10
	Prosocial Behaviour	2.98±1.9	2.10±1.6	**0.028**	6-10	5	0-4
Teacher	Total Difficulties score	16.89±7.4	12.44±3.9	**0.035**	0-11	12-15	16-40
	Emotional symptoms	2.20±1.9	1.50±1.4	0.246	0-4	5	6-10
	Conduct Problems	2.85±2.4	1.77±1.4	**0.044**	0-2	3	4-10
	Hyperactivity	5.85±2.9	5.46±2.9	0.470	0-5	6	7-10
	Peer Problems	3.09±1.9	2.09±2.2	0.058	0-3	4	5-10
	Prosocial Behaviour	3.83±2.2	2.00±1.9	**0.012**	6-10	5	0-4

The strengths and difficulties questionnaire (SDQ) was filled by parents and teachers and was used to evaluate clinical severity of attention-deficit hyperactivity disorder (ADHD) symptoms as well as strengths and difficulties at the beginning of the study and after 3-months of intervention. Averages were calculated for compliant participants with complete questionnaires at both times. Values are reported as mean ±standard deviation. p-value ≤0.05 shown in bold, points to a significant difference. SDQ scores are categorized as 'normal', 'borderline' or 'abnormal'.

composition and the omega-3 index method have been shown to be altered in common chronic childhood diseases in Western countries, such as asthma [32], allergy [33], depression [34] and ADHD [35]. The mean omega-3 fatty acid in healthy Israeli children was found to be 5.06% in our previous study [22] and higher than the mean omega-3 index in healthy Israeli adults, which was 4.76% ±1.32 [36].

Out of 32 ADHD children, 75% completed 3 months of intervention and only 61% finished the 6-month period. Perhaps the study requirements might have been too demanding for some of the children. They may have been deterred by the size and quantity of pills they were required to take. In compliant children, there was a statistically significant increase in the omega-3 index and other alterations in the fatty-acid profile. The increase was from an average of 4.4% omega-3 index at baseline to 11.35% after 3 months. These results confirm that the omega-3 fatty acid supplementation was absorbed and digested. Interestingly, while the rise in DHA was 2-fold higher after 3 months of omega-3 fatty acid intervention, the increase in the EPA ratio was tenfold higher, although it was only twice higher in the pills. As the fatty acids are calculated by relative abundance, the omega-3 fatty acid supplement significantly altered the overall fatty-acid profile, including raising levels of most omega-3 components and lowering relative levels of omega-6 fatty acids, short and long saturated fats, monounsaturated fats, and trans-fatty acids. ALA was the only type of omega-3 which decreased following the intervention. It is important to note that our results present relative amounts, meaning that the percentage of each fatty acid is out of the total fatty acids measured in RBC membranes. Thus, the observed change in percentage does not necessarily indicate a change in the actual amount of that fatty acid. Our supplementation was given according to body mass, and was therefore normalized in all subjects. In order to minimize oxidation, supplementation included refrigerated omega-3 fatty acid capsules together with alpha tocopherol, the main natural antioxidant.

We found beneficial effects on ADHD symptoms as measured by 3 types of validated questionnaires: the CGI-S completed by a psychiatrist and the ADHD-RS and SDQ filled by both teachers and parents. Due to the limitations of questionnaire data in terms of accurateness, a variety of questionnaires were chosen in an attempt to strengthen our analysis and achieve overall accuracy. The ADHD Observational Research in Europe found that CGI-S correlates with the ADHD-RS inattention and hyperactivity scores and with the SDQ [37]. Indeed, all 3 types of questionnaires showed a reduction in ADHD symptoms following intervention. We found a slight discrepancy between the scores of parents versus teachers. Low concordance between teachers and parents is typical since parents may over-report improvement due to their involvement in the intervention [38].

Interestingly, most results at 3 and 6 months following the intervention did not differ statistically significantly. This could be due to reaching a plateau of treatment effect.

The findings of this study must be interpreted within some limitations. First, our study design was not randomized controlled with ADHD children receiving placebo treatment to compare the effectiveness of omega-3 fatty acid supplements and therefore other factors may be involved. One possibility is that interactions may exist between the subject genetic background and the effects of nutritional interventions, such as the individual capacity to adsorb and make the fatty acid bioavailable, the fat content of the diet, or the omega-3/omega-6 fatty acid dietary ratio and levels of antioxidants in cells. Second, limited statistical power was due to the small sample size that completed the intervention. Additionally, not all required questionnaires were attained from the children who completed the study, so the number of questionnaires for comparison decreased accordingly. Finally, from this type of research, it is not possible to draw a conclusion of causal relationship, only of association. A randomized control study including a larger cohort and a control group receiving placebo treatment would likely strengthen the results of this study.

CONCLUSION

Our study supported that dietary supplementation of omega-3 fatty acid to children with ADHD raises their omega-3 index in RBCs and leads to other alterations in the membrane fatty-acid profile. We found statistically significant improvement in ADHD symptoms following omega-3 fatty acid supplementation, as observed by the CGI-S and parent and teacher questionnaires, which showed improvement in inattention, hyperactivity, and various difficulties. Our pilot study showed that omega-3 fatty acid improved ADHD symptoms even at the midpoint of 3 months.

AUTHORS' CONTRIBUTIONS

Full access to study data and responsibility for the integrity of the data and the accuracy of the data analysis: A.A.O., E.A., and U.Y. Study concept and design: A.A.O., S.T., and U.Y. Methodology: A.A.O., E.A., and U.Y. Validation: A.A.O. Analysis and interpretation of data: A.A.O. Investigation: A.A.O., E.A., and U.Y. Drafting of the manuscript: A.A.O., and U.Y. Review and editing: A.A.O. Supervision: S.T. All authors read and approved the final manuscript.

LIST OF ABBREVIATIONS

ADHD	=	Attention Deficit Hyperactivity Disorder
ADHD-RS	=	ADHD Rating Scale IV
ALA	=	Alpha-linolenic
CGI-S	=	Clinical Global Impression-Severity scale
CNS	=	Central Nervous System
DGLA	=	Dihomo-Gamma-Linolenic Acid
DHA	=	Docosahexaenoic Acid
DSM-5	=	Diagnostic and Statistical Manual of Mental Disorders-5
EPA	=	Eicosapentaenoic
LA	=	Linoleic Acid
M	=	Mean
PUFAs	=	Polyunsaturated Fatty-Acids
RBC	=	Red Blood Cell
SD	=	Standard Deviation
SDQ	=	Strengths and Difficulties Questionnaire

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ziv Clinic Institutional Review Board and Israel Ministry of Health.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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