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ORIGINAL CONTRIBUTION

Behavioral and cognitive effects of docosahexaenoic acid in drug-naïve children with attention-deficit/hyperactivity disorder: a randomized, placebo-controlled clinical trial

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Abstract
This study aimed to investigate the efficacy of docosahexaenoic acid (DHA) dietary supplementation on behavior and cognition in school-aged, drug-naïve children with attention-deficit/hyperactivity disorder (ADHD). A total of 50 participants with ADHD aged 7 to 14 were enrolled in a 6-month randomized, placebo-controlled clinical trial and received either DHA or placebo. The primary outcome measure was the change in the ADHD rating scale IV Parent Version–Investigator (ADHD-RS-IV) after 4 and 6 months. Secondary outcome measures included Conners Parent Rating Scale-revised, other behavioral rating scales including quality of life and global functioning, and computerized cognitive tasks. Baseline assessment also addressed the blood fatty acids profile. No superiority of DHA supplement to placebo was observed on ADHD-RS-IV, the a priori primary outcome. DHA supplementation showed a significant, nonetheless quite small, effect on children's psychosocial functioning, emotional problems, and focused attention. Neither major nor minor adverse events were reported throughout the trial. This study shows that 6-month DHA supplementation has no beneficial effect on the symptoms of ADHD in school-aged, drug-naïve children with an established diagnosis of ADHD. Nevertheless, the 6 months treatment with supplemental DHA appears to have small positive effects on other behavioral and cognitive difficulties, which, in light of the absence of side-effects, could be reasonably followed up in future intervention studies. (https://clinicaltrials.gov/ct2/show/NCT01796262: The Effects of DHA on Attention Deficit and Hyperactivity Disorder (DADA)).

Keywords Attention-deficit/hyperactivity disorder (ADHD) · Docosahexaenoic acid (DHA) · Fatty acids · Cognition · Behavior

Introduction

Attention-deficit/hyperactivity disorder (ADHD), characterized by age-inappropriate and persistent inattention, excessive motor activity, and impulsivity, is one of the most common neurodevelopmental disorders with 7.2% of children affected worldwide ([1]; even though the prevalence in Italy is approximately 1%, based on the National Institute of Health' reports [2]). ADHD is a heterogeneous condition and its etiology has genetic and environmental components. With respect to the latter, the possible effect of nutrition on clinical manifestations of ADHD has attracted, during recent years, the attention of clinicians, researchers, and families. In particular, growing interest has been given to the potential role of polyunsaturated fatty acids (PUFAs) for understanding the pathogenesis of the disorder and as a possible coadjutant approach to pharmacological treatment [3]. At the biological level, omega-3 PUFAs are a crucial part of neuronal phospholipid membranes, and, as precursors of eicosanoids, can influence the quality of development [4]. Docosahexaenoic acid (DHA), in particular, is relevant for membrane fluidity and the release of neurotransmitters [5], and it can have anti-inflammatory properties through

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the production of a class of lipid mediators (the "specialized pro-resolving mediators," [6]). Moreover, DHA is the most abundant PUFA in brain gray matter, providing for the 15–20% of the total fatty acid composition in the frontal areas. This is particular meaningful with respect to ADHD because DHA could potentially be linked to executive functions, such as focused attention, planning, and inhibition, which are primarily located in the frontal cortex and are known to be impaired in ADHD (see, for example, [7]).

Although these findings could motivate clinical trials that used DHA, the large majority of studies that have explored the efficacy of PUFA supplementation in ADHD utilized mixed omega-3 fatty acids, such as DHA, eicosapentaenoic acid, and alpha-linoleic acid (for systematic meta-analyses of available data, see [8–12]). Indeed, to the best of our knowledge, the only clinical trial that used DHA in ADHD was the study of Voigt and colleagues [13]. In that work, 54 children between the ages of 6 and 12 years received either DHA supplementation (345 mg per day) or placebo for 4 months. The study did not show improvements in any measures of ADHD symptoms, in spite of a significant increase of plasma phospholipid DHA level in the supplemented group. It is worth mentioning that Voigt and colleagues used DHA as augmentation therapy to the stimulant medication, because all participants were receiving maintenance therapy throughout the trial, withheld 24 h before the testing session. Therefore, no studies have examined yet the effect of DHA supplementation as monotherapy in ADHD. Given the lack of literature and the above-mentioned considerations about the plausible role of DHA in ADHD, we aimed to investigate the efficacy of DHA dietary supplementation on behavior and cognition in school-aged, drug-naïve children with ADHD in a randomized, placebo-controlled clinical trial. Based on the previous observation that it may take up to 3 months for the cerebral membranes to recover a normal fatty acid composition from a PUFA deficiency [14], we extended the trial duration from four to 6 months compared to the study of Voigt and colleagues.

Method

The present work is a 6-month, randomized, placebo-controlled, double-blind intervention trial investigating the efficacy of supplementation with DHA in children aged 7 to 14 with ADHD ('The Effects of DHA on Attention Deficit and Hyperactivity Disorder (DADA)'). The trial was registered at ClinicalTrials.gov as NCT01796262. This study received approval by the ethics committee of our institute and was, therefore, performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments, with written informed consent and assent from all caregivers and participants, respectively. Data collection began in June 2012 and ended in October 2014. Figure 1 shows the schematic overview of the design of the present study, including all the measures collected and the flows of participants through the study.

Participants

Participants aged 7 to 14 were recruited from the Child Psychopathology Unit at our institute over a 22-month period. The study coordinator contacted 128 parents by phone to invite children to participate in the study protocol. Of these, 50 children with ADHD and their parents agreed to participate. The main reason for declining to participate was a child's refusal to have his or her blood sampled. All participants were diagnosed by a child neuropsychiatrist in accordance with the diagnostic and statistical manual of mental disorders criteria (fourth ed., text rev.; [15]). A child psychologist experienced in the diagnosis of ADHD (AC) confirmed independently the diagnosed by through direct observation and the administration of the semi-structured interview Development and Well-Being Assessment (DAWBA; [16]). According to the clinical assessment, 15.7% of children met the criteria for the ADHD inattentive subtype, 33.3% fulfilled criteria for the hyperactive–impulsive subtype, and 51% had the combined subtype. The Wechsler Intelligence Scale for Children–III or –IV [17, 18] was used to obtain the Full Scale Intelligence Quotient (FSIQ) or FSIQ scores. Only participants with FSIQ or estimated FSIQ scores higher than 80 were included. Moreover, all children were required to be drug-naïve and not have consumed omega-3/omega-6 supplements during the 3 months prior to the recruitment. Exclusion criteria were a history of seizures, other neurological disorders, or diagnosed genetic disorders. All participants were Caucasian and had normal or corrected-to-normal vision.

Procedure

Participants were assigned a study number and randomly allocated by an independent third person to either the supplement or the placebo group using a computer-generated randomization scheme. Children, parents, and study investigators were blinded to the randomization until completion of data collection and analysis. All participants were assessed at our institute's Child Psychopathology Unit at baseline and after 4 and 6 months. At baseline, blood samples were obtained by collecting drops of blood from a fingertip after a minimum 1-h fast. The participants filled out the Pubertal Developmental Scale [19]. Weekly frequency of fish consumption was then collected. Last, data on parental employment were used as a measure of socioeconomic status

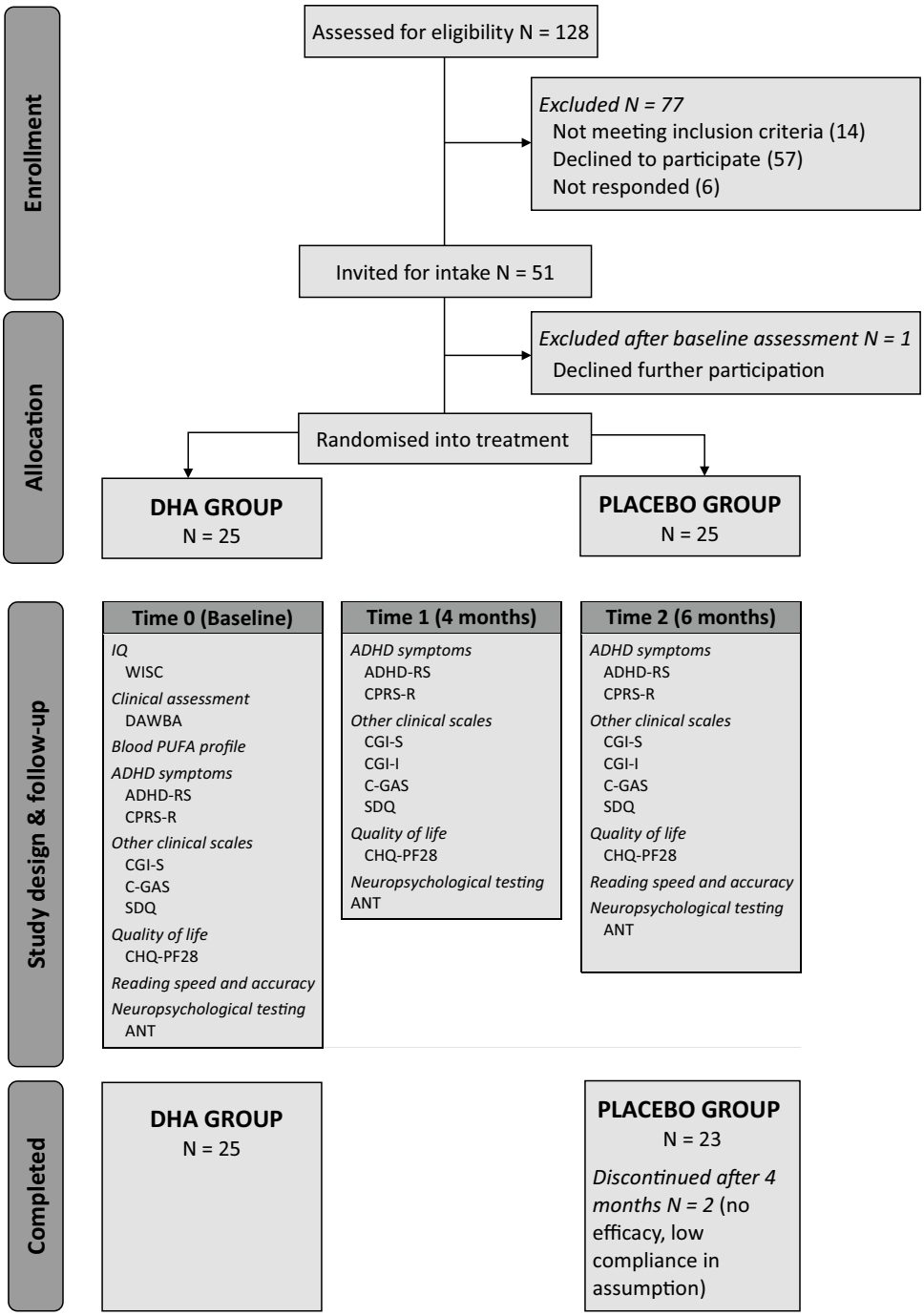

Fig. 1 Schematic overview of the study design, with a list of measures collected at each time point. ADHD-RS, ADHD Rating Scale; ANT, Amsterdam Neuropsychological Tasks; C-GAS, Children Global Assessment Scale; CGI-I, Clinical Global Impression-*Improvement scale;* CGI-S, Clinical Global Impression-Severity scale; CHQ-PF28, Child Health Questionnaire-Parent Form; CPRS-R, Conners' Parent Rating Scale-Revised; DAWBA, Development and Well-Being Assessment; DHA, docosahexaenoic acid; SDQ, Strengths and Difficulties Questionnaire; WISC, Wechsler Intelligence Scale for Children

and coded according to the Hollingshead 9-point scale for parental occupation [20].

At each visit, measurement of clinical parameters including height without shoes, weight in light clothing, and blood pressure (systolic and diastolic) were taken. After that, participants completed a battery of cognitive tests in a single session of approximately 50 min. While children were completing these tasks, parents and investigators filled out the behavioral and clinical rating scales, respectively. Between each visit, parents visited our institute monthly to receive the treatment supply for the following four weeks, check compliance, and report any adverse events.

Intervention

Active supplement consisted of two soft gelatin pearls per day providing a dose of 500 mg algal DHA. Placebo treatment consisted of two pearls per day containing 500 mg wheat germ oil. The placebo was stabilized with a low concentration of Vitamin E. The placebo pearls matched the

DHA ones in touch, smell, and size. Either the supplement or the placebo was provided in six identical boxes labeled with an identifying code and in compliance with good manufacturing process. Duration of treatment was 6 months. This period was chosen as long-chain PUFA levels in the brain can take up to 3 months to recover from a deficiency state [14, 21]. Compliance was assessed by weighting the leftover products that parents returned to the investigators on a monthly basis and was defined as taking more than 70% of the provided capsules. Participants were asked to maintain their usual diet throughout the intervention period and, in particular, to avoid foods enriched with EPA or DHA during the supplementation.

Outcome measures

The a priori primary outcome was the ADHD rating scale IV Parent Version–Investigator [22], used to assess parent ratings of ADHD behaviors.

As secondary outcomes, different measures of behavior and functioning, and cognition were used. Indeed, parents also completed the Conners' Parent Rating Scale–R [23] to investigate ADHD symptoms. For this questionnaire, ADHD index, Conners' Global Index restless–impulsive, Conners' Global Index emotional lability, Conners' Global Index total, DSM-IV inattentive, DSM-IV hyperactive–impulsive, and DSM-IV total were considered as dependent measures. Modifications of the Strengths and Difficulties Questionnaire (SDQ; [24]) score were considered possible improvements regarding the emotional and behavioral difficulties associated with the disorder. To rate the impact of ADHD on quality of life, the Child Health Questionnaire–Parent Form 28 item [25] was completed by the parents. The Child Health Questionnaire (CHQ) is a well-validated measure of quality of life, comprising an overall summary score for psychosocial functioning, as well as subscales that assess self-esteem, impact of the disorder on the parents, and participation in family activities. The children's global functioning was evaluated by a clinician using the Clinical Global Impression—severity Scale (CGI; [26]) and the Children's Global Assessment Scale (C-GAS; [27]). Moreover, the clinician used the CGI– improvement [26] at 4 and 6 months after supplementation had been initiated to compare the patients' general clinical condition to the period before the introduction of supplement use. Finally, an abbreviated battery of cognitive tests from the Amsterdam Neuropsychological Tasks (ANT; [28]) program was used to assess executive function domain. Participants completed four computerized tasks, always administered in the same order: baseline speed, focused attention 4 letters, shifting attentional set–visual, and sustained attention. Baseline speed measured simple response times to stimulus presence. In the focused attention test, participants had to respond (pressing the "yes" key) to one target letter among four letters presented on the screen at the same time, only when it was displayed in the relevant diagonal positions. The visual set-shifting task investigated three basic cognitive variables: vigilance, inhibition, and cognitive flexibility. Lastly, the sustained attention task assessed the fluctuation of attention over time. For further details about the dependent measures considered for these tasks, the reader is referred to Crippa et al. [29]. Reading skills were also assessed at baseline, and after 6 months of supplementation using word and non-word reading subtests from the Italian standardized Battery for the Assessment of Developmental Reading and Spelling Disorders [30]; both reading speed (syllables/seconds) and reading accuracy (number of errors) were registered.

Blood collection and fatty acid profiles analysis

Drops of blood from fingertips were collected at baseline from all children to evaluate the fatty acid profile. This method was chosen because whole blood is more easily obtainable than other components such as plasma and red blood cells, and the whole blood fatty acid composition offers a more balanced picture of the status of circulating PUFA in relation to fat dietary intakes [31, 32]. Whole blood samples were directly subjected to transmethylation for gas chromatography analysis, using a well-validated protocol [33]. Fatty acids from 14 to 24 carbons were detected, and fatty acid values were then expressed as a percentage of total fatty acids. We report here single fatty acid data for main omega-3 and omega-6, expressed as percentage of total fatty acids. The arachidonic acid(AA)/EPA and AA/DHA ratios were calculated as reliable indexes of the functional effects of long-chain PUFAs [34]. Last, the sum of EPA and DHA (the "omega-3 index"; [35]) and the sum of saturated fatty acids, monounsaturated fatty acids, and PUFAs, respectively, were also reported. For further details about the fatty acid profile analysis in relation to patients with neurodevelopmental disorders, the reader is referred to Crippa and colleagues [29].

Statistical analysis

Primary analyses were intent-to-treat, including all randomized study participants, and were conducted using SAS statistical software package, version 9.4 (SAS Institute Inc., Cary, NC, USA). Baseline between-group differences on the demographic variables, clinical questionnaires, cognitive measures, and blood fatty acid levels were analyzed using Chi square analysis, Mann–Whitney, or independent-samples *t* test, according to the distributional nature of the data. Effect of DHA versus placebo supplementation on outcome variables was investigated using linear mixed modeling. This statistical technique allows participants with missing data or

dropouts to be included in the analysis. Each outcome variable was individually assessed with the same mixed model design, including a fixed treatment group effect, a fixed time effect, and a treatment by time interaction, estimating the average group specific intercepts, rates of change over time, and group specific differences in those rates, respectively. In order to determine effect size estimates of DHA effect across the trial visits, eta-squared values for both main effects and for treatment by time interaction were computed and reported, with values of 0.01 considered small effects, 0.06 considered medium effects, and 0.14 considered large effects [36]. Detection of possible outliers was based on median value and interquartile range (IQR), namely every value lower than the 25th quartile minus 3 × IQR or greater than the 75th quartile plus 3 × IRQ was identified as an outlier and therefore removed. Between-group differences on CGI-improvement score were assessed using Kruskal–Wallis analysis. The significance level was two tailed ($p < 0.05$) for all analyses. No correction was applied for family wise error rate, as comparisons were strictly planned before the study's initiation and only the comparisons associated with a significant main effect on the linear mixed-model analysis were calculated. The present study was designed to detect a change in performance of 0.8 standard deviation, with 25 participants assigned to each group (power = 80%; $p < 0.05$). Cohen's d effect sizes were calculated to define effect size of DHA versus placebo supplementation between baseline and the end of treatment using the formula:

$$d = M_{\text{change-DHA}}/\text{SD}_{\text{DHA}} - M_{\text{change-placebo}}/\text{SD}_{\text{placebo}}$$

where $M_{\text{change-DHA}}$ is the change score (i.e., the mean of difference between pretest and posttest means) for the DHA group, $M_{\text{change-placebo}}$ is the mean of the change scores for the placebo group, SD_{DHA} and $\text{SD}_{\text{placebo}}$ are the standard deviation of DHA group scores and placebo group scores, respectively [37]. Effect size was interpreted as small with Cohen's d values between 0.2 and 0.5, medium with values between 0.5 and 0.8, and larger above 0.08, following the benchmarks proposed by Cohen [36].

Results

Baseline characteristics

Data on the demographic variables and blood fatty acid levels at baseline are summarized in Table 1. DHA and placebo group did not significantly differ in any of the demographic variables (all $p > 0.05$). Fish consumption per week before the supplementation was also not different between groups, as reported by parents ($\chi^2(3) = 1.032$, $p > 0.05$).

With respect to the fatty acid profile, two children (both in the DHA group) among the 50 participants recruited could not be analyzed due to insufficiency of the absorbed blood sample. When compared with participants in the placebo group, children in the DHA group had significantly higher levels of AA/EPA ratio (DHA group: 16.80 ± 18.14 vs. placebo group: 12.18 ± 9.66, $p = 0.045$). No other difference was found in fatty acid concentrations. All the participants had abnormally lower blood level of DHA at baseline compared to a control group of 22 healthy developing children matched by gender, age, and IQ [29]. Ratings of ADHD symptoms and other associated difficulties that often co-occur with the ADHD were similar in the two groups at baseline, with the exception of higher impact of symptoms on functioning evaluated by SDQ in DHA group ($p = 0.045$). Lastly, with respect to cognitive measures, children in the DHA group showed more false alarms in the focused attention task (false alarms relevant non-target, $p = 0.032$; false alarms irrelevant target, $p = 0.013$), and less flexibility in the visual set-shifting task compared to participants in the placebo group ($p = 0.029$).

Table 1 Demographics and blood fatty acid levels of the participants at baseline assessment

	DHA	Placebo
N	25	25
Females: males	2: 23	2: 23
Age	11.06 ± 1.85	10.91 ± 1.42
IQ	103 ± 13.045	104.48 ± 13.79
SES	58 ± 17.02	50.2 ± 19.82
BMI	18.51 ± 2.42	19.46 ± 3.13
% 18:2n-6 (LA)	22.34 ± 2.58	22.28 ± 2.27
% 20:4n-6 (AA)	9.34 ± 1.78	9.38 ± 2.27
% 20:5n-3 (EPA)	0.81 ± 0.38	1.24 ± 0.99
% 20:6n-3 (DHA)	1.68 ± 0.40	1.73 ± 0.53
DHA/EPA	2.49 ± 0.68	3.16 ± 1.58
AA/EPA	16.80 ± 18.14	12.18 ± 9.66
AA/DHA	5.72 ± 1.07	5.72 ± 1.70
SFA	34.07 ± 3.92	33.82 ± 4.03
MUFA	27.43 ± 3.59	27.56 ± 4.15
PUFA	37.38 ± 4.48	37.98 ± 3.77

IQ intelligence quotient, *SES* socio economic status, *BMI* body mass index, *LA* linoleic acid, *AA* arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Adverse events and treatment adherence

Over the course of the 6 months, no instances of either major or minor adverse events were reported. The mean compliance over the study, determined by monthly pearl counts of the number returned divided by number of pearls prescribed, was 83.2%. Two children, both in the placebo group, discontinued taking the study pearls after 4 months, due to no

observed efficacy and increasing difficulty in swallowing the capsules. Three further participants, one in the DHA group and two in the placebo group, did not reach the requested level of compliance (i.e., taking at least 70% of the prescribed pearls), and were therefore excluded from the efficacy outcomes analyses.

Efficacy outcomes: primary outcome measures

As can be seen in Table 2, the linear mixed-model analysis did not find any significant difference on ADHD rating scale, the a priori primary outcome, between the treatment groups ($p > 0.05$).

Indeed, a main effect of time was observed on the hyperactivity–impulsivity scale and on total score of the ADHD rating scale, with participants in both groups showing improvements over the 6 months, as shown in Fig. 2.

Efficacy outcomes: secondary outcome measures

With respect to the secondary behavioral outcomes, the linear mixed-model analysis further showed a main effect of time on the SDQ Hyperactivity scale and on SDQ total difficulties score, on the CGI severity, on the C-GAS, and on Conners' ADHD index, Conners' Global Index restless–impulsive, Conners' Global Index total, DSM-IV hyperactive–impulsive scale, and DSM-IV total (see Table 2). This suggests that children across groups displayed ameliorations of both symptoms and general functioning over the study. Moreover, as depicted in Fig. 3, significant interactions occurred between treatment condition and time. The linear mixed-model analysis revealed that children in the DHA group showed amelioration in CHQ Psychosocial summary between the baseline and the end of supplementation ($p < 0.01$) and improved in parental ratings of emotional problems on SDQ over the study (baseline vs. 4-month visit, $p < 0.05$; baseline vs. end of treatment, $p < 0.05$). The effect size of these interaction effects (eta squared) was 0.04 and 0.05, respectively, denoting small interaction effects. Consistently, pre-post effect sizes (Cohen's d) were also small, 0.013 and − 0.23, respectively. Children in placebo group showed improvement in the CHQ Parental impact-Time scale between the baseline and the 4-month visit ($p < 0.05$), but this amelioration was not sustained at the end of supplementation.

With respect to the other secondary cognitive endpoints, the linear mixed-model analysis revealed a main effect of time on several ANT scores: reaction time in baseline speed task; reaction time of correct responses, false alarms relevant non-target, and false alarms irrelevant target in the focused attention 4-letters task; reaction time of inhibition, reaction time of flexibility, number of errors inhibition, and number of errors flexibility in visual set-shifting; tempo × series and false alarms in the sustained attention task. Not surprisingly, a main effect of time was also found on non-word reading speed (see Table 3).

Finally, the linear mixed-model analysis showed a significant interaction between treatment condition and time on number of misses and false alarms irrelevant target in focused attention 4-letters task. As can be seen in Fig. 4, participants supplemented with DHA showed a decrease of misses in the focused attention 4-letters task at the 6-month visit (end of treatment vs. baseline, $p < 0.01$; end of treatment vs. 4-month visit, $p < 0.05$), whereas children in the placebo group displayed a lower number of misses at the 4-month ($p < 0.05$) but not at the 6-month visit ($p > 0.05$). Likewise, children in the DHA group showed a reduction of false alarms irrelevant target in the focused attention 4-letters task at the end of supplementation (end of treatment vs. baseline, $p < 0.001$; end of treatment vs. 4-month visit, $p < 0.05$). These interaction effects ranged small to medium in size, with eta-squared values of 0.7 and 0.3, and Cohen's d values of − 0.30 and − 0.28, respectively.

Discussion

The objective of the present clinical trial was to investigate the efficacy of 6-month supplementation with DHA as only medication on behavior and cognition in school-aged children with ADHD. To date, this is to the best of our knowledge the first study that explored the effect of DHA as monotherapy in a drug-naïve clinical sample. Differently from previous studies investigating the efficacy of PUFA supplementation in ADHD by means of mixed omega-3 fatty acids, we decided to use exclusively DHA because it is relevant for regulating both membrane fluidity and synaptic transmission [5] and it represents the 15–20% of the total fatty acid composition in the frontal lobes.

Overall, this is a substantially negative study. The results of the present randomized, placebo-controlled clinical trial did not show evidence of benefit on the a priori primary outcome measure—the ADHD rating scale IV—. Furthermore, the results did not show any significant treatment effects on other measures of ADHD symptoms, such as Conners Parents Rating Scale.

However, with respect to the secondary behavioral outcome variables, supplementation with DHA led to a slight but significant amelioration of children's psychosocial functioning as judged by parents, and to a decrease of parent-rated emotional problems in the selected study population. The size of these effects of 0.13 and 0.23, while statistically significant, was nevertheless quite small. These results are in line with those reported by previous meta-analyses, suggesting an absent or marginal effect of PUFA supplementation on ADHD behavioral manifestations [8–11]. Lastly,

Table 2 Behavioral measures per treatment group

	Baseline		4-month visit		6-month visit		Treatment		Time		Treatment × Time		Pre-post effect size
	DHA	Placebo	DHA	Placebo	DHA	Placebo	*F*	η^2	*F*	η^2	*F*	η^2	*d*[a]
Primary outcome measure													
ADHD rating scale													
Hyperactivity—impulsivity scale	15.36 (5.45)	14.48 (5.23)	11.42 (5.02)[b]	10.86 (6.63)[b]	11.29 (5.28)[b]	11.38 (7.61)[b]	0.15	0.00	14.74***	0.20	0.22	0.00	− 0.19
Inattention scale	14.20 (5.11)	17.28 (5.98)	14.38 (4.51)	14.95 (6.55)	13.63 (5.86)	14.95 (7.55)	0.82	0.01	1.17	0.02	0.79	0.01	0.06
Total	29.56 (9.46)	31.76 (9.76)	25.79 (8.67)[b]	25.81 (11.89)[b]	24.92 (10.28)[b]	26.33 (14.19)[b]	0.08	0.00	8.00**	0.11	0.20	0.00	− 0.09
Secondary outcome measures													
Strengths and difficulties questionnaire													
Emotional problem scale	3.36 (2.68)	2.56 (1.50)	2.79 (2.08)	2.81 (0.98)	2.33 (1.52)	2.38 (2.25)	0.62	0.01	2.03	0.03	3.78*	0.05	− 0.23
Conduct problem scale	3.52 (1.64)	3.80 (1.76)	3.71 (1.68)	3.76 (2.23)	3.25 (1.70)	3.43 (2.18)	0.13	0.00	1.47	0.02	0.20	0.00	0.03
Hyperactivity scale	8.12 (3.09)	7.80 (1.76)	6.96 (2.53)[b]	7.26 (2.28)[b]	6.74 (2.05)[b]	6.89 (2.26)[b]	0.01	0.00	5.16**	0.05	0.01	0.01	− 0.19
Peer problems scale	3.16 (4.63)	2.40 (1.68)	2.17 (2.08)	2.14 (1.68)	2.09 (2.24)	2.10 (1.79)	0.17	0.00	1.16	0.02	0.03	0.00	− 0.04
Prosocial scale	7.56 (2.20)	7.24 (2.37)	7.00 (1.89)	7.48 (2.42)	7.00 (1.93)	7.38 (2.52)	0.14	0.00	1.17	0.02	1.07	0.02	− 0.10
Impact	3.72 (2.95)[c]	2.28 (1.84)[c]	3.00 (2.48)	3.24 (2.59)	2.38 (2.28)	2.86 (2.37)	0.13	0.00	1.38	0.02	3.06	0.04	− 0.32
Total difficulties score	18.16 (8.47)	16.56 (3.64)	15.75 (5.76)[b]	15.65 (4.65)[b]	14.54 (5.02)[b]	14.05 (6.34)[b]	0.15	0.00	5.54**	0.03	0.98	0.02	− 0.25
Conners' parents rating scales													
ADHD index	72.24 (8.53)	75.00 (11.63)	69.58 (10.78)[b]	69.33 (10.34)[b]	66.54 (10.21)[b]	70.67 (12.77)[b]	0.41	0.00	3.48*	0.05	1.61	0.02	− 0.24
CGI: restless-impulsive	68.16 (9.72)	70.96 (11.46)	66.00 (11.17)[b]	64.48 (10.77)[b]	62.04 (10.14)[b]	64.81 (13.71)[b]	0.21	0.00	5.99**	0.08	1.54	0.02	− 0.09
CGI: emotional lability	59.88 (14.08)	58.88 (14.55)	55.54 (8.55)	55.76 (13.02)	51.92 (8.01)	57.10 (15.97)	0.18	0.00	2.63	0.04	2.02	0.03	− 0.41
CGI: total	65.48 (14.09)	69.52 (12.49)	63.91 (10.66)[b]	63.95 (11.52)[b]	60.30 (9.31)[b]	64.05 (14.25)[b]	0.45	0.01	5.73**	0.05	1.11	0.02	− 0.03
DSM IV: inattentive	68.64 (15.09)	73.48 (13.78)	69.48 (10.46)	70.57 (11.48)	67.22 (10.84)	69.90 (14.37)	0.40	0.01	1.77	0.01	0.29	0.01	0.02
DSM IV: hyperactive-impulsive	67.36 (14.10)	69.96 (12.28)	64.48 (13.20)[b]	61.52 (13.11)[b]	60.17 (10.03)[b]	63.43 (17.77)[b]	0.01	0.00	8.35***	0.06	2.57	0.04	− 0.09
DSM IV: total	70.79 (12.34)	74.08 (12.28)	69.04 (12.19)[b]	68.10 (12.14)[b]	65.48 (10.67)[b]	69.48 (15.64)[b]	0.14	0.00	4.16*	0.04	1.76	0.03	− 0.14
Child Health Questionnaire													
Physical functioning	0.31 (0.62)	0.18 (0.92)	0.56 (0.00)	0.46 (0.32)	0.36 (0.84)	0.53 (0.15)	0.07	0.00	0.82	0.02	0.90	0.02	− 0.03
Role—physical	0.24 (0.58)	0.17 (0.83)	0.45 (0.00)	0.11 (0.90)	0.23 (1.08)	0.37 (0.38)	0.23	0.00	0.01	0.00	1.03	0.02	− 0.03
General health	0.74 (0.91)	0.89 (0.64)	0.95 (0.62)	0.84 (0.67)	0.89 (0.86)	0.82 (1.13)	0.04	0.00	0.80	0.01	1.07	0.01	0.04
Bodily pain	− 0.13 (1.25)	0.06 (0.84)	0.33 (0.74)	0.29 (0.80)	0.27 (0.77)	0.20 (0.98)	0.33	0.00	1.07	0.02	0.41	0.01	0.08
Role—emotional/behavioral	− 1.08 (1.77)	− 0.74 (1.94)	− 0.77 (1.48)	− 1.46 (1.73)	− 0.65 (1.09)	− 1.14 (1.75)	0.36	0.00	1.16	0.02	2.87	0.04	0.18
Parental impact—time	− 0.06 (0.99)	− 0.09 (1.34)	0.19 (0.67)	− 0.26 (1.04)	0.18 (0.74)	0.14 (0.87)	0.00	0.00	1.55	0.03	3.53*	0.03	0.02
Parental impact—emotional	− 0.70 (0.87)	− 0.51 (0.99)	− 0.36 (0.82)	− 0.87 (0.95)	− 0.24 (0.52)	− 0.45 (1.06)	0.73	0.01	2.42	0.01	3.01	0.05	0.10
Self-esteem	− 0.69 (1.21)	− 0.61 (0.54)	− 0.39 (0.86)	− 0.82 (0.96)	− 0.48 (0.99)	− 0.66 (1.03)	0.83	0.00	0.20	0.00	1.87	0.03	0.03
Mental health	− 2.02 (1.32)	− 1.65 (1.33)	− 1.62 (1.28)	− 1.82 (1.40)	− 1.28 (0.96)	− 1.41 (1.48)	0.01	0.00	2.70	0.04	1.64	0.02	0.11
Behavior	− 1.52 (0.86)	− 1.17 (0.91)	− 1.28 (1.00)	− 1.16 (1.18)	− 1.07 (0.90)	− 1.29 (1.41)	0.39	0.00	1.33	0.01	0.69	0.02	0.12

Table 2 (continued)

	Baseline		4-month visit		6-month visit		Treatment		Time		Treatment × Time		Pre-post effect size
	DHA	Placebo	DHA	Placebo	DHA	Placebo	F	η^2	F	η^2	F	η^2	d^a
Physical summary	0.77 (0.64)	0.70 (0.80)	1.00 (0.35)	0.85 (0.74)	1.01 (0.41)	0.91 (0.55)	0.08	0.00	0.31	0.01	2.14	0.01	− 0.01
Psychosocial summary	− 1.46 (1.01)	− 1.19 (0.99)	− 1.20 (1.00)	− 1.63 (1.24)	− 1.05 (0.86)	− 1.46 (1.06)	0.32	0.00	1.11	0.02	4.21*	0.04	0.13
Children' Global Assessment Scale	67.52 (9.97)	68.76 (7.68)	72.21 (11.39)[b]	69.29 (7.91)[b]	73.25 (10.06)[b]	69.80 (8.04)[b]	0.84	0.01	3.99*	0.06	1.70	0.02	0.23
Clinical Global Impression-Severity	3.8 (0.87)	4.08 (0.91)	3.58 (0.93)[b]	3.67 (0.97)[b]	3.25 (0.74)[b]	3.71 (1.10)[b]	1.06	0.01	3.63*	0.05	1.10	0.02	− 0.06

CGI Conners' Global Index

[a]Cohen's d

[b]Main effect of time

[c]Between-group baseline difference

*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

as regards the secondary cognitive outcome measures, the results indicated a small, significant benefit of DHA on focused attention, as shown by the decrease of misses and false alarms in the supplemented group. Again, the effect size of these modifications, ranging from 0.28 to 0.30, was rather small. Although limited in size, we feel that this result of the present trial confirms the conclusion of the meta-analysis of Cooper et al. [12], which disclosed limited evidence of benefit of omega-3 in cognition only in children who had deficient levels of PUFAs. Indeed, all the children recruited in this trial had abnormally lower blood levels of DHA at baseline compared to 22 healthy developing peers matched by gender, age, and IQ [29]. Future studies assessing the impact of DHA on cognitive functioning should focus on subgroups of children with ADHD who are omega-3 deficient at baseline. Nonetheless, compared to pharmacological treatment effect size ranging from 0.6 for non-stimulant medication to 1.52 for stimulant medication [38], the effect of DHA supplementation on secondary outcomes disclosed in this trial is overall quite modest.

Comparison of results of the present trial with previous findings about effect of PUFA supplementation is limited by the fact that only one clinical trial has previously used DHA in ADHD [13]. The present results deviate from findings of Voigt and colleagues, where no statistical between-group differences were reported in any of the behavioral or cognitive performances evaluated after 4 months of DHA supplementation. There are several possible reasons for these differences. First, the measures of outcome used in the two studies were not identical. With regard to behavior, we found an effect of DHA on difficulties frequently associated with the disorder, such as the CHQ and SDQ, not included in the study of Voigt and colleagues. Indeed, the authors used two parental rating scales, the Child Behavior Checklist and the Conners' Rating Scales. It is important to note that, in line with Voigt and colleagues, the results of the present study did not reveal an effect of DHA on parents' ratings of behavior on Conners' Rating Scales. With respect to cognition, we did not find an effect of DHA supplementation on sustained attention (assessed by ANT), in agreement with Voigt and colleagues (measured by Test of Variable Attention). The present significant findings about the benefit of DHA on cognitive functions are restricted to focused attention. Another cause of difference in findings might be that the dose of the DHA supplementation in the present study was higher (500 mg vs. 345 mg per day) and given for a longer period (6 vs. 4 months), compared to the trial of Voigt et al. There is still some controversy whether larger doses of DHA further improve ADHD symptoms and over the role of the trial duration [8–12]. Although the meta-analytical results are not entirely concordant, we feel that differences in intervention between

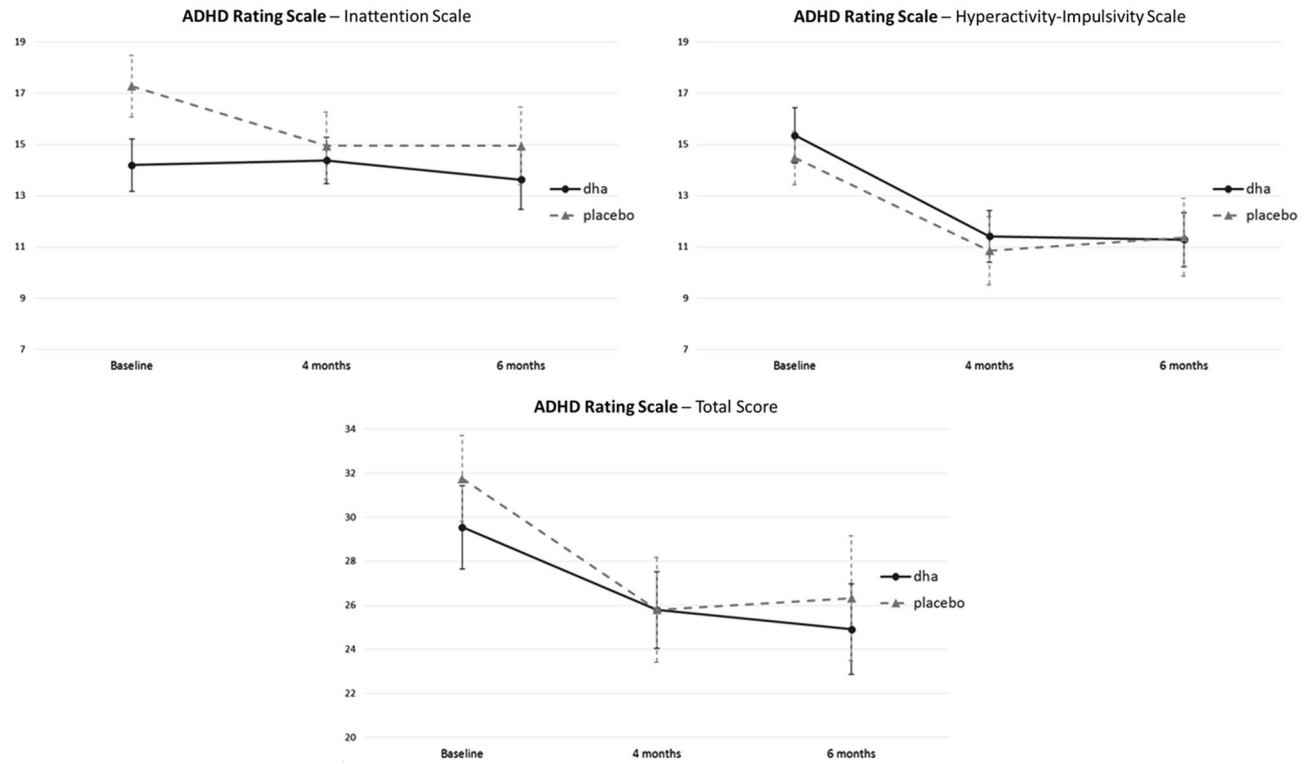

Fig. 2 Change in the a priori primary outcome measures, ADHD Rating Scale, in DHA and in placebo group. The linear mixed-model analysis demonstrated a main effect of time on the hyperactivity–impulsivity scale (higher right panel, $p < 0.001$) and on total score of the ADHD rating scale (lower panel, $p < 0.01$), with participants in both groups showing similar improvements over the 6 months. No significant effect of treatment condition was found ($p > 0.05$)

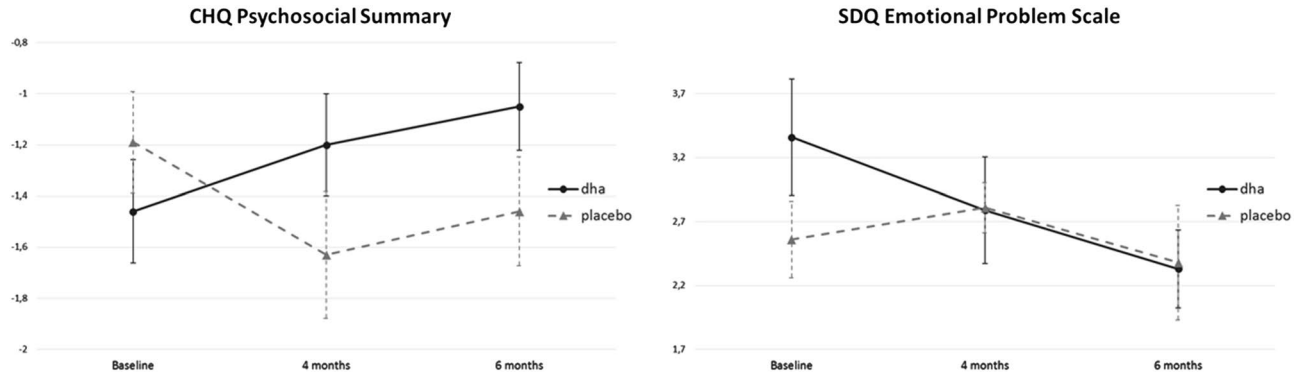

Fig. 3 Change in secondary behavioral outcome measures in DHA and in placebo group showing interactions between treatment condition and time. The linear mixed-effect analysis revealed that children in DHA group showed amelioration in the Child Health Questionnaire–Parent Form (CHQ) Psychosocial summary between the baseline and the end of supplementation (left panel; $p = 0.008$) and improved in parental ratings of emotional problem on the Strengths and Difficulties Questionnaire (SDQ) over the study (right panel; baseline versus 4-month visit, $p = 0.049$; baseline versus end of treatment, $p = 0.017$)

this study and the work of Voigt and colleagues might have led to divergent conclusions. Finally, it is worth remembering as a possible confounding factor that participants of Voigt and others were taking stimulant medication throughout the trial, whereas children recruited in the present study were drug-naïve. The present findings are also in line with the observations of a previous cross-sectional study, where we analyzed the relationship between PUFA status, cognitive, and behavioral traits in a mixed sample of children with ADHD—then recruited

Table 3 Cognitive measures per treatment group

	Baseline		4-month visit		6-month visit		Treatment		Time		Treatment × Time		Pre-post effect size
	DHA	Placebo	DHA	Placebo	DHA	Placebo	d^a	η^2	F	η^2	F	η^2	d[a]
Other outcome measures													
Reading Abilities													
Word reading speed (syll/sec)	3.03 (1.17)	2.68 (1.01)	–	–	3.32 (1.25)	2.96 (1.03)	1.62	0.01	1.30	0.01	0.07	0.00	0.02
Word reading accuracy (errors)	4.16 (4.44)	5.68 (4.49)	–	–	4.29 (3.95)	4.14 (4.60)	0.27	0.00	1.94	0.01	1.74	0.01	0.20
Non-word reading speed (syll/sec)	1.85 (0.78)	1.55 (0.45)	–	–	1.99 (0.63)[b]	1.73 (0.54)[b]	2.08	0.01	5.28*	0.04	0.01	0.00	0.00
Non-word reading accuracy (errors)	6.4 (4.65)	7.04 (6.27)	–	–	7.38 (5.01)	7.38 (6.97)	0.11	0.00	0.53	0.00	0.97	0.01	0.13
ANT—baseline speed													
RT (msec)	351.88 (70.16)	342.68 (74.74)	377.79 (93.80)[b]	366.48 (79.24)[b]	351.13 (64.70)[b]	357.81 (58.83)[b]	0.08	0.00	3.97*	0.06	0.66	0.01	− 0.20
SD of RT	136.42 (78.52)	129.6 (87.79)	159.13 (114.93)	152.05 (115.29)	124.46 (64.52)	141.86 (100.58)	0.00	0.00	1.28	0.02	0.38	0.01	− 0.24
ANT—focused attention 4 letters													
RT correct responses (msec)	1049.56 (347.56)	971.64 (332.64)	988.17 (393.38)[b]	951.05 (314.15)[b]	876.38 (237.87)[b]	921.00 (267.24)[b]	0.00	0.00	15.44***	0.11	1.61	0.03	− 0.30
SD of correct responses RT	484.75 (284.95)	395.73 (185.81)	421.46 (277.02)[b]	381.41 (216.19)[b]	341.36 (223.74)[b]	372.06 (199.03)[b]	0.30	0.00	5.70**	0.08	3.31	0.05	− 0.34
Misses	3.24 (2.82)	2.84 (2.59)	2.38 (1.66)	1.48 (1.44)	1.54 (1.32)	2.43 (2.25)	0.44	0.00	2.51	0.03	4.99*	0.07	− 0.30
False alarms relevant non-target	1.36 (1.38)[c]	0.56 (0.77)[c]	0.57 (0.73)[b]	0.29 (0.46)[b]	0.30 (0.56)[b]	0.19 (0.40)[b]	4.57*	0.04	9.13***	0.11	1.22	0.03	− 0.14
False alarms irrelevant target	2.56 (3.68)[c]	1.00 (0.91)[c]	1.43 (1.31)[b]	0.86 (1.46)[b]	0.78 (0.95)[b]	0.76 (1.00)[b]	4.05	0.03	5.88**	0.05	3.51*	0.03	− 0.28
ANT—visual set-shifting													
RT inhibition (msec)	376.08 (317.30)	333.96 (188.29)	162.09 (145.86)[b]	244.05 (153.71)[b]	164.68 (126.58)[b]	215.05 (154.72)[b]	0.73	0.00	8.83***	0.08	0.64	0.01	− 0.08
RT flexibility (msec)	672.72 (325.09)	616.98 (263.95)	511.23 (217.14)[b]	481.60 (174.16)[b]	445.85 (163.30)[b]	418.64 (171.86)[b]	0.22	0.00	11.42***	0.14	0.02	0.00	0.15
Number of errors inhibition	8.28 (5.49)	7.36 (7.31)	4.17 (4.53)[b]	4.30 (4.86)[b]	5.67 (6.20)[b]	3.90 (4.82)[b]	0.89	0.00	9.12***	0.09	1.38	0.01	0.01

Table 3 (continued)

	Baseline		4-month visit		6-month visit		Treatment		Time		Treatment × Time		Pre-post effect size
	DHA	Placebo	DHA	Placebo	DHA	Placebo	d^a	η^2	F	η^2	F	η^2	d^a
Number of errors flexibility	21.56 (12.18)[c]	14.96 (11.74)[c]	18.09 (13.35)[b]	12.85 (10.79)[b]	15.04 (12.39)[b]	9.75 (11.32)[b]	3.08	0.02	16.09***	0.20	0.36	0.01	− 0.15
ANT—sustained attention date													
Tempo X series	15.29 (3.28)	14.37 (4.06)	13.05 (2.80)[b]	13.19 (3.55)[b]	12.24 (4.02)[b]	12.04 (4.02)[b]	0.16	0.00	12.16***	0.16	2.07	0.02	− 0.11
SD	3.71 (1.55)	3.51 (1.51)	2.92 (1.24)[b]	3.14 (1.62)[b]	2.69 (1.49)[b]	2.85 (1.62)[b]	0.01	0.00	10.90***	0.17	0.96	0.01	− 0.06
Misses	34.80 (24.60)	37.68 (26.60)	29.09 (19.17)	35.57 (21.77)	32.26 (19.79)	37.14 (22.61)	0.24	0.00	0.34	0.00	0.01	0.00	− 0.06
False alarms	25.36 (18.12)	22.00 (16.67)	17.96 (11.68)[b]	17.38 (9.95)[b]	18.96 (18.10)[b]	12.86 (7.05)[b]	1.88	0.01	3.93*	0.05	0.68	0.01	0.23
Coefficient of variation	0.24 (0.07)	0.24 (0.08)	0.22 (0.06)	0.22 (0.07)	0.21 (0.07)	0.22 (0.06)	0.06	0.00	3.14	0.05	0.20	0.00	− 0.00

ANT Amsterdam neuropsychological task, *RT* reaction time, *SD* standard deviation

[a]Cohen's d

[b]Main effect of time

[c]Between-group baseline difference

*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

for the present clinical trial—and typically developing children [29]. In that study, we observed an association between higher level of DHA and lower parental rates of ADHD symptoms, lower clinical scores of severity, and a better global functioning measured by CHQ. In the present work, we extended those findings by suggesting that children with ADHD may benefit from DHA supplementation in terms of a better quality of life, as rated by their parents. Finally, with respect to the safety of the supplement used, no adverse events were reported by children and their parents, indicating a good tolerance for the dosage of both the DHA and the placebo.

The present work has several limitations. First, the study was limited by its small sample size. As elegantly calculated by Bloch and Qawasmi in their meta-analysis [8], a clinical trial should recruit approximately 330 participants to reliably detect the effect of omega-3 supplementation in light of an effect size of 0.31. Although the present study was underpowered, small but significant evidence of efficacy of DHA were ascertained. However, we cannot exclude that our sample size could have been unable to detect further significant benefits. In addition, we emphasize that in the present study the significance testing was not adjusted for multiple comparisons, because many of the outcome measures were intercorrelated. Small sample size could, therefore, have led to false-positive results, whereas one recent, well-powered study ($n = 162$) did not find any effect of mixed supplementation of DHA and eicosapentaenoic acid on behavioral symptoms or cognition [39]. Therefore, the results of the present study need to be replicated in a larger, independent sample. It is also fundamental to acknowledge the significant between-groups difference at baseline in the focused attention task that also showed an interaction between time and treatment condition. Children in the DHA group showed more false alarms of those in the placebo group, as shown in Fig. 4. This difference and other slight differences (although not statistically significant) on tests or questionnaires at baseline could have affected the findings of this trial, with children in the DHA group having more possibility of improvement. Finally, we cannot confirm the treatment adherence with blood samples throughout or at the end of the trial.

Keeping these limitations in mind, the present trial shows that 6-month DHA supplementation has no beneficial effect on the symptoms of ADHD in school-aged, drug-naïve children with an established clinical diagnosis. However, beyond the overall negative outcome, the 6 months treatment with supplemental DHA appears to have small positive effects on other behavioral and cognitive difficulties related to ADHD. In light of the absence of side-effects proved by this trial, these small benefits of DHA could be reasonably followed up in future intervention studies.

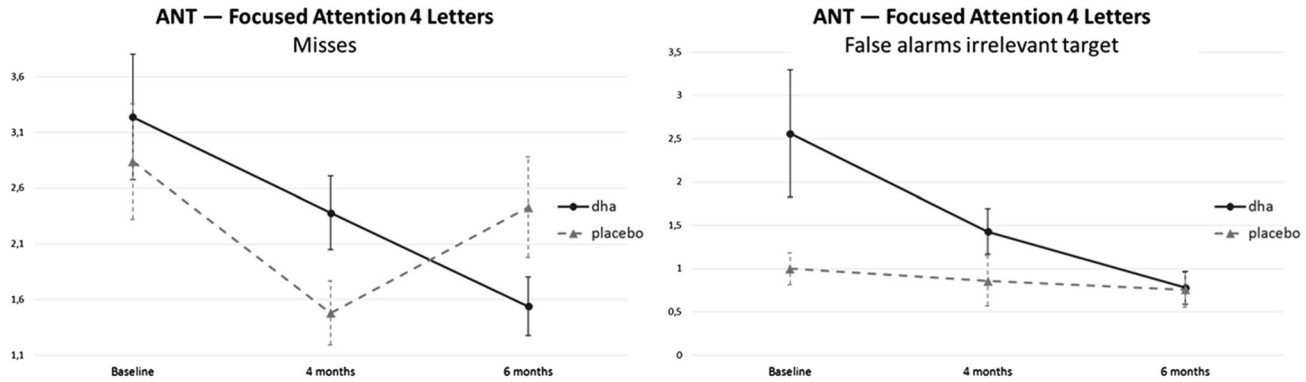

Fig. 4 Change in secondary outcome cognitive measures in DHA and in placebo group showing interactions between treatment condition and time. With respect to the Amsterdam Neuropsychological Tasks (ANT), participants supplemented with DHA showed a decrease of misses in focused attention 4-letters task at the 6-month visit (left panel; end of treatment versus baseline, $p=0.006$; end of treatment versus 4-month visit, $p=0.044$), whereas children in placebo group displayed a lower number of misses at the 4-month ($p=0.032$) but not at the 6-month visit ($p>0.05$). Furthermore, children in DHA group showed a reduction of false alarms irrelevant target in focused attention 4-letters task at the end of supplementation (right panel; end of treatment versus baseline, $p<0.001$; end of treatment versus 4-month visit, $p=0.028$). Children in the DHA group showed more false alarms in the focused attention task ($p=0.013$) also at baseline evaluation

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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