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Cognitive effects of polyunsaturated fatty acids in children with attention deficit hyperactivity disorder symptoms: A randomised controlled trial

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Abstract

This study investigated effects of PUFA and micronutrient supplementation on cognition in children with ADHD symptoms. In a randomised controlled trial, 7–12-year-old children with symptoms ≥ 2 S.D. on Conners' ADHD Index were given PUFA, PUFA + multivitamins/minerals (MVM), or placebo for 15 weeks, and then all children were given PUFA + MVM for an additional 15 weeks. After 15 weeks there were improvements in a test of the ability to switch and control attention (Creature Counting) in the PUFA groups compared to placebo (N = 129, p = 0.002). This improvement was also observed in the placebo group after taking PUFA from weeks 16 to 30 (N = 104). There were no significant improvements in other cognitive measures, or with additional micronutrient supplementation. However, improvements in cognitive performance mediated previous parent-reported improvements in inattention, hyperactivity and impulsivity [N. Sinn, J. Bryan, Effect of supplementation with polyunsaturated fatty acids and micronutrients on ADHD-related problems with attention and behaviour, J. Dev. Behav. Pediatr. 28 (2) (2007) 82–91], suggestive of a common neurological mechanism for these symptoms.

1. Introduction

Many school children have problems with inattention, hyperactivity and impulsivity, which may be diagnosed as attention deficit hyperactivity disorder (ADHD) when normal functioning starts to be impeded. ADHD prevalence rates vary, ranging between 3% and 11% in Western countries [1–3]. Cognitive deficits are commonly associated with ADHD symptoms, and it has been estimated that a quarter of children with ADHD have a learning disability [4]. Consistent with indications that developmental difficulties have a neurological basis are suggestions that cognitive deficits might be related to low levels of omega-3 (n-3) polyunsaturated fatty acids (PUFA) [5,6], and a small body of evidence that some children with developmental disorders such as ADHD, dyslexia, and dyspraxia may show improvements in symptoms following supplementation with n-3 PUFA [7–9]. The principle purpose of this study was to investigate effects of PUFA supplementation on cognition in children with ADHD symptoms.

Children with attention problems have been described as having a 'sluggish cognitive tempo' [10–12] and have been identified via psychophysiological assessments as having slower brain wave patterns, particularly in the frontal lobes. This may be associated with difficulties in executive functioning (EF), a cluster of higher order cognitive abilities associated with the frontal lobes that

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are broadly characterised by the regulation of attention, goal-directed behaviour, behavioural and response inhibition, processing speed, strategic planning and organisational abilities, working memory, and cognitive flexibility [13–15]. In support, children with ADHD often have difficulties with EF [14,16].

There is growing interest in the roles of n-3 PUFA docosahexaenoic acid (DHA) and precursor eicosapentaenoic acid (EPA) in brain structure and function and mental health [17–19]. The longest chain n-3 PUFA DHA is the most abundant PUFA in brain membrane phospholipids indicative of its role in membrane fluidity and associated metabolic and neural activities. DHA is particularly concentrated at neural synapses, sites of neurotransmitter signalling. Omega-6 PUFA arachidonic acid (AA) is also abundant in the brain reflecting a key role for brain structure and function. AA precursor, gamma-linolenic acid (GLA), and n-3 DHA precursor EPA are thought to be important for brain function via eicosanoid synthesis. EPA may be particularly important for production of eicosanoids with anti-inflammatory, anti-thrombotic, and vasodilatory properties. PUFA levels in neuronal membranes vary according to dietary intake. It is therefore of concern that intake of n-3 PUFA has declined in Western societies over recent decades.

PUFA have been proclaimed as critical for intellectual growth and development in the developing neonatal/ infant brain and in early childhood [20]. Given that brain development, particularly EF, continues throughout childhood [21,22], PUFA could also play an important role in cognitive function in older children. In addition, PUFA have been specifically associated with dopamine activity in the frontal lobes of the brain [23], which may impact directly on EF, and has been associated with ADHD [24].

A small number of studies have investigated effects of PUFA on cognition in this age group. An epidemiological survey found that PUFA intake was associated with better performance on the Digit Span subtest of the Wechsler Intelligence Scale for Children, 3rd edition (WISC-III; [10]) and, less robustly, on better reading after controlling for potential confounders [6]. Two early studies tested effects of n-6 PUFA in evening primrose oil on cognition in children with ADHD, and did not find any significant effects on a test of attention [25], or on a range of cognitive outcomes apart from short-term memory [26]. An American study using pure DHA but including children on stimulant medication found no improvements [27] and a Japanese study using oil-imbued bread also showed no effect [28]. Another small American study used a combination of n-3 and n-6 PUFA and found no significant improvements on cognitive outcomes [29]. However, a pilot study in the UK with children suffering from dyslexia and ADHD symptoms reported improved parent ratings of attention

and cognition in children [30] and this was supported by teacher ratings of improved attention and cognition in a larger study with children suffering from dyspraxia, both using a combined n-3 and n-6 PUFA supplement. In the latter study, significant improvements in reading and spelling were also reported [8]. A third of this sample had Conners' [31] ADHD scores in the clinical range ≥ 2 S.D. on teacher rating scales. Results of these studies were supported by a subsequent Australian study conducted by the present authors [9] that also found significant improvements in PUFA (combined n-3 and n-6) groups on parent ratings of cognitive problems/inattention compared to placebo over 15 weeks in a group of children all with ADHD scores ≥ 2 S.D. on Conners' ADHD Index [31]. The latter studies addressed some methodological issues in previous studies including selection criteria, length of supplementation, type and dosage of PUFA supplement, exclusion of children taking stimulant medication as a potentially confounding variable, a non-active placebo, and adequately powered sample sizes. Furthermore, it is important to examine whether parent/teacher observations are associated with objective measures of cognitive performance, selecting appropriately sensitive tests.

A test battery was designed for this study in consideration of frontal lobe brain development, cognitive deficits associated with ADHD, test sensitivity, and the role of PUFA in membrane fluidity, which may assist in speed of information processing. Additionally, speed of information processing is thought to underlie other cognitive abilities such as memory and learning [32]. Therefore, tests assessed global cognitive functioning using an IQ estimate, speed of processing, memory and learning, and EF, including tests of attention, inhibition, working memory, and distractibility. Because nutrients are synergistic and effective PUFA metabolism and eicosanoid synthesis are believed to rely on micronutrients such as zinc, magnesium and vitamins C, B1, B3, B6, and B12 [33,34], a micronutrient supplementation was also given along with PUFA to assess any possible additive benefits.

It was hypothesised that: (1) children taking PUFA would show improvements on cognitive outcomes compared to placebo after 15 weeks of supplementation; (2) there would be improvements with micronutrients over and above PUFA supplementation; (3) the placebo group would show similar improvements following switch to active treatment and that there would be further improvements in the PUFA treatment groups over an additional 15 weeks of supplementation; and (4) any changes in cognitive function would mediate parentreported improvements in attention and behaviour previously reported in this group of children following PUFA supplementation [9].

2. Method

2.1. Participants

A target sample size of 60 per group (total 180) was estimated using Cohen's recommendations [35] based on a medium effect size. Children were included if they were aged 7–12 and had ADHD symptoms, and were excluded if they were taking stimulant medication or had taken any PUFA supplements during the previous 3 months. A total of 201 South Australian children were registered by their parents, of which 182 attended their first appointment. Of those, 167 children (128 boys, 39 girls) had scores ≥ 2 S.D. above a US population average on Conners' ADHD Index [31]. Thirty-five of this group dropped out during the first 15 weeks (phase 1), and subsequently a further 23 dropped out. Therefore, 132 children completed phase 1 and 109 children completed the whole 30 weeks. Attrition was mainly due to non-compliance, and some children were prescribed stimulant medication, which was an exclusion criterion (see flowchart, Fig. 1). A Chi-square analysis showed no significant differences in dropout numbers between the groups, $\chi^2(4) = 1.91$, p = 0.75 during either phase of the study (N = 167).

Baseline demographics for those who completed phase 1 and those who completed both phases are given in Table 1. The mean IQ estimate across groups was lower than average (93.87), although slightly higher than the nine-point deficit identified in a meta-analysis of people with ADHD [36]. There were no significant differences between groups on demographic variables at baseline, except for parent ratings of health.

There was no difference in the age of children who pulled out before or during the study, F(2,179) = 0.99, p = 0.37. However, comparison of baseline parentreported scores on Conners' ADHD Index found that children who pulled out either before starting (M = 28.97) or before the end of phase one

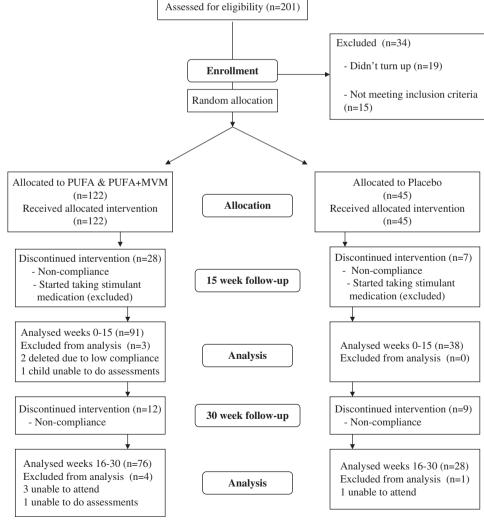


Fig. 1. Flowchart of participants through the study.

	Phase 1 (weeks 1-15)	ks 1–15)				Phase 2 (weeks 16-30)	sks 16–30)				
	PUFA/MVM (n = 46)	1	PUFA ($n = 45$)	Placebo $(n = 38)$	One-way ANOVA	PUFA/MVM $(n = 39)$		PUFA $(n = 37)$	Placebo ($n = 28$)	One-way ANOVA	
	$M\pm$ S.D.		M±S.D.	$M\pm$ S.D.	F(2, 126)	$M\pm$ S.D.	V	M±S.D.	$M\pm$ S.D.	F(2,114)	
A ge	9.28 ± 1.73		9.38+1.89	9.47 ± 1.83	0.12	8.97+1.61	6	9.38 + 1.91	9.64 ± 1.68	1.26	ĺ
BMI percentile	51.9 + 30.0		62.0 + 32.2	60.4 + 30.2	1.40	51.6 + 28.5	Ū.	59.3 + 32.9	63.0 + 30.1	1.23	
Child's health ^a	4.09 ± 0.88		3.90 ± 0.96	$+.37\pm0.59$	3.26^{*}	$+0.03\pm0.87$	ŝ	3.85 ± 0.98	$+.46\pm0.58$	4.34*	
IQ estimate ^b	96.3 ± 19.5		92.3 ± 17.1	93.0 ± 17.5	0.63	97.0 ± 20.9	.6	92.7 ± 18.7	96.9 ± 16.4	0.60	
Doctor ^c	2.76 ± 2.58		4.00 ± 6.56	3.03 ± 3.40	0.00	2.85 ± 2.67	4	4.40 ± 6.90	3.04 ± 3.84	1.11	
Hospital ^d	0.13 ± 0.40		0.14 ± 0.42	0.18 ± 0.39	0.20	0.15 ± 0.43	0	0.11 ± 0.39	0.11 ± 0.32	0.15	
School absences ^e	5.27 ± 6.03		5.79 ± 7.09	5.08 ± 5.56	0.14	4.91 ± 5.81	9	6.26 ± 7.18	4.46 ± 5.25	0.77	
Weeks breastfed	32.3 ± 33.0		30.3 ± 30.9	26.3 ± 29.6	0.38	33.6 ± 35.0		30.8 ± 31.4	29.2 ± 31.6	0.16	
PC^{f} age	40.4 ± 6.45		42.0 ± 6.65	38.4 ± 8.75	2.51	40.8 ± 6.70	4	42.0 ± 6.93	39.0 ± 9.78	1.21	
PC years	13.8 ± 2.69		13.5 ± 2.36	13.9 ± 5.35	0.17	14.1 ± 2.48	1.	13.6 ± 2.28	14.2 ± 6.06	0.25	
education ^g											
PC education	3.72 ± 1.21		3.68 ± 1.14	3.47 ± 1.20	0.50	3.85 ± 1.18	3	3.72 ± 1.15	3.54 ± 1.17	0.58	
PC occupation ¹	50.1 ± 13.6		51.2 ± 15.0	51.0 ± 13.2	0.07	50.6 ± 14.2	5.	52.3 ± 14.5	50.7 ± 14.0	0.16	
Child's gender	М	ц	M	F	F $\chi^2(2)$	(2) M	ш	М	F M	Ц	$\chi^2(2)$
	34	12	34 1	11 28	10 0.05	5 28	11	27	10 22	9	0.42
^a Parent rating of child', ^b IQ estimate taken fron ^c No. of visits in past 12 ^d No. of visits in past 12 ^d No. of visits in past 12 ^f In past 12 months. ^f PC: primary caregiver. ^g No. of years education leve ^h Highest education leve ⁱ Occupation rated on D *0005	^a Parent rating of child's health: 1 = poor; 5 = excellent. ^b IQ estimate taken from WISC dyad: Vocabulary and F ^c No. of visits in past 12 months. ^d No. of visits in past 12 months. ^d P. primary caregiver. ^f PC: primary caregiver. ^b Highest education level: 1 = primary school; 6 = postg ^b Occupation rated on Daniel's (1983) prestige scale (12 = **.0.005)	1 = poor dyad: Vo ed. ed. 983) pres	^a Parent rating of child's health: 1 = poor; 5 = excellent. ^b IQ estimate taken from WISC dyad: Vocabulary and Block Design. ^c No. of visits in past 12 months. ^d No. of visits in past 12 months. ^e In past 12 months. ^f PC: primary caregiver. ^b FOC: primary caregiver. ^b Highest education level: 1 = primary school; 6 = postgraduate degr ^b Cocupation rated on Daniel's (1983) prestige scale (12 = high; 69 = *Off	t Design. ate degree. gh; 69 = low).							

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(M = 28.74) had higher scores than children who completed phase 1 (M = 26.27) or the whole 30 weeks (M = 25.65), F(3,195) = 3.37, p = 0.02.

2.2. Materials

Measures comprised parent ratings of ADHD-related symptoms on Conners' Parent Rating Scales (CPRS)— Revised [31], as previously reported [9], a parent background questionnaire and a battery of cognitive assessments. The cognitive tests provided an interesting variety of fairly short duration tasks. Three different protocols used, where possible, alternate forms (i.e. Digit-Symbol Coding, Creature Counting, Rey Auditory Verbal Learning Test, and the Stroop test). Each group was given these protocols in three different sequences to avoid any possible order effects. Note that the test–retest reliability (or alternate form where different versions of the test were administered) and Cronbach's alpha coefficients given below were calculated with the placebo group from baseline to week 15 (n = 38).

2.2.1. IQ estimate

General cognitive ability was estimated via a short dyad form of the WISC-III [10], comprising the Vocabulary and Block Design subtests. Reliability (test-retest) and validity (correlation with full-scale IQ) coefficients for this combination are reported as 0.91 and 0.86, respectively [37]. The test-retest reliability for the vocabulary subtest is reported as 0.82 and 0.88 for 6–7 and 10–11 year olds, respectively [10], and in the current study was 0.89.

Block Design assesses visuo-spatial organisation, problem-solving ability, non-verbal abstract reasoning, and concept formation [37]. To avoid ceiling effects due to practice over repeated administration, the nine-block designs from the Wechsler Adult Intelligence Scale— Third Edition (WAIS-III; [38]) were added to the WISC-III version, although only the WISC-III totals were used to calculate the IQ estimate. The test–retest reliability is reported as 0.69 and 0.75 [10], and in the current study was 0.80 for the WISC-III subtest and 0.85 for the total test with additional WAIS-III designs.

2.2.2. Speed of processing

Speed of processing was measured via Inspection Time [39] and the Digit-Symbol Coding subtest of the WISC-III [10]. The test-retest reliability for Inspection Time in the present study was 0.77. The test-retest reliability for Digit-Symbol Coding is reported in the manual as 0.70–0.78. The alternate form reliability in the present study was 0.90.

2.2.3. Learning and memory

Recognition and recall memory were assessed using the Rey Auditory-Verbal Learning Test (RAVLT) [40]. The alternate form reliability in this study for each of the scores ranged from 0.68 to 0.78. Short-term recall memory was also assessed by the forward Digit Span subtest of the WISC-III [10]. The test–retest reliability in the present study was 0.71.

2.2.4. Executive functioning

2.2.4.1. Attention. The Creature Counting subtest of the Test of Everyday Attention for Children (TEA-ch) has been found to differentiate children with ADHD from controls [41] and was used a test of the ability to control and switch attention. The test-retest reliability coefficients for Creature Counting are reported as 0.71 for accuracy and 0.57 for the Creature Counting score [41]. In the present study the alternate form reliabilities were 0.54 for accuracy, 0.54 for number of correct switches and 0.10 for the Creature Counting score. Because the reliability of the latter score was so low, it was not included in analyses.

2.2.4.2. Working memory. Working memory was assessed by the Digit Backwards subtest of the WISC-III [10]. The test-retest reliability for the Digit Span score (total of Digits Forward and Backward) is reported in the manual as 0.67–0.75. In the present study the test-retest reliability for Digits Backward was 0.76 and for the total score was 0.85.

2.2.4.3. Ability to inhibit responses. Motor inhibition was assessed using the Knock and Tap subtest from the NEPSY, a developmental neuropsychological assessment battery [42]. The test-retest reliability was 0.42 for the first set, 0.26 for the second set, and 0.13 for the total of both sets. The second, more difficult set is the target test for assessing inappropriate inhibition. This reliability was unacceptably low, and the test also produced marked ceiling effects. Results were therefore not included in analyses.

2.2.4.4. Distractibility. Mental flexibility and the ability to ignore distraction from task-irrelevant information was assessed with the Stroop colour-word test [43,44]. Many of the children had difficulty reading the entire list of words, so only half the list was used. The test-retest reliability of the half-test with alternate forms in the present study was 0.39.

2.3. Supplements

The PUFA capsules, 'eye-q', were supplied by Equazen Neutraceuticals and Novasel Australia. Each contained 400 mg fish oil and 100 mg evening primrose oil with active ingredients EPA (93 mg), DHA (29 mg), GLA (10 mg), and vitamin E (1.8 mg). Placebo capsules contained palm oil, containing predominantly saturated/ monounsaturated fats: 44.3% palmitic acid C16, 4.6%

stearic acid C18, 1% myristic acid C14, 38.7% oleic acid C18, and 10.5% linoleic acid C18. These were imbued with a fishy aroma and flavour, and PUFA and placebo capsules were identical in appearance and colour. Children were required to take six active or six placebo capsules daily.

The multivitamin/mineral (MVM) supplement, 'multivitamins and minerals for kids' supplied by Blackmores Australia, consisted of chewable tablets with fruit flavours, each containing active ingredients vitamin A 175 IU, thiamine nitrate 700 mcg, vitamin B2 1.1 mg; vitamin B6 1.3 mg, nicotinamide 12 mg, vitamin C 60 mg, vitamin D3 100 IU, vitamin B12 1.5 mcg, vitamin E6 IU, biotin 50 mcg, vitamin B5 2.7 mg, folic acid 100 mcg, calcium hydrogen phosphate anhydrous 33.9 mg, ferrous fumarate 7.5 mg, magnesium oxide 8.32 mg, manganese sulphate 77 mcg, zinc oxide 1.25 mg, copper gluconate 178.6 mcg, and potassium iodide 118 mcg. Those in the LC-PUFA + MVM treatment condition, and all children from 16 to 30 weeks were required to take one MVM tablet daily.

Both supplements are available for purchase in pharmacies and health food shops.

2.4. Procedure

Participants were recruited via media releases, newspaper advertisements, and school newsletters advertising for children aged 7–12 with ADHD-related learning and behavioural difficulties (not necessarily diagnosed). Parents were given the 12-item Conners' ADHD Index [31] upon registration to determine their child's eligibility for the study. Supplements were independently coded by the manufacturer and blinding maintained until data was analysed. As children were registered, they were matched on age and gender before being randomly allocated to one of three conditions identified only by number.

Parents were required to complete a background questionnaire and the CPRS long version (CPRS-L) at baseline (week 0), and the CPRS-L at weeks 15 and 30. Children's height and weight were taken at weeks 0, 15, and 30 to calculate their BMI, which was converted into age- and gender-appropriate percentiles [45]. About 20-25 children commenced per week during March-May 2004, resulting in $3 \times (8-9)$ -week testing periods 15 weeks apart. Parents brought children into the Commonwealth Scientific and Industrial Research Organisation (CSIRO) division of Human Nutrition cognitive testing rooms at 0, 15, and 30 weeks. Cognitive assessments took 50-60 min per visit and were administered by a trained psychology graduate. If parents remained present, they were seated behind the child and encouraged to remain quiet throughout the testing.

At baseline, parents were given supplements or placebo according to randomised allocation with appropriate instructions. It was not possible to obtain a placebo tablet for the MVM tablets, so boxes were packed and sealed by an independent researcher in a separate division of CSIRO to maintain the doubleblind status during phase 1. At week 15, all parents were given LC-PUFA and MVM supplements. To monitor compliance, parents were asked to return all unused capsules and children were given calendars with stickers to record each time they took the capsules. The study was approved by the Human Research Ethics Committees of CSIRO and the University of South Australia.

2.5. Design

The study was a randomised, placebo-controlled intervention trial providing within- and between-group comparisons over 30 weeks with a one-way crossover to active supplements at 15 weeks. During weeks 1–15 (phase 1), participants were given either active LC-PUFA capsules with a MVM tablet, active LC-PUFA capsules alone, or placebo oil capsules. At 15 weeks all children were given the active LC-PUFA capsules and MVM tablets for weeks 16–30 (phase 2). The first phase of the study was double-blind; phase 2 was single-blind in that the researcher knew that all children were receiving active treatment after 15 weeks.

Mixed-design 2×2 ANCOVAs were used to test hypotheses 1 and 2 from weeks 1 to 15, and withingroup one-way repeated measures ANOVAs with two levels to test both parts of hypothesis 3 from weeks 16 to 30. Dependent variables were cognitive test outcomes, outlined under measures. Parent ratings of children's health were used as a covariate in analyses involving the placebo group because this group differed from the PUFA groups at baseline on this variable (see Table 1). Mixed design 2×2 ANCOVAs were used to investigate whether changes in cognitive performance mediated parental ratings of improved ADHD symptoms via reduced effect sizes (hypothesis 4).

3. Results

3.1. Data preparation and screening

All analyses were conducted using SPSS 11.5.0. To correct for a Type I error from multiple analyses, a Bonferroni adjustment was calculated for an alpha of p < 0.05 with 16 variables with an average correlation of 0.294. The resulting alpha level was set at p < 0.003 for all analyses. During phase 1, one child was unable to do the cognitive assessments and two were deleted due to unacceptably low compliance with taking supplements. A total of 129 cases were available for analysis of phase 1 data and 104 cases for phase 2 data (see Fig. 1). A variety of methods were used for dealing with small numbers of random missing data including predicted

values based on regression equations [46]. Four missing Stroop scores at times 1, 2, and 3 (for the same participants at each time) were not replaced because these participants had been unable to perform the Stroop test. Histograms were inspected for outliers; those ≥ 3.29 S.D. above or below the mean were replaced with the value closest to the next highest or lowest score in the distribution. Following treatment of missing values and outliers, skewness and kurtosis statistics were within normal parameters (± 1.96) and histogram distributions showed no notable deviations from normality.

3.2. Normative data

Where possible, baseline scores were scaled (M = 10; S.D. = 1.5) using information provided in respective test manuals. The sample was well below average (>1 S.D.) on Creature Counting (6.88 ± 3.33) and Digit-Symbol Coding scores (7.22 ± 3.53). Vocabulary scores were just below the average range (8.32 ± 3.31); Block Design (9.59 ± 3.90) and Digit Span (9.00 ± 3.00) scores were lower than the mean but within the average range.

3.3. Baseline analyses

Chi-square analyses conducted within each data set (1–15 and 1–30 weeks) showed no differences between groups on the order of the three different testing protocols, $\chi^2(4,1) = 1.27$, p = 0.87 and $\chi^2(4,1) = 1.35$, p = 0.85, respectively. One-way ANOVAs showed no significant baseline differences between groups on the cognitive assessments, with *F* values ranging from 0.13 (p = 0.88) to 1.75 (p = 0.18).

3.3.1. Phase 1 results: 0–15 weeks

Mixed design ANCOVAs, with health as a covariate, were performed to test whether there was a significant treatment effect on cognitive performance over 15 weeks in the PUFA groups compared with placebo, represented by a treatment × condition interaction. ANCOVA results are shown in Table 2 with means, standard deviations, and effect sizes. There was a significant improvement in the PUFA groups compared to placebo on Creature Counting (p = 0.002) with a large effect. Improvements in vocabulary scores showed a medium effect size but were not significant with the adjusted alpha level (p = 0.015).

Mixed design ANOVAs were performed to investigate whether there was any additional improvement with the MVM over and above the PUFA alone. There were no significant differences on cognitive outcomes between the PUFA groups with and without MVM. Therefore, the PUFA groups were combined for subsequent analyses.

One-way ANOVAs comparing changes in Creature Counting and Vocabulary scores from baseline to 15 weeks between males and females did not show any significant gender effects. 3.3.2. Phase 2 results: treatment crossover 16–30 weeks

Repeated measures ANOVAs were conducted to test the hypotheses that the placebo group would show similar improvements from weeks 16 to 30 following their switch to PUFA, and that the PUFA groups would continue to improve. ANOVA results and effect sizes are presented in Table 3 with means and standard deviations. As in the PUFA groups from 0 to 15 weeks, the placebo group showed improvements on Creature Counting from weeks 16 to 30 (p < 0.001) and Vocabulary (p < 0.001), and also on Coding (p < 0.001), Block Design (p < 0.001) and Inspection Time (p < 0.001), probably due to practice effects. PUFA groups showed continued significant improvement on Creature Counting and Vocabulary scores, as well as on RAVLT recall, Digit-Symbol Coding, and Block Design, also likely to be further practice effects as observed in both groups during phase 1. There were large improvements on Stroop scores in the PUFA groups (p < 0.001) not seen in the placebo group from weeks 16 to 30; however, the significance of this is unclear as all groups had similar Stroop scores at week 30.

3.4. Mediation of parent-reported improvements

Analyses were then performed to investigate whether changes in cognitive function mediated parent-reported improvements in inattention, hyperactivity and impulsivity. Firstly, correlations were conducted to investigate relationships between CPRS scores previously reported [9] and cognitive assessments from the present study at baseline, including cases for which parent data were available during phase 1 (N = 114). These are presented in Table 4. ADHD Index scores showed significant inverse correlations with better performance on attention control (Creature Counting), speed of processing (Digit-Symbol Coding and Inspection Time), and memory (RALVT total recall and recognition, and Digits Forward). Vocabulary scores were inversely correlated with hyperactivity/impulsivity. The cognitive problems/inattention subscale was only inversely correlated with the IQ estimate, memory (RAVLT total recall) and attention control (Creature Counting).

ANCOVAs were conducted to assess whether changes in cognitive scores from weeks 1 to 15 mediated treatment \times condition interactions in Conners' ADHD Index, hyperactivity/impulsivity and cognitive problems/ inattention scales (see Table 5). Mediation was determined by assessing whether effect sizes¹ were reduced when cognitive change scores were added as covariates to

¹*Note*: Treatment effect sizes presented in Tables 3 and 4 were recalculated from partial eta squared to Cohen's *d* to enable easier comparison with other studies. Effect sizes for Tables 5 and 6 are presented as partial eta squared because their function is merely relative comparison with one another.

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Table 2

Means, standard deviations and ANCOVAs between PUFA groups combined and placebo group on cognitive assessments at baseline and 15 weeks, N = 129

Cognitive assessments	Group	Baseline	15 weeks	2×2 ANCOVA	As ^a
		$M \pm$ S.D.	$M\pm$ S.D.	<i>F</i> (1,126) ^b	Effect
IQ estimate					
Block Design	PUFA	31.91 ± 18.55	39.75 ± 21.12		
	Placebo	32.42 ± 14.39	39.11 ± 19.57	0.29	0.06
Vocabulary	PUFA	21.88 ± 7.04	23.33 ± 7.16		
	Placebo	21.42 ± 7.93	21.29 ± 7.47	6.15*	0.31
IQ estimate	PUFA	94.31 ± 18.36	99.23 ± 19.21		
	Placebo	93.03 ± 17.49	94.87 ± 16.55	2.82	0.21
Speed of processing					
Digit-Symbol Coding	PUFA	34.27 ± 12.29	37.99 ± 13.45		
	Placebo	34.08 ± 11.59	36.55 ± 13.36	1.38	0.14
Inspection Time ^c	PUFA	101.2 ± 27.29	80.88 ± 22.16		
	Placebo	98.63 ± 31.77	83.09 ± 26.97	0.82	0.11
Recall and recognition memory					
RAVLT ^d total recall	PUFA	37.13 ± 10.06	39.29 ± 9.45	0.55	0.09
	Placebo	37.32 ± 10.21	38.50 ± 10.32		
RAVLT delayed recall	PUFA	7.63 ± 2.90	7.71 ± 2.95	0.02	0.02
	Placebo	6.92 ± 2.83	7.16 ± 2.75		
RAVLT 20-min delayed recall	PUFA	7.08 ± 2.99	7.50 ± 3.03	0.00	0.00
	Placebo	6.68 ± 2.95	7.05 ± 3.17		
RAVLT intrusions ^c	PUFA	3.63 ± 4.15	2.51 ± 3.41	0.81	0.11
	Placebo	2.61 ± 3.51	2.32 ± 2.33		
RAVLT recognition list A	PUFA	12.75 ± 2.14	12.53 ± 2.38	2.11	0.18
	Placebo	12.53 ± 2.57	11.74 ± 2.89		
Digits Forward	PUFA	7.98 ± 1.99	8.13 ± 2.05	0.05	0.03
	Placebo	8.32 ± 2.36	8.58 ± 2.70		
Executive functioning—attention					
Creature Counting: no. correct	PUFA	3.21 ± 2.34	4.77 ± 1.93		
-	Placebo	3.53 ± 2.46	3.82 ± 2.48	11.73**	0.43
Creature Counting: no. switches	PUFA	11.16 ± 8.47	16.32 ± 7.17		
-	Placebo	12.34 ± 9.03	13.37 ± 8.95	8.92**	0.37
Working memory					
Digits Backward	PUFA	3.56 ± 1.46	3.75 ± 1.67		
-	Placebo	3.87 ± 2.04	3.74 ± 2.06	0.75	0.11
Distractibility					
Stroop errors	PUFA	5.90 ± 8.28	4.26 ± 5.07		
•	Placebo	4.36 ± 5.18	4.11 ± 5.13	0.98	0.12
Stroop score ^c	PUFA	2.45 ± 0.73	2.34 ± 0.64		
-	Placebo	2.49 ± 0.84	2.17 ± 0.43	2.23	0.19

Note: Effect size = Cohen's *d*. Groups: PUFA, n = 91; placebo, n = 38.

^aHealth was a covariate (the placebo group differed significantly from the PUFA groups on baseline health ratings).

^bF values represent interaction effects between group condition and time.

^cLower scores represent better performance on these tests.

^dRAVLT: Rey Auditory Verbal Learning Test.

p < 0.05.**p < 0.01.

the analyses. Firstly, effects were investigated with and without health as a covariate. It can be seen that health ratings did remove some of the error variance in CPRS scores by increasing the effect size. However, these were not used as a covariate in remaining analyses as they would have counteracted any detectable mediating effects

(lower effect sizes) of the cognitive change scores. Changes in attention control from baseline to week 15 mediated treatment effects in the PUFA groups compared to placebo on the ADHD Index, hyperactivity/ impulsivity, and cognitive problems/inattention. Immediate recall memory (RAVLT total) mediated a small

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Table 3

Means and standard deviations at 0, 15 and 30 weeks (N = 104) plus one-way RM ANOVAs (with two levels) for placebo group^a and PUFA groups from 16 to 30 weeks

Cognitive assessment	Group	0 week	15 weeks	30 weeks	RM ANOVA	16–30 weeks
		$M \pm$ S.D.	$M\pm$ S.D.	$M \pm$ S.D.	F^{b}	Effect
IQ estimate						
Block Design total	PUFA	31.03 ± 19.21	38.64 ± 21.44	45.01 ± 22.80	28.598**	0.29
-	Placebo	35.18 ± 12.85	42.14 ± 18.65	49.86 ± 17.55	13.407**	0.43
Vocabulary score	PUFA	21.74 ± 7.27	23.18 ± 7.35	24.13 ± 6.78	8.763**	0.13
-	Placebo	23.54 ± 7.16	23.29 ± 7.12	25.57 ± 7.19	15.568**	0.32
IQ estimate	PUFA	94.91 ± 19.86	99.95 ± 20.82	101.88 ± 20.23	3.295	0.09
	Placebo	96.86 ± 16.43	98.25 ± 15.35	103.32 ± 14.99	12.245**	0.33
Speed of processing						
Digit-Symbol Coding	PUFA	33.41 ± 11.78	37.28 ± 13.16	39.89 ± 13.19	16.270**	0.20
	Placebo	35.07 ± 10.60	37.14 ± 11.65	41.43 ± 11.50	16.419**	0.37
Inspection Time ^c	PUFA	103.41 ± 27.32	82.97 ± 21.82	71.39 ± 15.95	27.138**	0.61
	Placebo	93.09 ± 31.62	78.96 ± 26.35	68.44 ± 29.10	5.036*	0.38
Learning and memory						
RAVLT ^d total recall A1–A5	PUFA	36.08 ± 9.89	38.34 ± 9.31	40.78 ± 9.74	6.861*	0.26
	Placebo	38.71 ± 9.70	38.86 ± 9.63	41.29 ± 10.15	3.103	0.25
RAVLT delayed recall	PUFA	7.25 ± 2.87	7.33 ± 8.18	6.74 ± 2.95	10.439**	0.10
	Placebo	7.46 ± 2.76	7.39 ± 2.57	8.14 ± 2.95	3.011	0.27
RAVLT 20-min delayed recall	PUFA	6.74 ± 2.95	7.16 ± 2.82	7.26 ± 2.96	0.640	0.03
2	Placebo	7.18 ± 2.97	7.43 ± 2.77	7.29 ± 3.04	0.181	0.05
RAVLT intrusions ^c	PUFA	3.61 ± 4.04	2.63 + 3.61	1.54 ± 1.97	6.702*	0.37
	Placebo	1.71 ± 2.36	2.29 ± 2.40	1.61 ± 2.39	1.689	0.28
RAVLT recognition list A	PUFA	12.63 ± 2.25	12.30 ± 2.43	12.09 ± 2.30	0.576	0.09
	Placebo	12.29 ± 2.80	11.43 ± 3.17	11.39 ± 2.95	0.007	0.01
Digits Forward	PUFA	8.07 ± 2.10	8.22 ± 2.18	8.50 ± 2.18	2.700	0.13
	Placebo	8.50 ± 2.43	8.79 ± 2.74	9.21 ± 2.62	2.141	0.16
Executive functioning—attention						
Creature Counting: no. correct	PUFA	3.03 ± 2.44	4.67 ± 2.04	5.11 ± 1.94	5.464*	0.22
e	Placebo	3.86 ± 2.31	4.14 ± 2.34	5.82 ± 1.49	23.640**	0.86
Creature Counting: no. switches	PUFA	10.49 ± 8.78	15.88 ± 7.53	18.30 ± 7.16	11.837**	0.33
	Placebo	13.61 ± 8.44	14.29 ± 8.32	20.89 ± 5.64	27.625**	0.93
Working memory						
Digits Backward	PUFA	3.50 ± 1.46	3.58 ± 1.53	3.75 ± 1.53	1.688	0.11
C	Placebo	4.07 ± 2.16	4.04 ± 2.13	4.39 ± 2.30	1.772	0.16
Distractibility						
Stroop errors ^c	PUFA	6.32 ± 8.54	4.45 ± 5.01	1.95 ± 3.03	20.923**	0.60
-	Placebo	4.15 ± 5.09	4.07 ± 5.55	1.71 ± 2.62	5.777*	0.54
Stroop score ^c	PUFA	2.42 ± 0.69	2.39 ± 0.67	2.09 ± 0.46	28.297**	0.52
*	Placebo	2.49 ± 0.61	2.18 ± 0.47	2.09 ± 0.42	1.395	0.20

Note: Groups: PUFA, n = 76; placebo, n = 28.

^aPlacebo group crossed over to PUFA at week 15.

^bd.f. for PUFA group = 1,74; d.f. for placebo group = 1,26. *F* values represent within-group improvement from weeks 16 to 30 following PUFA supplementation.

^cLower scores represent better performance on these tests.

^dRAVLT: Rey Auditory Verbal Learning Test.

**p*<0.05.

***p*<0.01.

proportion of treatment effects on the ADHD Index, hyperactivity/impulsivity, and cognitive problems/inattention, and the IQ estimate had a small mediating effect on cognitive problems/inattention. Improvements in vocabulary scores mediated some of the improvements in inattention and hyperactivity/impulsivity. It was then aimed to investigate whether combined changes in cognitive scores accounted for a greater proportion of these improvements. Therefore, further ANCOVAs were conducted using all of the cognitive scores as covariates that had individually to some degree reduced the effect of CPRS subscale treatment effects.

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	Cognitive problems/ inattention	Hyperactivity ADHD Index	y ADHD Index	Global: restless/ impulsive	Global: emotional lability	Global index: total	DSM-IV inattentive	DSM-IV e hyperactive/ impulsive	DSM- IV total	Opposition	al Anxious/ shy	Oppositional Anxious/ Perfectionism Social shy proble	n Social problems	Psychosomatic
Block Design	-0.04	-0.05	-0.03	-0.04	-0.10	-0.07	-0.03	-0.05	-0.04	0.01	-0.09	0.06	-0.14	-0.01
Vocabulary	-0.06	-0.15	-0.15	-0.16^{*}	-0.11	-0.16^{*}	-0.05	-0.21^{*}	-0.16^{*}	-0.10	-0.18^{*}	-0.08	-0.15	0.04
IQ estimate	-0.24^{**}	0.13	-0.11	-0.02	-0.02	-0.02	-0.14	0.05	-0.05	-0.07	-0.12	0.03	-0.18^{*}	-0.10
Digit-Symbol	-0.14	-0.13	-0.21^{*}	-0.12	-0.08	-0.12	-0.17^{*}	-0.13	-0.17^{*}	0.08	0.02	0.08	-0.09	0.10
Coding Inspection	0.16^{*}	0.21*	0.22*	0.15	-0.04	0.09	0.14	0.21^{*}	0.20^{*}	-0.03	-0.03	-0.06	0.04	-0.02
Time ^a RAVLT total	-0.24^{**}	-0.01	-0.23** -	-0.12	-0.03	-0.10	-0.19^{*}	-0.07	-0.15	0.03	-0.02	0.13	-0.04	0.05
recall A1–A5 RAVLT	-0.08	-0.09	-0.13	-0.12	-0.09	-0.12	-0.06	-0.11	-0.10	-0.03	-0.12	-0.00	-0.09	0.02
delayed recall RAVLT 20-	-0.06	-0.00	-0.03	-0.02	-0.02	-0.02	-0.04	-0.03	-0.04	0.04	-0.06	0.06	-0.14	0.03
min recall RAVLT	0.10	0.14	0.16^{*}	0.17^{*}	-0.04	0.11	0.11	0.21*	0.19*	0.03	0.02	0.03	0.03	-0.07
intrusions" RAVLT recognition list	-0.07	0.01	-0.16^{*}	-0.07	0.05	-0.03	-0.06	-0.05	-0.06	0.10	-0.02	0.09	0.03	0.06
A RAVLT recognition list	-0.13	-0.15	-0.19^{*}	-0.22^{**}	0.06	-0.14	-0.15	-0.21^{*}	-0.21^{*}	-0.04	0.04	0.15	0.00	0.09
B Digits	-0.13	-0.13	-0.22^{**}	$-0.22^{**} - 0.21^{*}$	-0.12	-0.20^{*}	-0.13	-0.21^{*}	-0.19^{*}	-0.06	-0.14	-0.10	-0.20^{*}	-0.02
Forward Creature Counting:	-0.17^{*}	-0.13	-0.23**	$-0.23^{**} - 0.19^{*}$	-0.04	-0.15	-0.17*	-0.18^{*}	-0.20^{*}	0.05	-0.05	-0.01	-0.18^{*}	0.05
correct Creature Counting: switches	-0.17*	-0.13	-0.24**	$-0.24^{**} - 0.18^{*}$	-0.01	-0.14	-0.18^{*}	-0.18^{*}	-0.21*	0.09	-0.04	-0.01	-0.13	0.06
Digits	-0.10	-0.09	-0.09	-0.07	-0.10	-0.09	-0.05	-0.12	-0.10	-0.02	0.06	-0.08	-0.11	0.20^{*}
Backward Stroop no. of	0.07	-0.05	0.10	0.02	-0.01	0.01	0.08	-0.01	0.03	-0.08	0.11	-0.01	0.07	0.08
Stroop score	0.13	0.02	0.12	0.07	0.03	0.06	0.15	-0.02	0.08	0.13	-0.02	-0.02	0.18^{*}	-0.05

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Table 5

ANCOVA results: treatment × condition interactions comparing PUFA and placebo groups from baseline to week 15 on Conners' parent ADHD Index, DSM-IV hyperactivity/impulsivity and cognitive problems/inattention scales, with and without health and cognitive change scores^a as covariates, N = 104

	$M \pm$ S.D.		$F(1,101)^{\rm b}$	Effect
	Baseline	15 weeks		
ADHD Index				
PUFA $(n = 77)$ Placebo $(n = 27)$	26.43 ± 5.98 26.99 ± 5.78	$21.53 \pm 7.22 25.29 \pm 5.27$	7.38**	0.067
Covariate	Parent ratings of child health ^d		9.09**	0.083
	Block Design		7.58**	0.070
	Vocabulary scores		7.94**	0.073
	IQ estimate		7.78**	0.072
	Speed of processing	Digit-Symbol Coding Inspection Time	7.31** 7.07**	0.067 0.065
	Memory	RAVLT total A1–A5	6.40*	0.060
		RAVLT delayed recall	7.13**	0.066
		RAVLT 20-min delayed recall	7.47**	0.069
		RAVLT intrusions	7.24**	0.067
		RAVLT recognition	7.22**	0.067
		Digits Forward	7.83**	0.072
	Executive functioning	Creature Counting: no. correct	5.26*	0.050
		Creature Counting: switches	4.98*	0.047
		Digits Backward	7.13**	0.066
		Stroop color-word test errors	7.91**	0.074
		Stroop color-word test score	7.65**	0.072
Cognitive problems/inattentia PUFA ($n = 77$)		20.89 ± 7.30		
POPA $(n = 77)$ Placebo $(n = 27)$	24.91 ± 6.42 25.30 ± 7.05	20.89 ± 7.30 24.51 ± 6.68	9.40**	0.084
	D () () () () () ()		10.07**	0.001
Covariate	Parent ratings of child health ^d		10.06**	0.091
	Block Design		9.57** 9.55**	0.086 0.086
	Vocabulary scores IQ estimate		9.33 8.74**	0.080
	Speed of processing	Digit-Symbol Coding	8.74**	0.080
	speed of processing	Inspection Time	9.57**	0.087
	Memory	RAVLT total A1–A5	8.49**	0.078
		RAVLT delayed recall	9.10**	0.083
		RAVLT 20-min delayed recall	9.69**	0.088
		RAVLT intrusions	9.61**	0.087
		RAVLT recognition	9.55**	0.086
		Digits Forward	9.64**	0.087
	Executive functioning	Creature Counting: no. correct	6.47*	0.060
		Creature Counting: switches	6.29*	0.026
		Digits Backward	9.09**	0.083
		Stroop color-word test errors Stroop color-word test score	10.45** 9.37**	0.095 0.086
DSM-IV inattention				
PUFA $(n = 77)$	20.05 ± 4.82	16.10 ± 5.97		
Placebo $(n = 27)$	19.85 ± 5.29	19.26 ± 4.25	11.76**	0.103
Covariate	Parent ratings of child health ^d		11.24**	0.100
	Block Design		11.97**	0.106
	Vocabulary scores		9.57**	0.087
	IQ estimate		10.93**	0.098
	Speed of processing	Digit-Symbol Coding	11.16**	0.100
		Inspection Time	12.16**	0.107
	Memory	RAVLT total A1–A5	10.92**	0.098
		RAVLT delayed recall	12.29**	0.108

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Table 5 (continued)

	$M \pm$ S.D.		$F(1,101)^{\rm b}$	Effect ^c
	Baseline	15 weeks		
		RAVLT 20-min delayed recall	12.15**	0.107
		RAVLT intrusions	12.10**	0.107
		RAVLT recognition	12.07**	0.107
		Digits Forward	12.07**	0.107
	Executive functioning	Creature Counting: no. correct	7.95**	0.073
	5	Creature Counting: switches	7.80**	0.072
		Digits Backward	10.95**	0.098
		Stroop color-word test errors	11.67**	0.105
		Stroop color-word test score	11.03**	0.100
DSM-IV hyperactive/im	pulsive			
PUFA $(n = 77)$	15.37+6.50	12.19+6.44		
Placebo $(n = 27)$	14.33 ± 5.16	13.40 ± 5.49	7.69**	0.070
Covariate	Parent ratings of child health	1	7.68**	0.071
corunate	Block Design		7.61**	0.070
	Vocabulary scores		5.59*	0.052
	IQ estimate		7.80**	0.072
	Speed of processing	Digit-Symbol Coding	7.84**	0.072
	Speed of processing	Inspection Time	7.40**	0.068
	Memory	RAVLT total A1–A5	7.24**	0.067
	incentory .	RAVLT delayed recall	8.46**	0.077
		RAVLT 20-min delayed recall	7.72**	0.071
		RAVLT intrusions	7.23**	0.067
		RAVLT recognition	8.13**	0.074
		Digits Forward	8.29**	0.076
	Executive functioning	Creature Counting: no. correct	6.33*	0.059
	······································	Creature Counting: switches	5.81*	0.054
		Digits Backward	7.48**	0.069
		Stroop color-word test errors	7.83**	0.073
		Stroop color-word test score	7.09**	0.067

^aCognitive change scores = scores at 15 weeks minus scores at baseline.

^bF values represent interaction effects between group condition and time on Conners' Parent Rating Scale subscale scores (9).

^cEffect size = partial eta squared (η_p^2) .

^dParent ratings of health was used as a covariate because groups differed in this variable at baseline.

p < 0.05.**p < 0.01.

Results of the treatment × condition interaction on the ADHD Index, cognitive problems/inattention, and DSM-IV hyperactivity/impulsivity subscales are presented in Table 6. Effects were further reduced with the combination of cognitive scores as covariates, particularly for cognitive problems/inattention and DSM-IV hyperactivity/impulsivity.

4. Discussion

In this study, 15 weeks of PUFA supplementation produced strong improvements in children's ability to switch and control attention compared to placebo. There were improvements in vocabulary with medium effects although they were not significant. No significant improvements were detected by assessments of general cognitive ability, speed of processing, learning and memory, working memory or distractibility. During phase 2 (weeks 16-30) when all groups switched to active treatment, the PUFA groups showed continued improvement on attention control and significant improvements in vocabulary. They also showed improvement on other measures, which are most likely practice effects as observed in phase 1. Contrary to hypotheses, there were no additional benefits of micronutrients over and above PUFA on cognitive outcomes that improved after PUFA supplementation. As discussed previously [9], the dosages of micronutrients may not have been sufficient and this may warrant further investigation. No adverse effects were reported apart from slight nausea by two parents and one report of nose bleeds.

Indications of improved vocabulary performance are plausible given that improved reading and spelling,

Table 6

ANCOVA results: treatment × condition interactions comparing PUFA and placebo groups from baseline to week 15 on Conners' parent ADHD Index, DSM-IV hyperactivity/impulsivity and cognitive problems/inattention subscales with combined cognitive change scores^a as covariates, N = 104

Covariates		$F^{b}(1,101)$	Effect $\eta_{\rm p}^2$
ADHD Index			
No covariate (ANOVA)		7.38**	0.067
Combined covariates	Creature Counting: no. correct		
	Creature Counting: no. switches		
	RAVLT total A1–A5	4.72*	0.045
Cognitive problems/inattention			
No covariate (ANOVA)		9.40**	0.084
Combined covariates	Creature Counting: no. correct		
	Creature Counting: no. switches		
	RAVLT total A1–A5	6.26*	0.059
DSM-IV inattention			
No covariate (ANOVA)		11.76**	0.103
Combined covariates:	Vocabulary scores		
	Creature Counting: no. correct		
	Creature Counting: no. switches		
	RAVLT total A1–A5	7.34**	0.070
DSM-IV hyperactive/impulsive			
No covariate (ANOVA)		7.69**	0.070
Combined covariates:	Vocabulary scores		
	Creature Counting: no. correct		
	Creature Counting: no. switches		
	RAVLT total A1-A5	4.51*	0.044

Note: η_p^2 = partial eta squared. Groups: PUFA, n = 77; placebo, n = 27.

^aCalculated as scores at 15 weeks minus scores at baseline.

 ${}^{b}F$ values represent interaction effects between group condition and time on CPRS subscale scores (9).

*p<0.05.

***p*<0.01.

which was not assessed in this study, were reported previously following PUFA supplementation [8]. Alternatively, improved vocabulary could result from improved attention and concentration, resulting in longer periods of reading and retention of information. In support of this possibility, a clear association has previously been identified between inattentive behaviour and language difficulties, particularly reading skills [47].

Importantly, improved scores in attention control, vocabulary, and to a lesser extent immediate recall memory were found to mediate a proportion of the treatment effects observed by parents [9] not only on inattention but also on hyperactivity/impulsivity. This lends support to the theoretical model of ADHD that links inattention with hyperactivity and impulsivity, although the underlying mechanism is not yet fully understood.

Objective measurements of attention in this study appear to support subjective parent and/or teacher reports of improved attention following PUFA supplementation in other studies. However, attention is a multifaceted cognitive ability. There are, for example, specific tests that measure factors of focussed, selective, divided and sustained attention as well as attentional control, all of which have all shown different patterns of performance [41] and appear therefore to tap into different aspects of attention. Therefore, future studies should explore the cognitive/attentional domains of functioning that respond to PUFA supplementation as reported by parents and teachers in more depth. Further operationalisation of objective cognitive assessments is required in order to identify specifically which aspects of attention PUFA assist, and which may mediate greater proportions of subjectively reported improvements in hyperactivity, impulsivity, and inattention.

Alternatively, Barkley's [16] cognitive model of ADHD suggests that the behavioural problems of hyperactivity, distractibility, and impulsivity are subsumed under the executive function of inhibition. The knock and tap test that was selected as a measure of inhibition in this study produced ceiling effects and was not adequate for differentiating between children. PUFA have been reported by parents and teachers to improve impulsivity (e.g. [8,9]); therefore, future studies could test effects of PUFA on more sensitive measures of inhibition such as 'Go/No-Go' to provide an

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objective assessment of the effect of PUFA on inhibition and a potential role in mediating co-occurring symptoms of ADHD.

The ability to ignore distracting information was assessed in this study by the Stroop colour-word test. Children with ADHD have consistently performed more poorly than controls on the Stroop test [16,36], which has traditionally been used to measure EF. Attention regulation is also believed to be an executive function, and improvements in the ability to switch and control attention could be indicative of improved frontal lobe performance. However, there was no treatment effect on working memory, another measure of EF. The issue remains as to what tests of EF are actually measuring, since it is difficult to isolate specific abilities without enlisting other cognitive functions [48]. Given that there are theoretical grounds for believing that PUFA may assist with frontal lobe performance, effects of PUFA on EF requires further investigation. The large improvements observed in the PUFA groups from weeks 16 to 30 on the Stroop task raises the possibility that extended PUFA supplementation might have improved frontal lobe activity and higher order cognitive function, given that if this were a practice effect, the same result would have been expected in the placebo group. The possibility that extended supplementation may assist in performance on the Stroop task, and therefore the ability to ignore distractions, would need to be investigated with a placebo control over 30 weeks before drawing any conclusions.

It should be noted that there are ambiguities in defining behaviour and cognition and in identifying specific functions related to physiological changes—i.e. changes in PUFA status may be reflected in some tests or measures but not in others, thereby highlighting the importance of accurate operationalisation [49]. The selection of tests to detect effects of nutritional intervention on cognitive function has received relatively little attention by researchers. This study and studies reviewed in the introduction to this paper failed to detect effects of n-3 supplementation on a range of specific cognitive measures. However, previous studies have found improvements in literacy in this group of children [8,30], indicating that these measures may be more able to detect meaningful cognitive outcomes. All in all, effects of PUFA on specific aspects of cognitive function in children with ADHD symptoms requires further exploration using tests of different types of attention [41], EF [14], and functional academic outcomes.

These findings can broadly be generalised to children showing symptoms of hyperactivity, impulsivity and inattention ≥ 2 S.D. from the population mean. A potential limitation of the study was the lack of an intent-to-treat analysis, resulting in possible bias in the results due to non-compliance. Although results to date are inconsistent there is enough theoretical and research support to warrant further investigation. Specific subgroups of children who are most likely to benefit need to be identified. The relative benefits of the n-3 PUFA DHA and EPA, and inclusion of the n-6 PUFA GLA also need to be investigated in this group as it is not clear from studies to date which, if not all, may be most important. Blood analyses of PUFA levels before and after supplementation and correlations with outcomes will also provide more definitive information.

To address future research directions indicated here, our Centre is currently conducting a 12-month threeway controlled crossover trial to investigate different measures of attention and literacy outcomes following n-3 PUFA supplementation with EPA- versus DHArich oils compared with placebo in children who have ADHD and learning difficulties. We are also measuring erythrocyte PUFA levels to assist in gaining better understanding of relationships between baseline levels and degree of response to supplementation.

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