Nutrition 28 (2012) 670–677

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Eicosapentaenoic and docosahexaenoic acids, cognition, and behavior in children with attention-deficit/hyperactivity disorder: A randomized controlled trial

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ARTICLE INFO

Article history:
Received 20 May 2011
Accepted 15 December 2011

Keywords:
Attention-deficit/hyperactivity disorder
Children
Learning difficulties
ω-3 Fatty acids
Docosahexaenoic acid
Eicosapentaenoic acid

ABSTRACT

Objective: To determine the effects of an eicosapentaenoic acid (EPA)–rich oil and a docosahexaenoic acid (DHA)–rich oil versus an ω-6 polyunsaturated fatty acid–rich safflower oil (control) on literacy and behavior in children with attention-deficit/hyperactivity disorder (ADHD) in a randomized controlled trial.

Methods: Supplements rich in EPA, DHA, or safflower oil were randomly allocated for 4 mo to 90 Australian children 7 to 12 y old with ADHD symptoms higher than the 90th percentile on the Conners Rating Scales. The effect of supplementation on cognition, literacy, and parent-rated behavior was assessed by linear mixed modeling. Pearson correlations determined associations between the changes in outcome measurements and the erythrocyte fatty acid content (percentage of total) from baseline to 4 mo.

Results: There were no significant differences between the supplement groups in the primary outcomes after 4 mo. However, the erythrocyte fatty acid profiles indicated that an increased proportion of DHA was associated with improved word reading ($r = 0.394$) and lower parent ratings of oppositional behavior ($r = 0.392$). These effects were more evident in a subgroup of 17 children with learning difficulties: an increased erythrocyte DHA was associated with improved word reading ($r = 0.683$), improved spelling ($r = 0.556$), an improved ability to divide attention ($r = 0.676$), and lower parent ratings of oppositional behavior ($r = 0.777$), hyperactivity ($r = 0.702$), restlessness ($r = 0.705$), and overall ADHD symptoms ($r = 0.665$).

Conclusion: Increases in erythrocyte ω-3 polyunsaturated fatty acids, specifically DHA, may improve literacy and behavior in children with ADHD. The greatest benefit may be observed in children who have comorbid learning difficulties.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is commonly diagnosed in childhood and has a high comorbidity with other disorders of behavior and mood such as conduct disorder and developmental coordination disorder [1]. At least one-fourth of children with ADHD also have a learning disorder resulting in difficulties with reading, spelling, and writing [2].

The ω-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) and, to a lesser extent, the ω-6 PUFA arachidonic acid (AA) constitute a large proportion of the lipid in the brain [3]. The ω-3 PUFAs DHA and eicosapentaenoic acid (EPA) are involved in many aspects of brain function including cell growth, neural signaling, and gene expression [4]. The low intake of ω-3 PUFAs in children from Western societies such as Australia is therefore of concern [5,6], not only for physical health such as blood

Ms. Milte is supported by a Ph.D. scholarship from the South Australian Health Department. Drs. Sinn, Young, and Howe are recipients of an Australian Research Council Linkage Project grant (LP0776922) in partnership with Novasel Australia ("Cognitive and Behavioral Benefits of Omega-3 Fatty Acids Across the Lifespan").

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doi:10.1016/j.nut.2011.12.009

pressure [7] but also for brain function, with implications for developmental disorders such as ADHD.

Some randomized controlled trials have investigated effects of ω-3 PUFA supplementation on behavior in volunteers with ADHD, with varying success [8]. The inconsistent results seen in these trials could be attributed to methodologic differences including variations in the type of supplement used [9]. Most studies have used supplements containing a combination of EPA and DHA in varying ratios. One study used pure DHA 345 mg [10] in medicated boys with ADHD and reported no effects; another study used a supplement containing only EPA 500 mg and reported improved ADHD symptoms [11], suggesting that pure EPA supplementation will assist in alleviating ADHD symptoms. The effects of the long-chain ω-3 PUFAs, EPA and DHA, on behavior, cognition, and literacy in children with ADHD have not been compared within a single randomized, controlled, intervention trial.

Variations among trials could also be attributed to the choice of volunteers recruited and the outcomes assessed. In the UK, improvements in cognitive and behavioral symptoms were observed after PUFA supplementation in children with dyslexia and ADHD symptoms [12] and in children with dyspraxia (one-third with ADHD symptoms in the clinical range) who were also on average 1 y behind in reading and spelling [13]. An Australian study found improved parent ratings of attention and behavior in children with ADHD symptoms in the clinical range [14,15]. A more recent Swedish study with children diagnosed with ADHD found that the subgroup with reading and writing difficulties were among the strongest responders [16]. In contrast, a study that included only boys who met strict *Diagnostic and Statistical Manual, Fourth Revision* criteria for ADHD and excluded comorbid disorders failed to find a treatment effect [10]. Therefore, the effects of ω-3 PUFA supplementation on behavior and literacy in children with ADHD symptoms and comorbidities need further investigation.

Importantly, few of these trials have included blood PUFA analysis [10,17]. Serum, plasma, or erythrocyte PUFA contents are biomarkers of PUFA intake and bioavailability [18], with the latter being most indicative of long term consumption and tissue incorporation. In the present sample of children, we found that higher ω-6 PUFA and lower ω-3 PUFA levels in erythrocytes were predictive of poorer outcomes at baseline, particularly word reading [19]. The inclusion of PUFA status in supplementation trials could be used to determine if the treatment is efficacious in children with a lower baseline ω-3 PUFA intake or in those who exhibit the greatest increase in PUFA levels over the supplementation period.

The aim of this study was to investigate effects of a selective supplementation with EPA or DHA versus the ω-6 PUFA linoleic acid (LA) on behavior, cognition, and literacy in children 7 to 12 y old with ADHD symptoms in a randomized, placebo-controlled trial. The erythrocyte PUFA status (percentage of total fatty acids) was used to determine if those with lower ω-3 PUFA levels were the greatest responders. In addition, the changes in PUFA levels and their correlation with changes in behavior and literacy/cognition were examined over 4 mo.

Materials and methods

A 4-mo parallel comparison of effects of a DHA-rich or an EPA-rich fish oil with an LA-rich safflower oil was undertaken as stage 1 of a 12-mo randomized, controlled, three-way crossover trial in children 7 to 12 y old with ADHD symptoms. The study was conducted in Adelaide and Brisbane, Australia and was approved by the human research ethics committees of the University of South Australia and the Queensland University of Technology. It was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12607000332426).

Participants

Children were eligible to participate if they had a diagnosis of ADHD or parent-rated symptoms higher than the 90th percentile on the Conners Parent Rating Scale (CPRS) [20] and parent-reported learning difficulties (described as literacy performance behind their year level at school). Children were excluded if they had consumed ω-3 PUFA supplements during the 3 mo before the study or were taking any ADHD medication. A required sample size, to provide greater than 80% power to detect a medium effect size [21] and allow for a 30% dropout rate, was calculated for the 12-mo three-way crossover intervention study, aiming for 120 participants (40 per group).

Procedure

The recruitment and assessments occurred from June 2007 through June 2009. Figure 1 shows the flow of participants through the study. Children were recruited through media releases and television interviews, newspaper advertisements, school newsletters, and flyers. Information was sent to interested parents and guardians of 115 children who were deemed eligible through a brief screening interview over the telephone. If they did not have an official diagnosis of ADHD, parents were asked to complete the ADHD index, a 12-item subscale from the CPRS, to determine if their children were rated above the 90th percentile [20]. The study was explained to the children and their parent(s) or guardian and written informed consent was obtained.

Ninety children were recruited for the baseline assessments and were independently allocated to one of three conditions (EPA-rich oil, DHA-rich oil, or LA-rich oil) using the process of randomization by minimization [22] based on age and gender. Study investigators involved in the data collection, parents, and children were blinded to the randomization until completion of the data collection and analysis. Children visited the Nutritional Physiology Research Centre at the University of South Australia (Adelaide, Australia; $n = 52$) or the Institute of Health and Biomedical Innovation (Brisbane, Australia; $n = 38$) with their parents or guardians at baseline and after 4 mo. Capsule containers were returned by the parents at the 4-mo visit for a compliance assessment by capsule counting. At each visit, the blood samples were collected by venipuncture into 6-mL tubes containing ethylenediaminetetraacetic acid. After the giving a blood sample, the children were given a small snack (toast and fruit juice or water) and underwent 30 to 45 min of cognitive assessments. All researchers were trained in the assessment tasks by the same person initially and the same instructions and protocol were used for each volunteer's assessments.

Supplements

Based on previous reports of improved symptoms in children with total long-chain ω-3 PUFAs 750 mg/d and considering that compliance is variable in children, we chose to supplement with at least 1 g/d to achieve an optimal response (the upper safety limit is 3 g/d). Participants consumed 4 × 500-mg capsules per day containing an EPA-rich fish oil providing EPA 1109 mg and DHA 108 mg, a DHA-rich fish oil providing EPA 264 mg and DHA 1032 mg, or a safflower oil (control) providing LA 1467 mg/d. All oils were stabilized with a low concentration of vitamin E. Capsules were provided by Novasel Australia (Mudgeeraba, Qld, Australia).

Assessment tools

Primary outcomes: literacy and behavior

Literacy was assessed using the word reading and spelling subtests from the Wechsler Individual Achievement Test III [23]. We also investigated performance on the vocabulary subtest from the Wechsler Scale of Children's Intelligence III [24]. The raw scores were converted to age-scaled scores. The parent ratings of ADHD symptoms were assessed by completion of the long version of the CPRS [20]. The subscales were transformed to age- and gender-adjusted *t* scores.

Secondary outcomes: attention and inhibition

Different forms of attention were assessed as a secondary outcome measurement using an abbreviated test battery from the Test of Everyday Attention for Children [25]. Focused attention was measured using Sky Search, a timed subtest in which children are asked to circle as many "target" spaceships as they can on a sheet filled with similar distracter spaceships. In part 2, they are timed while they circle as many as they can without distracters. The second score is subtracted from the first score to eliminate the effects of motor slowness. Score! measures the ability to sustain attention during a relatively simple non-stimulating task by asking children to keep count of the number of "scoring" sounds they hear on a tape. Creature Counting measures the ability to switch and control attention. In this test, children are asked to count creatures in a burrow,

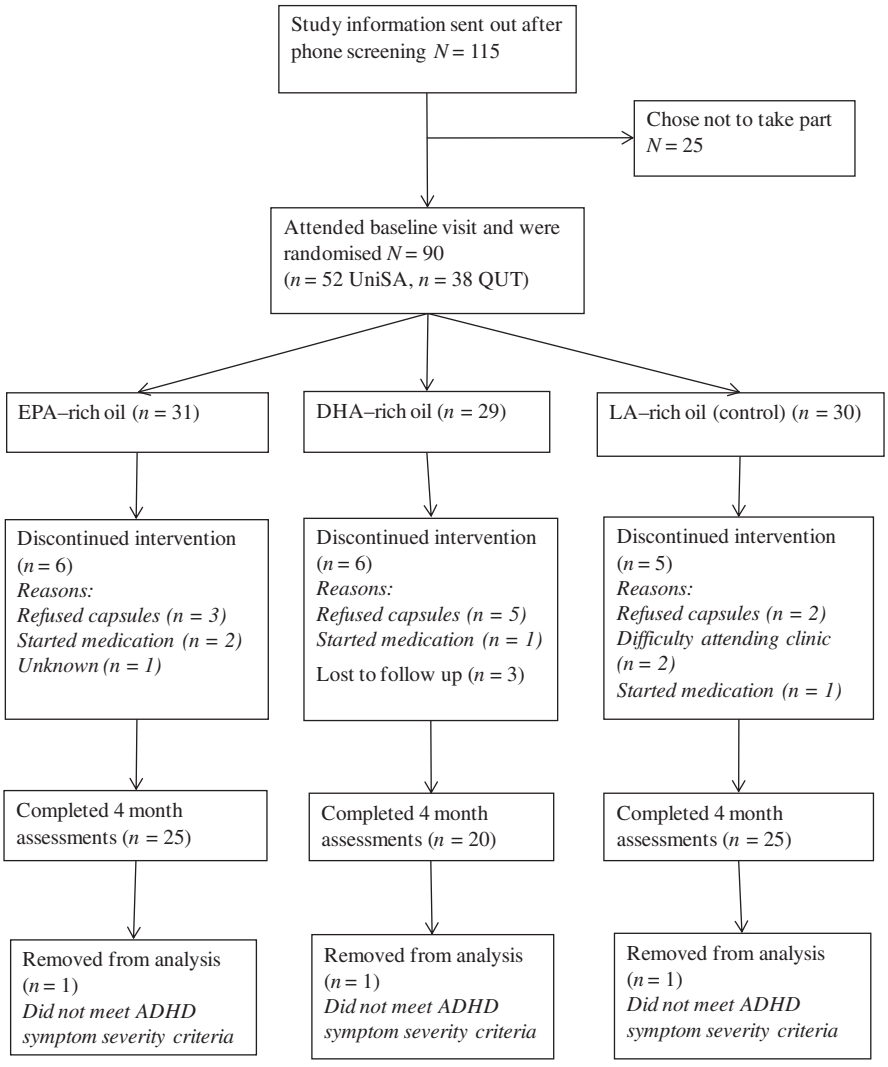

Fig. 1. Flow of participants through the study. ADHD, attention-deficit/hyperactivity disorder; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; QUT, Queensland University of Technology; UniSA, University of South Australia.

and when they come to an arrow, they must switch their counting up or down according to the direction of the arrow. Sky Search DT measures divided attention by asking children to combine the Sky Search and the Score! tasks.

Inhibition, or the ability to hold back a response, was assessed using a computerized go/no-go task [26]. This task involved pressing the "h" key to respond to predefined stimuli on a computer screen (e.g., a green man) and to withhold the response and press the spacebar instead when a specific stimulus appears (e.g., a red man). In this task, 171 green men were presented with 45 red men randomly dispersed among them. The number of errors was calculated as a measurement of response inhibition.

While the children were completing the tasks, the parents/guardians completed the CPRS and questionnaires that collected information about the child and parent demographics.

Assessment of fatty acid profiles

Relative proportions of individual fatty acids in erythrocyte phospholipids were assessed as described previously [27]. Erythrocytes were isolated within 2 h of collection by centrifugation, washed in isotonic saline, and stored at −80°C. Erythrocytes were thawed and the lipids extracted and trans-esterified. The resultant fatty acid methyl esters were separated and quantified using a Shimadzu 2010 gas chromatograph (Nakagyo-ku, Kyoto, Japan) equipped with a 50-m capillary column (0.32 mm inner diameter) coated with BPX-70 (0.25-μm film thickness; SGE Pty Ltd., Melbourne, VIC, Australia). The retention times were compared with those of authentic lipid standards (GLC-463, Nu-Chek Prep, Inc., Elysian, MN, USA) for identification.

Statistical assessment

The data analysis was conducted using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance and chi-square analysis were conducted on parametric and non-parametric data, respectively, to determine if the demographic, physiologic, and behavior measurements were evenly distributed between the groups at baseline. An analysis of intervention effects on the primary outcome measurements (literacy and CPRS) was conducted using raw scores. The effects of EPA and DHA versus LA (control) supplementation on the outcome variables were investigated using linear mixed modeling. Data presented include the 95% confidence interval and intraclass correlation for each model. A Pearson correlation analysis was conducted to determine significant associations between the changes in outcome measurements and the changes in erythrocyte PUFA status (percentage of total fatty acids) from baseline to 4 mo. The significance level was set at $P < 0.05$. Linear mixed modeling and the Pearson correlations were repeated in a subgroup of children who also had learning difficulties.

Results

Description

Of the 90 children 6 to 13 y old who commenced the study (52 at the University of South Australia and 38 at the Queensland University of Technology), three were excluded owing to a lack of

an ADHD diagnosis or ADHD symptom severity scores below the 90th percentile on the Conners Global Scale. Seventy children completed the 4-mo assessments. Blood samples were obtained from 75 volunteers at baseline and 52 volunteers at 4 mo and from 48 children at the two time points. There were no differences in CPRS behavior ratings between those who provided blood samples and those who did not (data not shown). Forty-three children (48%) had an official diagnosis of ADHD at baseline. However, there were no differences in the ratings of ADHD symptom severity between those with and those without an official diagnosis. Seventy percent had word reading scores below their age level, 79% performed behind their age level in spelling, and 91% performed behind their age level in vocabulary.

Twenty-five parents reported the following additional medical conditions in their children: asthma (eight; two of these also had allergies, one had hypermobility/low muscle tone, and one had eczema), eczema (three), three Indigenous foster children had fetal alcohol syndrome (block randomized to different treatment conditions), speech impairment, central auditory processing disorder, dyspraxia/sensory disorder, global delay, developmental delay, epilepsy (two; one also had cerebral palsy and obsessive–compulsive disorder), Asperger's syndrome (two), autism spectrum disorder, migraines (two, including one with motor tics), and oppositional–defiant disorder. Twelve children were taking the following non-ADHD medications: salbutamol (Ventolin, GlaxoSmithKline, London, UK), amitriptyline (Endep, Merck & Co. Inc., NJ, USA), sodium valproate (Epilim, Sanofi S.A., Paris, France), cyproheptadine (Periactin, Merck & Company Inc., New Jersey, USA), montelukast (Singulair, Merck & Co. Inc., NJ, USA), cetirizine (Zyrtec, Sydney, Australia), and carbamazepine (Tegretol, Novartis International A.G., Basel, Switzerland).

Thirty-three percent of parents ($n = 29$) reported other instances of mental illness in their family, including anxiety, depression, bipolar disorder, mood disorder, ADHD, nervous disorder, schizophrenia, drug and alcohol abuse, panic attacks, and dementia. Twenty-two mothers reported smoking during pregnancy (2–45 wk) and 26 reported drinking alcohol while pregnant (six for the entire pregnancy, nine only during the first 3 mo). Table 1 presents a detailed outline of further demographic variables, erythrocyte PUFA levels, CPRS *t* scores, and age-adjusted literacy scores at baseline.

Table 1
Baseline demographics, erythrocyte PUFA, CPRS, and age-adjusted literacy

	EPA ($n = 30$)	DHA ($n = 28$)	Control (LA) ($n = 29$)
Demographics ($N = 87$)			
Age (y)	8.77 (1.76)	8.89 (1.60)	9.14 (2.03)
Gender (percent male)	80	75	83
Birth weight (g)	4.76 (2.18)	5.06 (2.58)	6.03 (2.12)
Parent-rated health*	4.17 (0.76)	3.89 (0.80)	4.14 (0.83)
Number of weeks breast-fed	22.57 (34.06)	25.58 (27.73)	14.28 (18.65)
Length of gestation (wk)	38.63 (3.39)	39.42 (3.58)	39.48 (3.51)
Level of primary parent's education†	2.31 (1.31)	2.23 (1.24)	2.76 (1.43)
CPRS *t* scores ($N = 87$)			
ADHD index	75.70 (6.75)	75.96 (6.97)	77.62 (5.59)
Conners restless/impulsive	77.40 (8.37)	75.43 (7.97)	77.41 (7.90)
Conners emotional lability	60.60 (18.96)	67.39 (12.12)	62.52 (17.58)
Conners global	75.27 (9.22)	72.46 (13.32)	76.31 (8.92)
DSM-IV inattentive	72.70 (8.25)	74.89 (12.69)	75.03 (11.46)
DSM-IV hyperactive/impulsive	77.13 (11.31)	75.00 (10.17)	76.21 (11.61)
DSM-IV total	75.60 (9.91)	76.68 (10.11)	78.21 (8.31)
Literacy (age-scaled scores, $N = 87$)			
Word reading	93.27 (18.42)	88.57 (18.20)	91.03 (20.18)
Spelling	91.00 (16.34)	83.79 (20.72)	86.39 (17.08)
Vocabulary	6.07 (2.30)	6.32 (1.96)	7.00 (2.44)
PUFAs (percentage of red blood cell membrane, n = 75)			
EPA	0.61 (0.23)	0.66 (0.30)	0.56 (0.17)
DHA	3.64 (0.95)	3.71 (0.75)	3.42 (0.79)
Total ω-3 PUFAs	6.79 (1.27)	6.94 (1.16)	6.36 (1.12)
AA	11.28 (1.39)	11.45 (0.89)	10.92 (1.82)
Total ω-6 PUFAs	24.01 (3.30)	24.40 (1.51)	23.84 (3.05)

AA, arachidonic acid; ADHD, attention-deficit/hyperactivity disorder; CPRS, Conners Parent Rating Scale; DSM-IV, *Diagnostic and Statistical Manual, Fourth Revision*; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid

Parametric data presented as mean (SD). There were no significant differences between the groups.

* Rated on a scale whereby 1 = poor and 5 = excellent.

† Level of education ranged from 1 = grade 10 to 6 = postgraduate degree.

Adverse events

The treatments were well tolerated on the whole, with only six cases of minor adverse events reported during the 4 mo. From the LA (control group), one volunteer reported bad breath. Three from the EPA group each reported flatulence, yellow teeth, and/or an unpleasant taste. From the DHA group, one reported an unpleasant taste and one reported occasional nose bleeds. Thirty-six completers (51%) correctly guessed the supplement they had been taking (11 or 44% from the EPA group, 14 or 70% from the DHA group, and 11 or 44% from the LA group). This would be expected by chance, although the rate of 70% in the DHA group may be attributed to the fact that most parents who correctly guessed their child's supplement stated as the reason for their guess that they were/were not deriving benefits from the treatment (i.e., some parents who guessed correctly that their children were taking fish oil stated it was because they perceived improvements in their child; conversely, some who guessed correctly that their child was taking placebo stated their reason as no perceived improvements). Because only one volunteer each from the EPA and DHA groups guessed they were taking fish oil because of a reported "fishy" taste, the treatment blinding was considered successful.

Completers and compliance

Seventy children returned for visit 2 after 4 mo, resulting in a dropout rate of 20%. There were 31, 29, and 30 completers in the EPA, DHA, and LA groups, respectively ($P = 0.225$). The mean compliance according to the capsule count of the returned bottles by the parents was 89% across the groups (EPA group 91%, standard deviation 12.06; DHA group 92%, standard deviation 9.75; LA group 86%, standard deviation 10.91). Changes in erythrocyte ω-3 and ω-6 PUFAs after 4 mo of supplementation corresponded to the supplement that was taken in each group (Fig. 2). The greatest increases in erythrocyte EPA and DHA were observed in the EPA and DHA groups, respectively, although the two fish oil supplements increased the total ω-3 PUFA status to a similar level compared with the control.

Primary analysis

There were 58 missing observations in the demographic data and 25 missing observations in the baseline data owing to incomplete questionnaire completion or an inability to complete the cognitive test. Birth weight had the most missing values (43%). In the follow-up data, there were 461 missing observations and an average of 23 missing observations per outcome variable; 17 were from dropouts and the remainder was from

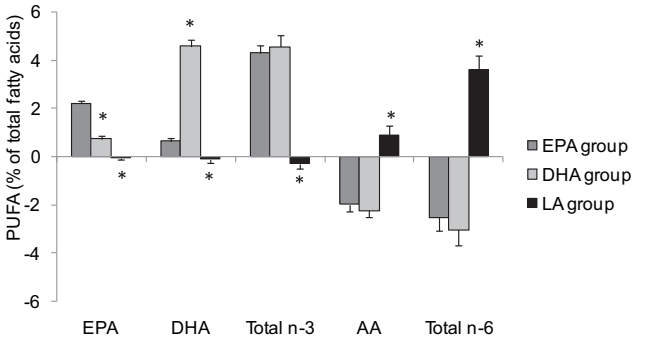

Fig. 2. Mean 4-mo change in erythrocyte polyunsaturated fatty acids in the three supplement groups ($N = 44$; LA, $n = 17$; DHA, $n = 10$; EPA, $n = 17$). * $P < 0.05$. AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; n-3, ω-3; n-6, ω-6; PUFA, polyunsaturated fatty acid

incomplete questionnaires or an inability to complete the cognitive tests. Analysis using the missing completely at random (MCAR) test of Little [28] showed that the data were likely to be missing at random. The application of a linear multilevel model was appropriate for this missing pattern. One-way analyses of variance showed that the treatment groups did not differ in demographic variables, CPRS subscales, or cognition or literacy scores at baseline. Treatment effects on the outcome variables were assessed by visit (baseline and 4 mo) by treatment interactions on the outcome variables for the DHA and EPA groups compared with LA using linear mixed modeling (Table 2), thus taking all 87 cases into consideration.

There were no significant treatment effects in the linear mixed model analysis for literacy or parent-reported behavior with the CPRS. Similarly, an analysis of cognitive assessments completed by the children showed no significant treatment effects of DHA and EPA on the measurements of attention or inhibition (Table 2).

Associations between changes in erythrocyte PUFA and changes in outcome variables

Pearson correlation analyses were used to identify changes in outcome measurements that were associated with changes in erythrocyte PUFA levels, including EPA, DHA, total ω-3 PUFA, AA, and total ω-6 PUFA, over 4 mo (Table 3). The strongest relations were between increased erythrocyte DHA and improvements in word reading and parent-rated oppositional behavior after 4 mo. Improved parent-rated anxiety/shyness was associated with increased erythrocyte EPA and total ω-3 PUFA and decreased total ω-6 PUFA.

Subgroup analysis in children with learning difficulties

Thirty-seven children were behind their age level in reading and spelling (performing on average 2 y behind their age level) and were included in the subgroup analyses. We had complete blood data for 17 children in this subgroup. The linear mixed model analysis showed no significant treatment effects of DHA and EPA on any outcome measurements (data not shown). Pearson correlations (Table 4) showed that increased DHA was associated with improvements in a range of outcome measures, including oppositional behavior, hyperactivity, restlessness, overall ADHD behavior, Sky Search DT, spelling, and word reading. Increased total ω-3 PUFA was also associated with improved parent-rated anxiety/shyness.

Table 2
Treatment effects (time by treatment interactions) for linear mixed model analysis of all cases as randomized to treatment group (EPA versus LA, DHA versus LA) on literacy, CPRS, and cognitive assessments over 4 mo ($N = 87$; LA, $n = 30$; DHA, $n = 28$; EPA, $n = 29$)

	Estimate	SEM	*t* Score	*P*	95% CI	ICC*
Literacy						
Word reading				0.34		0.95
EPA versus LA	1.62	2.23	0.73	0.47	−2.83 to 6.07	
DHA versus LA	3.48	2.35	1.48	0.14	−1.22 to 8.18	
Spelling				0.67		0.89
EPA versus LA	−0.66	1.22	−0.54	0.59	−3.11 to 1.79	
DHA versus LA	−1.13	1.27	−0.89	0.38	−3.68 to 1.42	
Vocabulary				0.60		0.84
EPA versus LA	1.28	1.26	1.02	0.31	−1.23 to 3.80	
DHA versus LA	0.56	1.31	0.43	0.67	−2.06 to 3.18	
CPRS						
Oppositional				0.80		0.69
EPA versus LA	0.07	1.51	0.05	0.96	−2.94 to 3.08	
DHA versus LA	−0.90	1.61	−0.56	0.58	−4.12 to 2.32	
Cognitive problems				0.79		0.50
EPA versus LA	1.20	1.75	0.69	0.50	−2.29 to 4.70	
DHA versus LA	0.60	1.87	0.32	0.75	−3.12 to 4.33	
Hyperactivity				0.58		0.78
EPA versus LA	0.98	1.13	0.87	0.39	−1.27 to 3.23	
DHA versus LA	1.12	1.21	0.92	0.36	−1.30 to 3.53	
Anxious/shy				0.62		0.80
EPA versus LA	−0.88	0.90	−0.98	0.33	−2.67 to 0.91	
DHA versus LA	−0.44	0.96	−0.46	0.64	−2.36 to 1.48	
Social problems				0.78		0.81
EPA versus LA	−0.52	0.74	−0.70	0.49	−2.00 to 0.96	
DHA versus LA	−0.24	0.79	−0.31	0.76	−1.82 to 1.33	
ADHD index				0.60		0.37
EPA versus LA	1.56	1.77	0.88	0.38	−1.96 to 5.09	
DHA versus LA	1.64	1.90	0.86	0.39	−2.15 to 5.43	
Conners restless				0.62		0.63
EPA versus LA	0.90	0.95	0.96	0.34	−0.98 to 2.79	
DHA versus LA	0.66	1.01	0.66	0.51	−1.35 to 2.68	
Conners emotional				0.95		0.72
EPA versus LA	−0.15	0.54	−0.28	0.78	−1.24 to 0.93	
DHA versus LA	−0.15	0.58	−0.27	0.79	−1.30 to 1.00	
Conners global				0.85		0.73
EPA versus LA	0.67	1.18	0.57	0.57	−1.69 to 3.03	
DHA versus LA	0.45	1.26	0.36	0.72	−2.06 to 2.96	
DSM-IV inattention				0.59		0.42
EPA versus LA	1.23	1.47	0.84	0.41	−1.70 to 4.16	
DHA versus LA	−0.20	1.57	−0.13	0.90	−3.32 to 2.92	
DSM-IV hyperactivity				0.97		0.79
EPA versus LA	0.18	1.12	0.16	0.87	−2.05 to 2.42	
DHA versus LA	0.30	1.22	0.25	0.81	−2.13 to 2.74	
DSM-IV total				0.83		0.57
EPA versus LA	1.20	2.30	0.52	0.60	−3.39 to 5.79	
DHA versus LA	−0.08	2.49	−0.03	0.98	−5.04 to 4.89	
Cognitive assessments						
Sky Search				0.01		0.77
EPA versus LA	−0.07	0.04	−1.81	0.08	−0.15 to 0.01	
DHA versus LA	0.06	0.04	1.40	0.17	−0.02 to 0.14	
Creature Counting				0.90		0.59
EPA versus LA	0.26	0.61	0.43	0.67	−0.96 to 1.48	
DHA versus LA	0.02	0.62	0.03	0.98	−1.23 to 1.27	
Score!				0.68		0.46
EPA versus LA	−0.68	0.76	−0.89	0.38	−2.20 to 0.85	
DHA versus LA	−0.37	0.78	−0.47	0.64	−1.92 to 1.18	
Sky Search DT				0.54		0.71
EPA versus LA	−3.06	3.49	−0.88	0.39	−10.11 to 3.98	
DHA versus LA	1.00	3.91	0.26	0.80	−6.86 to 8.85	
Go–no-go				0.99		0.21
EPA versus LA	3.04	31.53	0.10	0.92	−59.71 to 65.79	
DHA versus LA	0.21	32.64	0.01	0.99	−64.72 to 65.14	

ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; CPRS, Conners Parent Rating Scale; DHA, docosahexaenoic acid; DSM-IV, *Diagnostic and Statistical Manual, Fourth Revision*; EPA, eicosapentaenoic acid; ICC, intraclass correlation; LA, linoleic acid; PUFA, polyunsaturated fatty acid

A positive slope indicates an improvement in all outcome measurements.

* The ICC provides a percentage of the variance explained by the individual subject differences, and a value above 50% indicates a reasonable goodness of fit.

Table 3
Correlations between changes in erythrocyte PUFAs and changes in outcome variables from baseline to 4 mo ($n = 44$–47^{*})

	EPA	DHA	Total ω-3 PUFAs	AA	Total ω-6 PUFAs
Oppositional	−0.194	0.392†	0.056	0.074	0.125
Cognitive problems	−0.159	0.149	−0.045	0.240	0.272
Hyperactivity	−0.028	0.093	0.017	0.071	0.149
Anxious/shy	0.328†	0.169	0.340†	−0.272	−0.309†
ADHD index	−0.197	0.164	−0.098	0.051	0.073
Conners restless	−0.216	0.203	−0.069	0.155	0.136
Conners emotional	−0.030	0.164	0.120	0.162	0.162
Conners global	−0.178	0.242	0.012	0.198	0.184
DSM-IV inattentive	−0.162	0.229	−0.003	0.219	0.222
Sky Search	0.254	0.021	0.198	−0.149	−0.266
Score!	−0.202	−0.266	−0.249	−0.043	0.112
Sky Search DT	−0.066	0.205	0.080	−0.035	0.018
Word reading	−0.133	0.394‡	0.120	−0.067	−0.147
Spelling	−0.073	0.103	0.005	−0.112	−0.082

AA, arachidonic acid; ADHD, attention-deficit/hyperactivity disorder; DSM-IV, *Diagnostic and Statistical Manual, Fourth Revision*; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids
A positive correlation coefficient indicates an improvement in the outcome measurement with an increase in PUFA level.
* The variable numbers represent missing, untreated data on the questionnaire or cognitive test items or blood samples.
† $P < 0.05$.
‡ $P < 0.01$.

Baseline PUFA levels and improved outcome measurements

Higher total ω-6 PUFA ($r = 0.373$, $P = 0.004$) and AA ($r = 0.326$, $P = 0.013$) levels at baseline were significantly correlated with lower parent ratings of anxiety/shyness after 4 mo. This may be a spurious finding because increased ω-3 and decreased ω-6 levels were associated with improved anxiety/shyness ratings over the 4 mo (Table 3). Baseline PUFA status was not significantly associated with improvements in any other outcome measures.

Discussion

This study investigated the effects of supplementation with EPA- or DHA-rich oil on literacy, cognition, and behavior of children with ADHD symptoms. No treatment effects were observed with the EPA or DHA-rich supplements compared with the LA control. However, an increased erythrocyte DHA over 4 mo was associated with improved word reading and oppositional behavior in the entire sample, and this effect was much stronger in the subgroup with learning difficulties, who also showed associations between increased DHA and measurements of divided attention and parent-rated behavior. Increased EPA and total ω-3 PUFA levels were associated with decreased anxiety/shyness.

Table 4
Correlations between changes in erythrocyte PUFAs and changes in outcome variables from baseline to 4 mo in a subgroup with learning difficulties ($n = 17$)

	EPA	DHA	Total ω-3 PUFAs	AA	Total ω-6 PUFAs
Oppositional	−0.241	0.777†	0.316	−0.057	−0.060
Cognitive problems	−0.261	0.335	0.032	0.225	0.187
Hyperactivity	−0.015	0.702†	0.429	−0.212	−0.247
Anxious/shy	0.450	0.250	0.536*	−0.270	−0.285
ADHD index	−0.400	0.425	−0.034	0.051	0.030
Conners restless	−0.097	0.705†	0.363	−0.154	−0.178
Conners emotional	−0.138	0.135	0.000	0.007	0.048
Conners global	−0.159	0.665†	0.304	−0.125	−0.122
DSM-IV inattentive	−0.243	0.427	0.095	0.131	0.108
Sky Search	0.410	−0.239	0.204	−0.142	−0.148
Score!	−0.410	−0.096	−0.329	0.409	0.348
Sky Search DT	−0.189	0.676†	0.248	−0.122	−0.136
Word reading	−0.064	0.683†	0.334	−0.291	−0.327
Spelling	−0.153	0.556*	0.220	−0.213	−0.167

AA, arachidonic acid; ADHD, attention-deficit/hyperactivity disorder; CPRS, Conners Parent Rating Scale; DSM-IV, *Diagnostic and Statistical Manual, Fourth Revision*; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids
A positive correlation coefficient indicates an improvement in the outcome measurement with an increase in PUFA level.
* $P < 0.05$.
† $P < 0.01$, significant correlations.

This study is the first to investigate the effects of EPA- and DHA-rich oils compared with a control on ADHD symptoms in children. To date, there has been a lack of consistent results between different trials of ω-3 PUFA supplementation and child behavior problems [8]. A range of supplement formulations has been tested, including high DHA [10,29] and mixtures of ω-3 and ω-6 PUFAs with a high EPA content [13,15], which could contribute to the inconsistencies seen in the literature. The present study failed to find a significant treatment effect of the EPA- or DHA-rich supplement compared with the control, which may be attributed to the difficulties in recruiting eligible children, resulting in only 52% power to detect significant treatment effects.

However, across the entire sample, within-individual increases in DHA status resulting from supplementation were associated with improvements in word reading and oppositional behavior, and in the subgroup with learning difficulties, increased DHA had even stronger associations with improved word reading, oppositional behavior, ability to divide attention and parent-rated hyperactivity and restlessness. Therefore, although high EPA supplements have shown beneficial treatment effects in the past, this may be attributed in part to the conversion of EPA to DHA. It may also be that EPA and DHA have differential effects because we found improved anxiety/shyness with increased EPA levels. Our study indicates that increasing erythrocyte DHA status by ω-3 PUFA intake may play a particularly important role in assisting behavior and cognition difficulties in this group. In this regard, another inconsistency in the literature pertains to dosage. In the one study that used a pure DHA supplement [10], boys were given only DHA 345 mg/d, considerably lower than studies that have found significant improvements with long-chain ω-3 PUFA 750 mg/d. Our findings with DHA 1 g/d therefore suggest that this warrants following up with higher doses (and unmedicated children, unlike the latter study).

Even stronger relations were seen between increased DHA in response to supplementation and improved word reading, spelling, attention, and parent-rated measurements of behavior in the subgroup of children who were performing behind their age in spelling and reading. Similarly, although no overall treatment effect on ADHD symptoms was found in a Swedish study of children diagnosed with ADHD, the subgroup with reading and writing difficulties were among the strongest responders [16]. Therefore, given the body of research to date, this study supports indications that children with learning difficulties as part of a constellation of developmental problems may be a subgroup of responders to ω-3 PUFA supplementation.

The assessment of erythrocyte PUFAs provided a measurement of adherence through changes in ω-3 PUFA status in the present study. It also importantly allowed relations between a change in the PUFA status and corresponding improvements in outcome measurements to be explored. Few ω-3 PUFA supplementation trials on child behavior and cognition have included blood PUFA status in their analysis, and a fraction of these have

reported relations between supplementation-induced changes in erythrocyte PUFAs and outcome measurements. Notably, Stevens et al. [30] reported correlations between increased DHA and improved teacher ratings of attention in a small sample of children with ADHD symptoms. A study in 60 children 8 to 13 y old with ADHD and impaired visual sustained attention performance also reported correlations between increased plasma DHA and improvements in measurements of attention after supplementation with EPA plus DHA 250 mg/d [17]. Another study indicated that, after an 8-wk supplementation period using EPA plus DHA 16.2 g, a lower AA:EPA ratio in plasma phospholipids was a significant correlate of improvements in severity-of-illness ratings in nine children 8 to 16 y old under treatment for ADHD [31]. Considering the valuable contribution that an assessment of PUFA status can make to the analysis of ω-3 supplementation trials in children with ADHD, this important measurement, although challenging, should be included where possible in future trials.

Assessment of erythrocyte PUFA also allowed us to determine the extent to which baseline PUFA status influenced the response to supplementation. Although higher levels of total ω-6 PUFA, AA, and ω-6 docosapentaenoic acid (DPA) at baseline were associated with greater improvements in parent ratings of anxiety/shyness after 4 mo, this effect was not observed across other outcome measurements. Therefore, despite growing evidence of a divide between responders and non-responders in the ω-3 PUFA treatment for child behavior and cognition [16], it appears that baseline erythrocyte PUFA status had relatively little influence in this cohort. The responders may have higher requirements for PUFAs than the other children, or the enzymes involved in the metabolism of PUFAs may play a role. In addition, given the low intakes of ω-3 PUFAs in Australian children [5,6], the low levels in our sample may not have allowed for enough variability to detect any influence of baseline levels on the degree of response.

This study is limited by a smaller sample than was originally proposed. Despite an intensive 12-mo recruitment phase over two sites, our final sample provided 52% power, thereby decreasing our ability to detect a significance difference between groups on our primary outcome measurement, word reading. This small sample can be attributed to difficulties in recruiting children with ADHD who were not taking stimulant medication [32] and the difficulty of obtaining blood samples in this population. In addition, it should be noted that half the children did not have a clinical diagnosis for ADHD; however, all children had symptom ratings above the threshold for a possible clinical diagnosis, and there were no differences in the parent ratings of symptoms between those with and those without a diagnosis.

Despite these limitations, the present study adds to evidence suggesting that increased ω-3 PUFA intake can improve attention, literacy, and behavior problems in some children with ADHD. Given the growing body of evidence, it appears that children with ADHD symptoms and comorbid reading and spelling difficulties may represent a subgroup of responders to ω-3 PUFA supplementation that should be explored in further trials. Given the low ω-3 PUFA intakes in Western populations generally, the variation in the diagnostic criteria between the studies to date and the recent evidence that DHA supplementation can improve sustained attention and frontal lobe function in healthy boys [33], future research should explore the benefits of ω-3 PUFA supplementation for children who have developmentally delayed school performance but not necessarily a clinically diagnosed developmental disorder.

Conclusion

An increase over 4 mo in erythrocyte ω-3 PUFAs, particularly DHA, was associated with improvements in literacy and the behavior of some children with ADHD. The greatest benefit was observed in children who had comorbid learning difficulties.

Acknowledgments

The authors acknowledge the assistance of Jacqueline Boon, Shelley Appleton, Suzanne Hegarty, and Liz Anderson with the recruitment and data collection in Brisbane and all the children and their parents for their participation.

References

[1] Dewey D, Kaplan BJ, Crawford SG, Wilson BN. Developmental coordination disorder: associated problems in attention, learning, and psychosocial adjustment. Hum Mov Sci 2002;21:905–18.
[2] Mayes SD, Calhoun SL, Crowell EW. Learning disabilities and ADHD: overlapping spectrum disorders. J Learn Disabil 2000;33:417–24.
[3] Sinclair AJ, Crawford MA. The accumulation of arachidonate and docosahexaenoate in the developing rat brain. J Neurochem 1972;19:1753–8.
[4] Sinclair AJ, Begg D, Mathai M, Weisinger RS. Omega 3 fatty acids and the brain: review of studies in depression. Asia Pac J Clin Nutr 2007;16(suppl 1):391–7.
[5] Meyer BJ, Kolanu N. Australian children are not consuming enough long-chain omega-3 polyunsaturated fatty acids for optimal health. Nutrition 2011;27:1136–40.
[6] O'Sullivan TA, Ambrosini G, Beilin LI, Mori TA, Oddy WH. Dietary intake and food sources of fatty acids in Australian adolescents. Nutrition 2011;27:153–9.
[7] Ramel A, Martinez JA, Kiely M, Bandarra NM, Thorsdottir I. Moderate consumption of fatty fish reduces diastolic blood pressure in overweight and obese European young adults during energy restriction. Nutrition 2010;26:168–74.
[8] Transler C, Eilander A, Mitchell S, van de Meer N. The impact of polyunsaturated fatty acids in reducing child attention deficit and hyperactivity disorders. J Atten Disord 2010;14:232–46.
[9] Sinn N, Milte CM, Howe PRC. Oiling the brain: a review of randomised controlled trials of omega-3 fatty acids in psychopathology across the lifespan. Nutrients 2010;2:128–70.
[10] Voigt RG, Llorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC. A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. J Pediatr 2001;139:189–96.
[11] Gustafsson PA, Birberg-Thomberg U, Duchén K, Landgren M, Malmberg K, Pelling H, et al. EPA supplementation improves teacher-rated behaviour and oppositional symptoms in children with ADHD. Acta Paediatr 2010;99:1540–9.
[12] Richardson AJ, Puri BK. A randomized double-blind, placebo-controlled study of the effects of supplementation with highly unsaturated fatty acids on ADHD-related symptoms in children with specific learning difficulties. Prog Neuropsychopharmacol Biol Psychiatry 2002;26:233–9.
[13] Richardson AJ, Montgomery P. The Oxford–Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. Pediatrics 2005;115:1360–6.
[14] Sinn N, Bryan J. Effect of supplementation with polyunsaturated fatty acids and micronutrients on learning and behaviour problems associated with child ADHD. J Dev Behav Pediatr 2007;28:82–91.
[15] Sinn N, Bryan J, Wilson C. Cognitive effects of polyunsaturated fatty acids in children with attention deficit hyperactivity disorder symptoms: a randomised controlled trial. Prostaglandins Leukot Essent Fatty Acids 2008;78:311–26.
[16] Johnson M, Ostlund S, Fransson G, Kadesjo B, Gillberg C. Omega-3/omega-6 fatty acids for attention deficit hyperactivity disorder: a randomized placebo-controlled trial in children and adolescents. J Atten Disord 2009;12:394–401.
[17] Vaisman N, Kaysar N, Zaruk-Adasha Y, Pelled D, Brichon G, Zwingelstein G, et al. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n-3 fatty acids containing phospholipids. Am J Clin Nutr 2008;87:1170–80.
[18] Rise P, Eligini S, Ghezzi S, Colli S, Galli C. Fatty acid composition of plasma, blood cells and whole blood: Relevance for the assessment of the fatty acid status in humans. Prostaglandins Leukot Essent Fatty Acids 2007;76:363–9.
[19] Milte CM, Sinn N, Buckley JD, Coates AM, Young RM, Howe PRC. Polyunsaturated fatty acids, cognition and literacy in children with ADHD with and without learning difficulties. J Child Health Care 2011;15:14–24.

[20] Conners CK. Conners' rating scales—revised. New York: Multi-Health Systems; 2000.
[21] Cohen J. A power primer. Psychol Bull 1992;112:155–9.
[22] Altman DG, Bland JM. Treatment allocation by minimisation. BMJ 2005;330:843.
[23] Wechsler D. Wechsler Individual Achievement Test screener manual. San Antonio, TX: Harcourt Brace & Company; 1992.
[24] Wechsler D. Manual for the Wechsler Scale of Children's Intelligence–III. New York: Psychological Corporation; 1991.
[25] Manly T, Robertson IH, Anderson V. Test of Everyday Attention for Children manual. Suffolk, UK: Thames Valley Test Company; 1999.
[26] Trommer BL, Hoeppner JA, Lorber R, Armstrong KJ. The go–no-go paradigm in attention deficit disorder. Ann Neurol 1988;24:610–4.
[27] Milte CM, Sinn N, Street SJ, Buckley JD, Coates AM, Howe PR. Erythrocyte polyunsaturated fatty acid status, memory, cognition and mood in older adults with mild cognitive impairment and healthy controls. Prostaglandins Leukot Essent Fatty Acids 2011;84:153–61.
[28] Little RJA. A test of missing completely at random for multivariate data with missing values. J Am Stat Assoc 1988;83:1198–202.
[29] Hirayama S, Hamazaki T, Terasawa K. Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder—a placebo-controlled double-blind study. Eur J Clin Nutr 2004;58:467–73.
[30] Stevens L, Zhang W, Peck L, Kuczek T, Grevstad N, Mahon A, et al. EFA supplementation in children with inattention, hyperactivity and other disruptive behaviors. Lipids 2003;38:1007–21.
[31] Sorgi PJ, Hallowell EM, Hutchins HL, Sears B. Effects of an open-label pilot study with high-dose EPA/DHA concentrates on plasma phospholipids and behavior in children with attention deficit hyperactivity disorder. Nutr J 2007;6:16.
[32] Hollingworth SA, Nissen LM, Stathis SS, Siskind DJ, Varghese JM, Scott JG. Australian national trends in stimulant dispensing: 2002–2009. Aust N Z J Psychiatry 2011;45:332–6.
[33] McNamara RK, Able J, Jandacek R, Rider T, Tso P, Eliassen JC, et al. Docosahexaenoic acid supplementation increases prefrontal cortex activation during sustained attention in healthy boys: a placebo-controlled, dose-ranging, functional magnetic resonance imaging study. Am J Clin Nutr 2010;91:1060–7.