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[1–3]

Nachum Vaisman, Nehemia Kaysar, Yahalomit Zaruk-Adasha, Dori Pelled, Gérard Brichon, Georges Zwingelstein, and Jacques Bodennec

ABSTRACT

Background: Increasing evidence supports n−3 fatty acid (FA) supplementation for patients with psychiatric disorders, such as attention deficit hyperactivity disorder. However, the exact metabolic fate of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on different glyceride carriers remains unclear.

Objective: We investigated whether conjugation of EPA and DHA to phospholipid (PL-n−3) or to triacylglycerol (fish oil; FO) affects their incorporation in blood compartments and influences executive functioning.

Design: Children aged 8–13 y with impaired visual sustained attention performance received placebo, 250 mg/d EPA + DHA esterified to PL-n−3 (300 mg/d phosphatidylserine), or FO for 3 mo in a randomized double-blind manner. Main outcome measures included plasma and erythrocyte FA profile and continuous performance test results (Test of Variables of Attention; TOVA).

Results: Sixty of the 83 children enrolled completed the interventions (n = 18–21 per group). There was an enrichment of EPA (1.5–2.2-fold), docosapentaenoic acid (DPA; 1.2-fold), and DHA (1.3-fold) in the PL fraction in the plasma of FO- and PL-n−3–fed children. In erythrocytes, only PL-n−3 resulted in a significant reduction (≈30%) of very-long-chain saturated FAs (C20-24) and in an increase (1.2- and 2.2-fold, respectively) in linoleic acid and DPA. Total TOVA scores increased in the PL-n−3 ($\bar{x} \pm$ SD: 3.35 ± 1.86) and FO (1.72 ± 1.67) groups but not in the placebo group (−0.42 ± 2.51) (PL-n−3 > FO > placebo; $P < 0.001$). A significant correlation between the alterations in FAs and increased TOVA scores mainly occurred in the PL-n−3 group.

Conclusion: Consumption of EPA+DHA esterified to different carriers had different effects on the incorporation of these FAs in blood fractions and on the visual sustained attention performance in children. This trial was registered at clinicaltrials.gov as NCT00382616. *Am J Clin Nutr* 2008;87:1170–80.

INTRODUCTION

The nervous system is the organ with the second largest concentration of lipids, exceeded only by adipose tissue. Approximately 35% of these lipids are long-chain polyunsaturated fatty acids (LC-PUFAs), such as arachidonic acid (AA; 20:4n−6) and docosahexaenoic acid (DHA; 22:6n−3) (1), which are known to play an essential role in brain development and functions (2). Both AA and DHA could be either provided in diet or synthesized in the body from the essential fatty acid (EFA) linoleic acid (LA; 18:2n−6) and α-linolenic acid (18:3n−3), respectively (3). Epidemiologic studies (4, 5) suggest that dietary consumption of n−3 LC-PUFAs, especially eicosapentaenoic acid (EPA; 20:5n−3) and DHA, affects neuropsychiatric disorders, presumably because of their structural and neurochemical involvement in pathophysiological processes (6–8). Moreover, interventions with n−3 LC-PUFAs ranging from 1 to 6.2 g/d EPA and from 0 to 3.4 g/d of DHA were associated with a therapeutic effect in a broad spectrum of psychiatric disorders (9–12). Similarly, supplementation of DHA-containing phospholipid (PL), such as the bovine brain cortex phosphatidylserine (PS), to animal models was shown to attenuate neuronal effects of aging (13) and to affect behavior as well (14, 15). In humans, administration of 300 mg/d PS for 3 mo to subjects with age-associated memory impairment resulted in a consistent improvement of performance in memory tests (16) and was also associated with beneficial effect on symptoms of depression in geriatric women (17).

Deficiencies of n−3 fatty acids (FAs) are associated with a wide range of neuropsychiatric and neurodevelopmental disorders, such as attention-deficit hyperactivity disorder (ADHD) (18–21), dyslexia (22), depression and aggression (6), and autism (23). The effect of DHA supplementation of 345 mg/d or 3.6 g/body wt, provided as ethyl esters (24) in capsules or as triacylglycerol in functional food (25), was tested in ADHD children. These interventions induced a pronounce increase in blood n−3 LC-PUFAs, but failed to affect ADHD symptoms. Results of recent studies, in which EPA- and DHA-enriched fish oils (FOs) were provided along with n−6 LC-PUFA containing oils and/or vitamins to ADHD children were conflicting (26–29).

To date, the role of the carrier in the metabolic fate of these FAs in schoolchildren, remains poorly studied. Current available reports, obtained mostly from infant formulas studies, compare

[1] From the Clinical Nutrition Unit (NV and YZ-A) and the Child and Adolescent Psychiatry Unit (NK), Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel; Enzymotec Ltd, Migdal-HaEmeq, Israel (DP); Université de Lyon, Université Lyon 1, Institut Michel Pacha (GB, GZ, and JB), UMR CNRS 5123 (JB), and CTRS-IDÉE, Hospices Civiles de Lyon (JB), Lyon, France.

[2] Supported by Enzymotec Ltd, Migdal-HaEmeq, Israel.

[3] Address reprint requests to N Vaisman, Clinical Nutrition Unit, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel-Aviv, 64239, Israel. E-mail: vaisman@tasmc.health.gov.il.

Received September 9, 2007.
Accepted for publication November 30, 2007.

DHA attached to the backbone of PL (eg, n−3–enriched egg yolk) to counter dietary intervention containing mostly fish or algal oils (30–32). Interestingly, early observations suggested that dietary LC-PUFAs esterified to PLs rather than triacylglycerols are more effective substrates for brain tissues accretion in term baboons (33).

In the current study, we investigated the differential incorporation of n−3 LC-PUFAs provided as either PL (mainly PS) or triacylglycerol (FO) into blood fractions compared with rapeseed oil in schoolchildren with impaired attention performance. The effect of these dietary supplements on the children's executive functions was also assessed.

SUBJECTS AND METHODS

Subjects

This study was conducted between July 2004 and January 2005 at Tel-Aviv Sourasky Medical Center (Tel-Aviv, Israel). The subjects were recruited during their summer vacation, when most schoolchildren with ADHD are voluntarily taken off stimulant medications. The 250 children or their surrogates who responded to a newspaper advertisement were first screened by telephone. Children were included if they were 8–13 y of age and had received a previous diagnosis of ADHD by a clinical psychiatrist, neurologist, or pediatrician. Children with significant sensory or neurological limitations, epilepsy, mental retardation, psychosis, or pervasive developmental disorder were excluded. Also excluded were children taking medications with known central nervous system effects, including stimulants or dietary supplements other than vitamins. Of these applicants (**Figure 1**), 102 (27 females) were screened with a continuous performance test (CPT), a Test of Variables of Attention (TOVA) (34) (*see* below), to determine their total TOVA score. A score lower than −1.8 (SD) from age- and sex-adjusted normal means suggests a high probability of impaired attention performance (34).

All children were white, and no socioeconomic data were collected. The protocol was approved by the Institutional Review Board at Tel Aviv Sourasky Medical Center (TASMC-03-NV-220-CTIL), and written informed consent was obtained from all parents or legal guardians.

Protocol and diets

Eighty-three of the 102 eligible participants were randomly assigned in a double-blind manner to receive n−3 LC-PUFA–containing PLs (PL-n−3; Enzymotec LTD, Migdal HaEmeq,

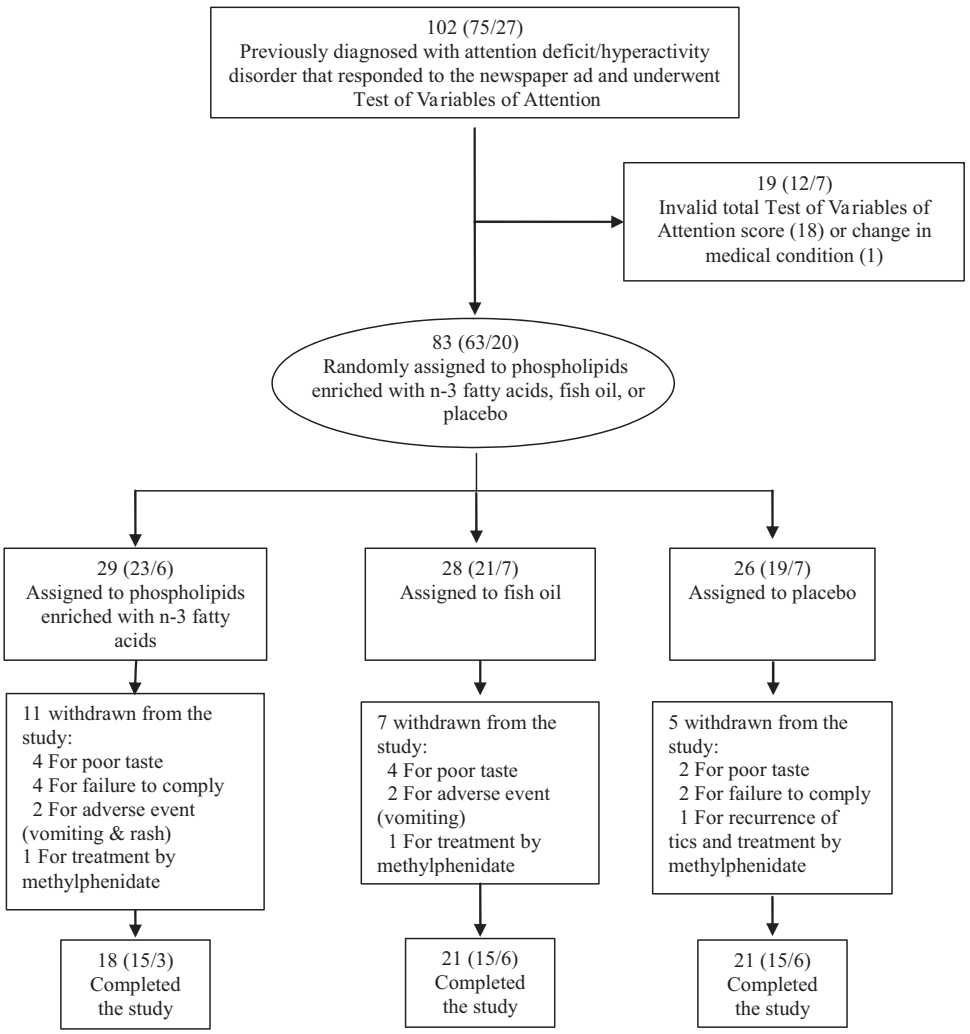

FIGURE 1. Flow diagram of subject (boys and girls) participation throughout the study.

Israel), FO (Ocean Nutrition, NS, Canada), or placebo (rapeseed oil; Milumor, Kiryat Bialik, Israel) for 3 mo. The randomization sequence was a block randomization process (block size 3), ensuring equal numbers of male and female applicants in each arm of the trial after every 3 randomized subjects and approximately equal numbers overall ($P = 0.8564$; Figure 1).

Supplements were formulated by emulsifying the different lipid preparations to a dairy chocolate-flavored spread (Hashachar Chocolate Flavored Spread; Hashachar Haoleh, Haifa, Israel) with 4–7 mg/d citrus oil extract (Ganir, Sde Gat, Israel) that was added to buffer taste discernment (total 25 g/d). Tocopherol mixtures (0.2% by wt) were added to the base stock FO (control FO treatment) by the manufacturer (Ocean Nutrition Ltd, Halifax, Canada). No antioxidant other than the original preparation was introduced to the formulated matrix. The PL-n−3 was synthesized by Enzymotec Ltd (Migdal HaEmeq, Israel) using different sources of marine species. Rosemary extract, ascorbyl palmitate, and mixed natural tocopherols (0.8% by wt) were added to the final formulation. The participant's parents or guardians were instructed to spread a daily aliquot of enriched chocolate-flavored paste on a single slice of bread. Compliance was monitored weekly by phone calls. In addition, following the baseline visit, 14 identically appearing spreads containers were allocated and collected at 10–12-d intervals. The chocolate spread containers were weighted, and the dosage was determined according to the container weight. The daily PL and FA composition of the different treatments are given in **Table 1**. The parents were instructed to not provide the spreads to their children until after the blood samples were collected.

The subjects' parents were regularly asked to instruct their children to maintain their usual level of physical activity and diet throughout the study and to report any symptoms, disease onset, medication or dietary supplement consumption, or change in usual habits. Concomitant use of prescription or nonprescription agents with potent psychotropic properties, including ADHD treatments and dietary supplements (except for vitamins), was prohibited within 4 wk before the initial evaluation and during the entire intervention. Noncompliance was defined as poor consumption (<70%) or discontinuation of the assigned experimental diet or a parallel use of medications and/or dietary supplements as specified under exclusion criteria.

TABLE 1
Phospholipid and main fatty acid composition of the study interventions

	Phospholipids enriched with n−3 fatty acids	Fish oil	Placebo
	mg/d		
Phospholipids			
Phosphatidylserine	300[1]	ND[2]	ND
Phosphatidylethanolamine	66	ND	ND
Phosphatidic acid	48	ND	ND
Lysophospholipids	24	ND	ND
Phosphatidylcholine	18	ND	ND
Phosphatidylinositol	12	ND	ND
Total	468	ND	ND
Fatty acids			
14:0	19	63	ND
16:0	141	147	30
18:0	7	31	13
20:0	0	2	5
22:0	1	2	3
24:0	0	4	1
16:1n−7	19	68	1
18:1n−9	37	95	415
18:1n−7	44	25	ND
20:1n−9	5	12	13
22:1n−9	5	7	4
18:2n−6	11	18	150
18:3n−6	1	2	0
20:4n−6	4	7	0
18:3n−3	7	25	69
20:5n−3	156	153	ND
22:5n−3	4	0	0
22:6n−3	95	96	ND
Rest	24	41	37
Total	580	799	742

[1] Typical daily dosage values.
[2] ND, none detected.

Blood lipid analysis

Nonfasting blood samples (5 mL) were drawn from a peripheral vein into heparinized tubes at baseline and at the end of the 3-mo intervention during the early morning hours. Erythrocytes were separated from plasma by Ficoll-Hypaque density-gradient centrifugation at 1260 × *g* for 30 min at 4 °C. The plasma and erythrocytes were immediately collected and stored at −80 °C until analyzed for lipids. The lipid analysis in plasma and erythrocytes, respectively, for subjects in the PL-n−3 ($n = 1, 4$), FO ($n = 3, 3$), and placebo ($n = 2, 2$) groups could not be performed at baseline or after 3 mo because of insufficient sample material.

Lipids were extracted from plasma and erythrocytes according to a modified Folch procedure (35). Briefly, the samples were homogenized in methanol and, after addition of chloroform [final chloroform – methanol ratio of (2:1, vol:vol)], extraction was allowed to occur at room temperature. The samples were filtered and washed twice with water containing 0.25% KCl (wt:vol). The chloroform lower phase evaporated, and the total lipid content was determined by weight. Total PLs were quantified after mineralization by using sulfuric acid:perchloric acid (2:1, vol:vol) containing 0.1% V_2O_4, followed by phosphate determination (36). Individual PLs were separated by 2-dimensional thin-layer chromatography (TLC) (silica gel plates G60; Merck, Darmstadt, Germany) by using tetrahydrofuran-acetone-methanol-water (50:20:40:6, vol:vol:vol:vol) as the first developing solvent and chloroform-acetone-methanol-acetic acid-water (50:20:10:15:5, vol:vol:vol:vol) as the second developing solvent (37). The TLC plate was subsequently dried, and the PLs were visualized with the Dittmer and Lester reagent (38) and quantified as described above.

Determinations of the PL and neutral lipid FA profiles from the indicated blood fractions were performed as follows: PLs were separated from neutral lipids by TLC using diisopropylether as the developing solvent, and FAs were converted to methyl esters using acetyl chloride (39). Neutral lipids were resolved from each other on TLC using hexane-diisopropylether–acetic acid (80:20:1, vol:vol:vol) as the developing solvent. Triacylglycerol and cholesterol esters plasma fractions, identified according to authentic standards, were transmethylated as described for PLs or first submitted to saponification and then methylated with acetyl chloride. FA methyl esters were quantified by a gas chromatography apparatus (Chrompack CP9000, Les Ulis, France) equipped with a flame ionization detector. Separation was achieved on a 30-m Omegawax 250

capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 μm (Supelco, Saint Quentin Fallavier, France). The carrier gas (nitrogen) flow rate was 15 mL/min at 60 kPa. Samples were injected at 250 °C. The oven temperature remained at 180 °C for 3 min after injection, was increased to 225 °C at a rate of 2 °C/min, and kept constant for 10 min, after which it rose to 235 °C at a rate of 10 °C/min. The temperature was then kept constant at 235 °C until 65 min, ie, until the end of data acquisition. The detector was set at 250 °C. FA methyl esters were identified by using authentic standards (Supelco).

Neuropsychological and behavioral tests

TOVA, an age- and sex-normalized computer-based assessment of inattention, was performed at baseline and after 3 mo (closure of the intervention). This computerized CPT includes a target stimulus and a nontarget stimulus (34). The stimuli are presented for 200 ms at the rate of 30/min. The duration of the test is 22.5 min. Targets are present on 22.5% of trials during the first half of the test and on 77.5% of the trials during the last half.

The variables measured were as follows: *1*) errors of omission or failure to respond to the designated target that is interpreted as a measure of inattention, *2*) errors of commission or erroneous response to the nontarget that is considered as a measure of impulsivity, *3*) response time (in ms) or the latency time required to respond correctly to the target stimulus that is interpreted as a measure of information processing, and *4*) response time variability (the SD of response times) that is interpreted as an index of consistency of attention. These results were further compared with normal, same-sex, same-age, and average intelligence quotient groups to be reported as SDs. The number of anticipatory responses (very short latency responses) that represent guessing is interpreted as a measure of impulsivity; this variable, however, is also used to ensure the validity of the results. A high anticipatory response score is interpreted as an invalid result according to the TOVA manual; thus, the total TOVA score could not be calculated. The SD indicates the extent of the disorder, ie, a more negative value points at specific parameter severity, whereas conversely, a more positive deviation from the normal value suggests a better than average performance. The total TOVA score, also referred to as the ADHD index score, includes the response time, *d* prime or response sensitivity, which is used to interpret the rate of performance change over time and response time variability. This score serves as an indication of the degree of similarity in performance between the assessed children and normative samples (34).

An additional behavior assessment included the Hebrew translation of the parental Abbreviated Conners Rating Scale (40), which is an easy-to-complete 10-item questionnaire graded on a Likert scale of 1–4 (not at all = 0, just a little = 1, pretty much = 2, and very much = 3). These items elicit observations of hyperactive-inattentive behaviors. A Hebrew translation of the 11-item mood or emotional liability subscale of Child Behavior Checklist was also assessed (41) and graded by the parents (0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true).

Statistical analysis

The statistical analyses were conducted for the subjects who completed the 3-mo intervention and had a minimal compliance of 70% throughout. The results are expressed as means ± SDs. All statistical tests were 2-tailed, and significance was set at a level of 0.05.

Baseline characteristics were compared using an analysis of variance (ANOVA) model for continuous variables and the Kruskal-Wallis test for nonparametric variables, except for randomization and sex, for which the Fisher's exact test was used. An arcsin transformation was made before the statistical analysis for the percentage values of the PL profile in plasma, and their changes were evaluated by using ANOVA followed by post hoc analysis based on Games-Howell comparisons. Baseline values were inserted into the model as covariates if they were found to be statistically different. The assumption of normal distribution and homogeneity of variance for plasma and erythrocyte lipids as well as TOVA variables were unmet. Accordingly, the effect of these interventions was analyzed using the Kruskal-Wallis nonparametric test followed by post hoc testing for differences between means with the use of Gabriel and Games-Howell tests, which adjust $\alpha = 0.05$ for multiple comparison. Likewise, the differences within groups were determined by using Wilcoxon's signed-rank test. The differences in the proportion of subjects with total TOVA scores within normative values in the assigned groups at the end of the intervention were tested with a chi-square test followed by comparisons between the treatments by using Fisher's exact test. Spearman's rank-order correlation coefficient was used to evaluate the associations between changes in TOVA variable scores and changes in blood PL FAs over the intervention period. The statistical analyses were carried out by using SPSS statistical software (SPSS Inc, Chicago, IL) version 13.0.

RESULTS

Study subjects

A flow chart of subject participation is shown in Figure 1. A total of 102 putative ADHD children were recruited for this study. Eighteen of the applicants were withdrawn from the study because of invalid total TOVA score (34). Another candidate was dropped for unrelated health reasons (Figure 1).

The final 83 subjects were randomly assigned into 3 groups; 23 dropped out because of a dislike of the preparation taste ($n = 10$), noncompliance with the dietary regime ($n = 6$), adverse effects ($n = 3$ for gastrointestinal-related discomfort and vomiting; $n = 1$ for rash), reassignment to methylphenidate ($n = 2$), and reoccurrence of tics ($n = 1$). Most of these dropouts occurred within the first 2-wk after enrollment. Both the PL-n−3 and FO treatments were generally well tolerated by the participants, and there were no differences between the attrition rates of the different intervention groups (PL-n−3, 11/29; FO, 7/28; and placebo, 5/26; $P = 0.1585$). Biochemical and neuropsychological endpoints were unavailable for the dropouts, so that any change from baseline values could not be calculated for these subjects.

No significant differences were detected between the baseline characteristics of the 60 schoolchildren in the PL-n−3, FO, and placebo groups who completed the intervention (**Table 2**). The scores for the parental assessment of child behavior ratings at baseline suggested that these children had symptoms of inattention.

TABLE 2
Baseline characteristics of the 60 children who completed the interventions

Characteristic[1]	Phospholipids enriched with n−3 fatty acids ($n = 18$)	Fish oil ($n = 21$)	Placebo ($n = 21$)
Age (y)[2]	9.17 ± 1.27[3]	9.40 ± 1.06	9.31 ± 1.28
Sex (male/female)	15/3	15/6	15/6
Behavioral assessments (parents)			
Abbreviated Conners Rating Scale	14.33 ± 6.67	17.10 ± 5.26	15.05 ± 6.2
Child Behavior Checklist for ages 4–18 y (subclass)	10.00 ± 5.10	8.95 ± 3.84	9.95 ± 4.47

[1] No significant differences were found, $P > 0.05$ (Kruskal-Wallis nonparametric test). The participants, all of whom had a previous diagnosis of attention deficit hyperactivity disorder (ADHD), had not taken prescription or nonprescription agents with potent psychotropic properties, including ADHD treatments and dietary supplements (except vitamins) ≥ 4 wk before enrollment.
[2] Age range was 8–13 y for all groups.
[3] $\bar{x}$ ± SD (all such values).

Plasma phospholipid profiles

The composition of serum PLs are shown in **Table 3**. The baseline PL profile analysis showed that the plasma phosphatidylcholine (PC) and phosphatidylethanolamine (PE) concentrations of the PL-n−3 group were statistically different from those of the FO group ($P = 0.044$ and 0.032, respectively) but not from the placebo group ($P = 0.066$ and 0.150, respectively). The tested interventions did not affect plasma PL concentration (≈32–42 mg/L; Table 3). Likewise, after the 3-mo consumption of lipid preparations, no differences were noted in plasma lysophosphatidylcholine, sphingomyelin, PC, phosphatidylinositol, and PE concentrations ($P = 0.448$, 0.278, 0.083, 0.432, and 0.317, respectively). Plasma PS concentrations were below the detection level for all groups, including PL-n−3–fed children. These results are consistent with previous observations (42), which suggest that within a few hours of dietary ingestion, PS is converted to other PLs via its lyso form and the subsequent decarboxylation in the mucosal cells.

Changes in the plasma FA profiles as a result of dietary interventions were found primarily in the PL rather than in the triacylglycerol or cholesterol ester fractions (data not shown). Plasma and erythrocyte PL FA compositions at baseline and the changes after 3 mo in the 3 intervention groups are listed in **Table 4**. There were no differences between the children's FA profiles in plasma and erythrocyte PLs at baseline.

Saturated fatty acids

Of the saturated fatty acids (SFAs) in plasma PLs, no differences across diets were detected (Table 4). In erythrocyte PLs, long-chain SFA, arachidonic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) concentrations, however, decreased significantly in the PL-n−3–fed children compared with the FO group ($P = 0.033$, 0.021, and 0.005, respectively) and, to a lesser extent, the placebo group ($P = 0.114$, 0.046, and 0.045, respectively). These alterations did not affect ($P = 0.162$) the proportion of total SFAs (data not shown).

cis Monounsaturated fatty acids

The largest difference between the n−3 dietary matrices and placebo was observed in the n−9 rather than in the n−7 FAs. There were no differences across diets in the plasma and erythrocyte eicosenoic acid (20:1n−9) and erucic acid (22:1n−9) concentrations (Table 4). In the erythrocyte PLs, an increase (≈230%) in nervonic acid (24:1n−9) concentrations was detected in response to PL-n−3 administration compared with the placebo ($P = 0.029$) but not the FO ($P = 0.389$) treatment. There

TABLE 3
Composition of phospholipids in the plasma of children with impaired visual sustained attention performance at baseline and changes after 3 mo of intervention[1]

Phospholipid	Baseline	Change after 3 mo	P for difference between groups[2]
	% of total		
Lysophosphatidylcholine			
PL-n−3	9.1 ± 2.4[3]	0.1 ± 3.9	0.448
Fish oil	9.5 ± 2.8	−1.0 ± 2.6	
Placebo	9.7 ± 3.8	0.8 ± 5.2	
Sphingomyelin			
PL-n−3	19.3 ± 2.2	2.4 ± 3.5	0.278
Fish oil	20.5 ± 2.8	0.9 ± 4.7	
Placebo	19.9 ± 3.0	−0.3 ± 6.3	
Phosphatidylcholine			
PL-n−3	68.7 ± 4.4[a]	−4.8 ± 7.0	0.081[4]
Fish oil	64.9 ± 4.8[b]	−0.4 ± 5.9	
Placebo	65.4 ± 4.5[a,b]	0.3 ± 7.8	
Phosphatidylinositol			
PL-n−3	2.5 ± 2.0	0.8 ± 2.8	0.432
Fish oil	2.7 ± 1.5	0.8 ± 3.0	
Placebo	2.6 ± 2.0	−0.3 ± 2.9	
Phosphatidylethanolamine			
PL-n−3	0.8 ± 1.2[a]	1.3 ± 1.9	0.317[4]
Fish oil	2.2 ± 2.3[b]	−0.5 ± 3.1	
Placebo	2.2 ± 3.4[a,b]	−0.5 ± 4.1	

[1] $n = 17$–18. PL-n−3, phospholipids enriched with n−3 fatty acids: baseline phospholipids were different between groups for phosphatidylcholine ($P = 0.023$) and phosphatidylethanolamine ($P = 0.031$) as analyzed with a Kruskal-Wallis nonparametric test followed by Gabriel and Games-Howell tests post hoc. Values not sharing a common superscript letter are significantly different ($P < 0.05$). No significant differences were detected between groups or within total plasma phospholipid concentrations at baseline (PL-n−3: 371 ± 174 mg/L; fish oil: 425 ± 155 mg/L; and placebo: 417 ± 204 mg/L) or after supplementations (PL-n−3: 317 ± 86 mg/L; fish oil: 372 ± 134 mg/L; and placebo: 343 ± 129 mg/L).
[2] Obtained by ANOVA with or without baseline concentrations included in the model as covariates followed by post hoc Games-Howell comparisons. Values of plasma phospholipids were normalized by using arcsin transformation.
[3] $\bar{x}$ ± SD (all such values).
[4] Baseline concentrations were included into the model as covariates.

TABLE 4
Fatty acid composition in plasma and erythrocyte phospholipids in children with abnormal total Test of Variables of Attention (TOVA) scores at baseline and the subsequent changes after 3 mo of treatment[1]

	Plasma phospholipids				Erythrocyte phospholipids			
Fatty acid	PL-n−3	Fish oil	Placebo	P^2	PL-n−3	Fish oil	Placebo	P^2
	% of total fatty acids				*% of total fatty acids*			
16:0								
Baseline	15.0 ± 4.9[3]	16.3 ± 4.0	16.8 ± 3.9		17.6 ± 6.8	17.5 ± 3.8	17.6 ± 5.5	
3-mo change	1.2 ± 2.8	1.1 ± 4.1	−0.7 ± 3.3	0.130	−1.8 ± 6.1	−1.8 ± 3.3	−1.1 ± 5.0	0.793
18:0								
Baseline	16.0 ± 1.4	16.1 ± 1.4	16.0 ± 1.1		15.3 ± 1.5	15.0 ± 1.7	15.3 ± 1.6	
3-mo change	−0.3 ± 1.6	−0.1 ± 1.0	−0.2 ± 1.6	0.852	−1.1 ± 1.5	−0.3 ± 1.9	−0.2 ± 2.2	0.188
20:0								
Baseline	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2		0.6 ± 0.3	0.5 ± 0.2	0.5 ± 0.2	
3-mo change	−0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1	0.158	−0.2 ± 0.3[4,a]	−0.0 ± 0.1[b]	−0.1 ± 0.2[a,b]	0.035
22:0								
Baseline	2.6 ± 0.9	2.2 ± 0.7	2.2 ± 0.7		2.7 ± 1.0	2.0 ± 0.9	2.3 ± 1.0	
3-mo change	−0.1 ± 0.6	−0.0 ± 0.7	−0.1 ± 0.7	0.928	−1.1 ± 0.9[4,a]	−0.2 ± 0.8[b]	−0.3 ± 1.0[b]	0.031
24:0								
Baseline	2.5 ± 0.8	2.2 ± 0.4	2.2 ± 0.5		7.0 ± 2.9	5.2 ± 2.6	5.9 ± 2.9	
3-mo change	0.1 ± 0.6	0.2 ± 0.6	0.1 ± 0.5	0.756	−3.1 ± 2.5[4,a]	−0.5 ± 2.0[b]	−1.0 ± 2.2[b]	0.009
16:1n−7								
Baseline	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1		0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	
3-mo change	−0.0 ± 0.1	−0.0 ± 0.1	−0.0 ± 0.1	0.121	−0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.2	0.370
18:1n−9								
Baseline	9.0 ± 1.6	8.0 ± 1.0	8.2 ± 1.6		11.2 ± 2.4	11.0 ± 1.9	11.1 ± 1.7	
3-mo change	−1.3 ± 1.5	−0.7 ± 0.9	−0.4 ± 1.4	0.218	−1.2 ± 2.2	−0.8 ± 1.7	−0.6 ± 1.8	0.838
18:1n−7								
Baseline	1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.2		1.2 ± 0.3	1.2 ± 0.2	1.2 ± 0.3	
3-mo change	−0.2 ± 0.4	−0.1 ± 0.2	0.1 ± 0.3	0.052	−0.1 ± 0.3	−0.1 ± 0.2	0.0 ± 0.3	0.743
20:1n−9								
Baseline	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1		0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.5	
3-mo change	−0.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	0.843	−0.1 ± 0.2	−0.1 ± 0.1	−0.2 ± 0.5	0.083
22:1n−9								
Baseline	1.3 ± 0.9	0.9 ± 0.3	0.9 ± 0.7		1.0 ± 0.3	0.9 ± 0.5	0.9 ± 0.5	
3-mo change	−0.3 ± 0.9	0.1 ± 0.6	0.1 ± 0.8	0.592	−0.4 ± 0.4	−0.3 ± 0.5	−0.2 ± 0.6	0.456
24:1n−9								
Baseline	ND	ND	ND		1.4 ± 1.2	2.7 ± 1.8	2.3 ± 1.6	
3-mo change	ND	ND	ND		1.9 ± 1.1[6,a]	1.2 ± 1.5[4,a,b]	0.2 ± 2.2[b]	0.036
18:2n−6								
Baseline	18.7 ± 3.3	19.9 ± 2.4	19.4 ± 3.2		9.3 ± 1.6	9.6 ± 1.8	9.1 ± 1.5	
3-mo change	2.9 ± 4.0[5,a]	0.1 ± 2.7[b]	0.8 ± 2.3[a,b]	0.036	1.8 ± 1.3[4,a]	0.4 ± 1.5[b]	0.7 ± 1.5[a,b]	0.015
20:4n−6								
Baseline	10.9 ± 2.4	10.1 ± 2.4	10.4 ± 1.4		9.6 ± 5.0	11.1 ± 4.4	10.0 ± 4.6	
3-mo change	−1.5 ± 1.3[5,a]	−0.9 ± 1.9[a]	0.7 ± 1.6[b]	<0.001	3.3 ± 4.3	1.5 ± 3.9	1.9 ± 5.1	0.349
22:4n−6								
Baseline	0.7 ± 0.2	0.7 ± 0.3	0.6 ± 0.1		2.6 ± 1.5	2.8 ± 1.2	2.6 ± 1.4	
3 mo change	−0.3 ± 0.2[4,a]	−0.3 ± 0.3[6,a]	0.0 ± 0.2[b]	<0.001	0.3 ± 1.8	0.1 ± 1.2	0.4 ± 1.4	0.333
20:5n−3								
Baseline	0.7 ± 1.2	0.6 ± 0.6	0.7 ± 0.5		0.8 ± 0.6	1.0 ± 0.7	1.2 ± 0.6	
3-mo change	0.3 ± 1.2[6,a]	0.3 ± 0.8[4,a]	−0.0 ± 0.5[b]	0.003	0.5 ± 0.5	0.2 ± 0.8	−0.0 ± 0.5	0.058
22:5n−3								
Baseline	1.0 ± 0.3	0.9 ± 0.3	1.0 ± 0.3		1.0 ± 0.7	1.3 ± 0.6	1.2 ± 0.7	
3-mo change	0.2 ± 0.3[5,a]	0.2 ± 0.3[a]	−0.1 ± 0.2[b]	0.014	1.0 ± 0.7[4,a]	0.7 ± 0.6[6,a,b]	0.2 ± 0.8[b]	0.016
22:6n−3								
Baseline	4.4 ± 1.5	4.0 ± 1.4	4.8 ± 2.0		4.1 ± 2.1	3.4 ± 1.5	3.7 ± 1.5	
3-mo change	0.9 ± 1.8[5,a]	1.3 ± 1.8[5,a]	−0.0 ± 1.4[b]	0.012	−1.4 ± 1.8	−0.7 ± 1.2	−0.4 ± 1.9	0.479

[1] $n = 14–19$; PL-n−3, phospholipids enriched with n−3 fatty acids; ND, not detected. No significant differences in baseline fatty acid concentrations were found between the study groups ($P > 0.05$). Values not sharing a common superscript letter are significantly different ($P < 0.05$).

[2] Changes between treatments were analyzed by the Kruskal-Wallis nonparametric test followed by the Gabriel and Games-Howell tests post hoc.

[3] $\bar{x}$ ± SD (all such values).

[4–6] Within-group comparisons of changes over the study intervention period (Wilcoxon signed-rank test): [4] $P < 0.01$, [5] $P < 0.05$, [6] $P < 0.001$.

was a tendency toward a reduction in total monounsaturated fatty acid (MUFA) concentrations in plasma PLs ($P = 0.063$), but the dietary matrices did not significantly affect ($P = 0.140$) total MUFAs in the erythrocyte fraction (data not shown).

n−6 Polyunsaturated fatty acids

In both plasma and erythrocyte PLs, LA concentrations significantly increased (17 ± 23% and 22 ± 17%) from baseline after consumption of PL-n−3 compared with FO (1 ± 14% and 6 ± 17%; $P = 0.029$ and 0.008, respectively), and, to a lesser degree, compared with placebo (5 ± 12% and 10 ± 21%; $P = 0.221$ and 0.046, respectively) (Table 4). In contrast, a significant decrease in the AA and adrenic acid (22:4n−6) content in the PL-n−3– and FO-fed groups compared with the placebo group was detected in the plasma but not in the erythrocyte PLs.

n−3 Polyunsaturated fatty acids

The response of n−3 LC-PUFAs in plasma PLs to the diets mirrored that of the n−6 LC-PUFAs, although the magnitude of the effect was more pronounced. In plasma PLs, EPA, docosapentaenoic acid (DPA; 22:5n−3), and DHA concentrations increased significantly at the end of the interventions in both the PL-n−3 and FO groups compared with the placebo group (Table 4). The combined effects of the PL-n−3 and FO supplements on the n−3 and n−6 FA concentrations in plasma PLs resulted in a distinct increase (≈190–220%) in the ratio of EPA to AA (change from baseline values of 0.06 ± 0.05 and 0.04 ± 0.11, respectively) as compared with placebo (−0.01 ± 0.04; $P < 0.0001$). In erythrocyte PLs, the PL-n−3 and, to a lesser extent, the FO supplements significantly increased DPA concentrations compared with the placebo ($P = 0.022$ and 0.133, respectively). Erythrocyte EPA and DHA concentrations were not altered by the dietary treatments at the end of the feeding interventions (Table 4).

Computerized test of attention

Changes in sustained attention and impulsivity before and after the interventions, as measured by the TOVA, are summarized in **Table 5**. No significant differences were detected between the baseline variables of attention in this objective analysis. The baseline total TOVA scores indicate that these children were markedly different (total mean: −5.1 SD) from those of age- and sex-matched normative values (≥ −1.8 SD). The total TOVA score was significantly affected by the treatments to a different extents: PL-n−3 > FO > placebo ($P < 0.001$). Accordingly, the ratio of subjects with total TOVA scores within the normative range at the end of the treatment was significantly different between the interventions (**Figure 2**). The proportion of subjects with normative scores in the PL-n−3 group (11/18) was statistically different from that of the placebo group (3/21; Figure 2), but not from the FO group (7/21; $P = 0.120$). Furthermore, a treatment effect was obtained in all TOVA-adjusted variables, except for the errors of omission (Table 5). The PL-n−3 and, to a limited extent, the FO treatment significantly improved the executive functioning in errors of commission, response time, and response time variability as compared with placebo intervention (PL-n−3: $P = 0.050$, 0.031, and 0.003, respectively; FO: 0.126, 0.673, and 0.028, respectively).

TABLE 5
Mean changes from baseline in Test of Variables of Attention (TOVA) scores of the children presenting abnormal executive functioning after the 3 mo of dietary interventions[1]

TOVA variables	Baseline	Change after 3 mo	P[2] for difference within group	P for difference between groups
Total TOVA score				
PL-n−3	−4.53 ± 2.41[3]	3.35 ± 1.86[a]	<0.001	<0.001
Fish oil	−5.69 ± 3.64	1.72 ± 1.67[b]	<0.001	
Placebo	−4.93 ± 2.96	−0.45 ± 2.51[c]	0.418	
Errors of omission				
PL-n−3	−1.31 ± 1.76	0.90 ± 1.43		0.125
Fish oil	−1.62 ± 1.69	0.37 ± 1.07		
Placebo	1.63 ± 1.71	0.33 ± 1.44		
Errors of commission				
PL-n−3	0.13 ± 0.81	0.75 ± 1.09[a]	0.006	0.046
Fish oil	−0.02 ± 1.34	0.53 ± 1.18[a,b]	0.264	
Placebo	0.12 ± 1.33	−0.06 ± 1.32[b]	0.896	
Total response time				
PL-n−3	−1.72 ± 1.22	0.72 ± 0.69[a]	0.039	0.036
Fish oil	−2.10 ± 1.20	0.24 ± 0.71[a,b]	0.046	
Placebo	−2.00 ± 1.06	−0.02 ± 1.17[b]	0.690	
Response time variability				
PL-n−3	−1.81 ± 1.07	1.01 ± 1.25[a]	0.022	0.002
Fish oil	−2.22 ± 1.08	0.63 ± 0.86[a]	0.014	
Placebo	−1.88 ± 1.18	−0.30 ± 1.44[b]	0.490	

[1] $n = 18$–20. PL-n−3, phospholipids enriched with n−3 fatty acids. A positive change indicates improvement. No baseline variable was found to be significantly different, $P > 0.05$ (Kruskal-Wallis nonparametric test).

[2] Within-group comparisons of changes over the study intervention period were obtained with the Wilcoxon signed-rank test, whereas changes between treatment comparisons were analyzed with the Kruskal-Wallis nonparametric test followed by the Gabriel and Games-Howell tests post hoc. Values not sharing a common superscript letter are significantly different ($P < 0.05$).

[3] $\bar{x}$ ± SD (all such values).

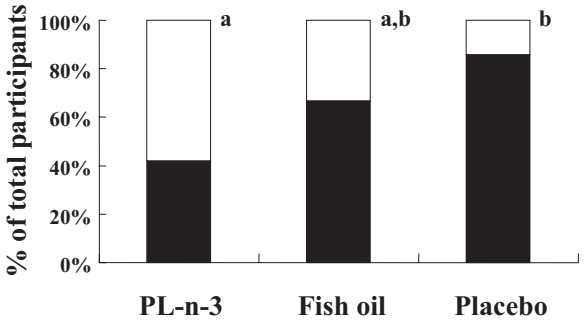

FIGURE 2. Proportions of children presenting normal (□, ≤ −1.8 SD) or abnormal (■, > −1.8 SD) total Test of Variables of Attention (TOVA) scores after the consumption of phospholipids enriched with n−3 fatty acids (PL-n−3; $n = 18$), fish oil ($n = 21$), or placebo ($n = 21$) for 3 mo. Chi-square analysis followed by comparison between the treatments with Fisher's exact test showed a significant effect of diet ($P = 0.015$) and a significant difference between placebo and PL-n−3 treatments ($P = 0.004$).

These alterations in the TOVA variables were shown to correlate with the FA profile changes in both the plasma and erythrocyte PLs (**Table 6**). Across the study interventions, there was a significant positive correlation with the changes in total TOVA scores only with the changes in plasma DHA concentrations (Table 6). In the PL-n−3 group, significant correlations were negatively associated between the changes in total TOVA scores and the changes in plasma PL EPA concentrations, EPA/AA ratios (−0.515; $P < 0.05$), and erythrocyte PL LA concentrations and positively associated with changes in erythrocyte PL lignoceric acid and DHA (0.560; $P < 0.05$) concentrations. Similarly, significant correlations were found for changes in adjusted TOVA variables and other biochemical alterations, such as plasma PL DHA, AA (data not shown), and erythrocyte PL behenic acid (data not shown), mostly in children who were assigned to the PL-n−3 treatment.

The parental Abbreviated Conners Rating Scale questionnaire failed to show a significant effect between the treatments. Rather, it showed a significant change from baseline in all treatment groups (PL-n−3: −5.00 ± 8.32; FO: −3.20 ± 6.05; and placebo: −2.35 ± 3.73). Minimal and insignificant changes from baseline values were noted by the parents in the subscale of Child Behavior Checklist after all 3 dietary regimens (PL-n−3: −1.47 ± 5.10; FO: −0.53 ± 3.10; and placebo: −0.78 ± 3.52).

DISCUSSION

Our main finding was that consumption of EPA and DHA in similar amounts, but in different carriers, to children with deviated attention performance for 3 mo resulted in different outcomes: in the incorporation in blood FAs, in executive functioning as assessed by computerized CPT, and in correlations between these effects. To the best of our knowledge, the randomized controlled trial presented herein is the first short-term intervention study with n−3 LC-PUFAs to show a correlation between these biochemical and cognitive function outcomes.

In the current study, supplementation with PL-n−3 and FO to children increased EPA, DPA, and DHA concentrations but decreased AA and adrenic acid concentrations in plasma PLs, but not in the triacylglycerol and cholesterol ester fractions. These findings agree with published observations (43), which showed that ≈60% of EPA and DHA from digested FO was selectively

TABLE 6
Spearman's rank-order correlations between changes in Test of Variables of Attention (TOVA) scores and changes in plasma and erythrocyte phospholipid fatty acid concentrations[1]

	Plasma phospholipids							Erythrocyte phospholipids						
	20:5n−3				22:6n−3			24:0				18:2n−6		
TOVA variables	Across 3 diets	PL-n−3	Fish oil	Placebo	Across 3 diets	PL-n−3	Fish oil	Placebo	Across 3 diets	PL-n−3	Fish oil	Placebo	Across 3 diets	PL-n−3	Fish oil	Placebo
Total TOVA score	0.140	−0.594[2]	0.163	−0.306	0.301[2]	−0.235	0.063	0.352	−0.033	0.587[2]	0.181	0.090	0.064	−0.749[3]	0.012	−0.082
Errors of omission	−0.002	−0.468[4]	0.271	−0.303	0.155	−0.506[2]	0.427[4]	0.189	−0.005	0.132	0.064	0.037	0.018	−0.259	0.044	−0.124
Errors of commission	0.008	−0.121	−0.184	−0.197	−0.035	−0.468[4]	−0.198	0.009	0.108	0.044	−0.079	0.347	−0.004	−0.137	0.235	−0.263
Total response time	−0.010	−0.368	0.341	−0.344	0.281[2]	−0.024	0.170	0.467[4]	−0.047	0.604[2]	0.266	−0.197	0.014	−0.577[2]	−0.234	0.160
Response time variability	−0.048	−0.412	−0.292	−0.396	0.113	−0.341	−0.183	0.357	0.102	0.676[2]	0.131	−0.044	−0.104	−0.813[3]	−0.089	−0.070

[1] $n = 14$–19 per group (total $n = 51$–53 for all treatments). PL-n−3, phospholipids enriched with n−3 fatty acids.
[2] $P < 0.05$.
[3] $P < 0.01$.
[4] $P < 0.1$.

incorporated into the PL fraction of plasma. Furthermore, it was shown previously (24, 44, 45) that n−3 LC-PUFA interventions in children increased the n−3 LC-PUFA and reduced the n−6 LC-PUFA concentrations in plasma PLs. These metabolic alterations could plausibly be associated with the balance in intake of n−3 and n−6 LC-PUFAs and the possible negative feedback mechanism on desaturase enzymes, such as Δ^6 (46) and Δ^5 (47) desaturase, which catalyze the conversion of both n−3 and n−6 from precursors to DHA and adrenic acid, respectively.

In erythrocytes, our findings suggest that PL-n−3 and FO had a limited effect on n−3 LC-PUFA concentrations, whereas the n−6 LC-PUFA concentrations were unchanged. These observations are inconsistent with previous work (29). The counterintuitive findings could be explained by the low n−3 LC-PUFA dose intervention, the different effect, and the kinetics of dietary EPA and DHA and/or the participants' metabolism. In the aforementioned study, 5-fold more DHA (480 mg/d) was provided to the putative ADHD children (Table 1), and n−6 LC-PUFA oils were added as well. Furthermore, the daily consumption of 1 or 0.2 g DHA by hyperlipidemic adults receiving statin therapy resulted in different kinetics of DHA in the plasma and erythrocytes (48). A new equilibrium was reached in the plasma within 1 mo, whereas in the erythrocytes it was observed only after 4–6 mo of supplementation. Moreover, consumption of the low-dose supplementation in that study was associated with a slightly slower incorporation rate for both of these blood compartments. Additionally, dietary EPA and DHA were suggested to affect FA metabolism differently (49). EPA or DHA supplementation may result in a distinct increase in the administered FAs, albeit via different kinetics, and with a minimal elevation of the counter fatty acid in the plasma PL fraction (48). Specifically, EPA and DHA interventions have shown that EPA accumulated faster than did DHA in plasma (50, 51), leukocytes (52), and erythrocytes (53, 54). Finally, early reports have associated ADHD children with symptoms of EFA deficiency and reduced blood concentrations of n−3 and n−6 LC-PUFAs, with no obvious lack of dietary intake when compared with control children (55). The underlying mechanism for this deficiency in ADHD subjects is still unclear. However, an increased breakdown of FAs via oxidation and/or a decrease in EFA metabolism could play, at least in part, a role in this pathophysiology. The latter was recently supported by preliminary findings associating a single nucleotide polymorphism in the EFA desaturase gene *FADS2* with ADHD (56). Our finding of an effect of PL-n−3 consumption on erythrocyte FA profile alterations, such as an increase in DPA rather than in DHA concentrations and a decrease in >C20 SFA and in LA concentrations, might be associated with the impaired FA metabolism of these children.

PL-n−3 consumption induced an increase of the total TOVA score of 94% or 3.35 SD (from −4.53 to −1.18 SD), from baseline. Changes in this score that exceeded 80% or 2.5 SD were previously reported for stimulants (57, 58). Introducing similar amounts of EPA+DHA via FO also had significant effect of 1.72 SD or 37% (from −5.69 SD to −3.97 SD), although to a limited extent (Figure 2). Our findings differ from previous reports in which DHA (345 mg/d) (24) or EPA+DHA (100 + 514 mg/d) (25) supplementation in ADHD children for 2–4 mo was shown to result in elevated plasma PL DHA concentrations and in no effect on the CPT scores. Likewise, in young patients with recurrent self-harm, the 12-wk administration of EPA+DHA (1220 + 908 mg/d) failed to affect impulsivity as assessed by computerized CPT (9). One possibility for resolving this discrepancy might be the use of different n−3 LC-PUFA carriers and/or food matrices. Earlier reports (*see* review in reference 59) suggest that PS affects memory, learning, and behavior. These observations were mainly associated with the stimulation of the cholinergic system. Few studies have suggested that the cholinergic system may also be involved in the pathophysiology of ADHD (60). Interestingly, nicotinic stimulation was shown to affect executive functioning, specifically sustained attention, and working memory in CPT. A second possibility could be the different n−3 LC-PUFA proportions used in the interventions. Recent reports favored EPA rather than DHA (EPA/DHA ratios ≈3; range: 1.2–6.8) for improving behavior in children with neurodegenerative or neurodevelopmental disorders (26, 28, 44, 45, 61, 62). Our n−3 LC-PUFA–containing interventions were characterized by a high EPA/DHA ratio (≈1.6). Moreover, the noted effectiveness was attained while providing a considerably modest daily dose of EPA+DHA (250 mg compared with ≈1200 mg/d; range: 345–4300 mg/d). Taken together, our findings suggest that providing preparations with a high EPA/DHA ratio could affect visual sustained attention performance, even at subgram amounts, in pediatric populations.

In the PL-n−3 group, plasma and erythrocyte PL FA alterations were correlated with changes in the TOVA indexes. Elevated concentrations of n−3 LC-PUFAs in blood membranes were previously shown to correlate with modest improvement in behavioral assessments in children (29, 44, 45, 63). Similarly, infants consuming n−3 LC-PUFAs showed a lower visual evoked potential latency that was correlated with an increase in DHA in erythrocyte lipids (44, 64). Interestingly, in our study the highest Spearman's rho value was calculated for the changes in erythrocyte LA concentrations after PL-n−3 intervention (Table 6). This finding could, at least in part, be associated with the induced cellular fatty acid metabolism alterations observed after n−3 LC-PUFA consumption. This may be associated with the difference in n−3 LC-PUFA carriers, because it was previously suggested that PL-n−3 is a better vehicle for n−3 LC-PUFA accretion (65, 66).

In conclusion, our findings indicate that providing n−3 LC-PUFAs esterified to PLs or triacylglycerols for 3 mo to children with impaired visual sustained attention performance results in different FA profiles in blood fractions and affects their executive functions differently. Moreover, the blood lipid alterations were correlated with the changes in the CPT scores, mainly in the PL-n−3 group. A better understanding of the physiology of n−3 LC-PUFAs and the role of their carriers throughout the metabolic process in children with n−3 LC-PUFA deficiency could place the results of the current investigation in clearer context. In the meantime, these observations could assist in planning future trials of the role of n−3 LC-PUFA carriers in psychiatric as well as in healthy pediatric populations.

We thank Doron Comaneshter (Statistics Services Unit, Tel Aviv Sourasky Medical Center) for help with the statistical analyses and Esther Eshkol for editorial assistance.

The authors' responsibilities were as follows—NV (Principal Investigator): designed the trial; NV, DP, GB, GZ, and JB: interpreted the data and wrote the manuscript; YZ-A: coordinated the study, responsible for patient recruitment and management, and assisted with the blood work; JB and GB: performed the laboratory analyses; and NK: performed the TOVA analyses and interpreted the corresponding data. All authors critically revised the manuscript and approved the final report. NV is a consultant at Enzymotec

LTD and DP is the Director of Clinical Studies at Enzymotec LTD. The other authors had no personal or financial conflict of interest.

REFERENCES

1. Benatti P, Peluso G, Nicolai R, Calvani M. Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties. J Am Coll Nutr 2004; 23:281–302.
2. Alessandri JM, Guesnet P, Vancassel S, et al. Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life. Reprod Nutr Dev 2004;44:509–38.
3. Horrobin DF. Fatty acid metabolism in health and disease: the role of delta-6-desaturase. Am J Clin Nutr 1993;57(suppl):732S–6S, discussion 736S–7S.
4. Hibbeln JR. Fish consumption and major depression. Lancet 1998;351: 1213.
5. Noaghiul S, Hibbeln JR. Cross-national comparisons of seafood consumption and rates of bipolar disorders. Am J Psychiatry 2003;160: 2222–7.
6. Hibbeln JR, Ferguson TA, Blasbalg TL. Omega-3 fatty acid deficiencies in neurodevelopment, aggression and autonomic dysregulation: opportunities for intervention. Int Rev Psychiatry 2006;18:107–18.
7. Hibbeln JR, Salem N Jr. Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. Am J Clin Nutr 1995;62:1–9.
8. Young G, Conquer J. Omega-3 fatty acids and neuropsychiatric disorders. Reprod Nutr Dev 2005;45:1–28.
9. Hallahan B, Hibbeln JR, Davis JM, Garland MR. Omega-3 fatty acid supplementation in patients with recurrent self-harm: Single-centre double-blind randomised controlled trial. Br J Psychiatry 2007;190: 118–22.
10. Stoll AL, Severus WE, Freeman MP, et al. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 1999;56:407–12.
11. Su KP, Huang SY, Chiu CC, Shen WW. Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. Eur Neuropsychopharmacol 2003;13:267–71.
12. Zanarini MC, Frankenburg FR. Omega-3 Fatty acid treatment of women with borderline personality disorder: a double-blind, placebo-controlled pilot study. Am J Psychiatry 2003;160:167–9.
13. Zanotti A, Valzelli L, Toffano G. Chronic phosphatidylserine treatment improves spatial memory and passive avoidance in aged rats. Psychopharmacology (Berl) 1989;99:316–21.
14. Drago F, Canonico PL, Scapagnini U. Behavioral effects of phosphatidylserine in aged rats. Neurobiol Aging 1981;2:209–13.
15. Castilho JC, Perry JC, Andreatini R, Vital MA. Phosphatidylserine: an antidepressive or a cognitive enhancer? Prog Neuropsychopharmacol Biol Psychiatry 2004;28:731–8.
16. Crook TH, Tinklenberg J, Yesavage J, Petrie W, Nunzi MG, Massari DC. Effects of phosphatidylserine in age-associated memory impairment. Neurology 1991;41:644–9.
17. Maggioni M, Picotti GB, Bondiolotti GP, et al. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. Acta Psychiatr Scand 1990;81:265–70.
18. Mitchell EA, Aman MG, Turbott SH, Manku M. Clinical characteristics and serum essential fatty acid levels in hyperactive children. Clin Pediatr (Phila) 1987;26:406–11.
19. Stevens LJ, Zentall SS, Deck JL, et al. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 1995; 62:761–8.
20. Chen JR, Hsu SF, Hsu CD, Hwang LH, Yang SC. Dietary patterns and blood fatty acid composition in children with attention-deficit hyperactivity disorder in Taiwan. J Nutr Biochem 2004;15:467–72.
21. Young GS, Maharaj NJ, Conquer JA. Blood phospholipid fatty acid analysis of adults with and without attention deficit/hyperactivity disorder. Lipids 2004;39:117–23.
22. Richardson AJ. Long-chain polyunsaturated fatty acids in childhood developmental and psychiatric disorders. Lipids 2004;39:1215–22.
23. Vancassel S, Durand G, Barthelemy C, et al. Plasma fatty acid levels in autistic children. Prostaglandins Leukot Essent Fatty Acids 2001;65: 1–7.
24. 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.
25. 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.
26. 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.
27. 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.
28. Sinn N, Bryan J. Effect of supplementation with polyunsaturated fatty acids and micronutrients on learning and behavior problems associated with child ADHD. J Dev Behav Pediatr 2007;28:82–91.
29. Stevens L, Zhang W, Peck L, et al. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 2003; 38:1007–21.
30. Carnielli VP, Verlato G, Pederzini F, et al. Intestinal absorption of long-chain polyunsaturated fatty acids in preterm infants fed breast milk or formula. Am J Clin Nutr 1998;67:97–103.
31. Amate L, Gil A, Ramirez M. Feeding infant piglets formula with long-chain polyunsaturated fatty acids as triacylglycerols or phospholipids influences the distribution of these fatty acids in plasma lipoprotein fractions. J Nutr 2001;131:1250–5.
32. Mathews SA, Oliver WT, Phillips OT, Odle J, Diersen-Schade DA, Harrell RJ. Comparison of triglycerides and phospholipids as supplemental sources of dietary long-chain polyunsaturated fatty acids in piglets. J Nutr 2002;132:3081–9.
33. Wijendran V, Huang MC, Diau GY, Boehm G, Nathanielsz PW, Brenna JT. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. Pediatr Res 2002;51:265–72.
34. Greenberg LM, Waldman ID. Developmental normative data on the test of variables of attention (T.O.V.A.). J Child Psychol Psychiatry 1993; 34:1019–30.
35. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226: 497–509.
36. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem 1959;234:466–8.
37. Bodennec J, Koul O, Aguado I, Brichon G, Zwingelstein G, Portoukalian J. A procedure for fractionation of sphingolipid classes by solid-phase extraction on aminopropyl cartridges. J Lipid Res 2000;41:1524–31.
38. Dittmer JC, Lester RL. A simple, specific spray for the detection of phospholipids on thin-layer chromatograms J Lipid Res 1964;15:126–7.
39. Guglielmo CG, Williams TD, Zwingelstein G, Brichon G, Weber JM. Plasma and muscle phospholipids are involved in the metabolic response to long-distance migration in a shorebird. J Comp Physiol [B] 2002;172: 409–17.
40. Conners CK. Conners' Parent Rating Scales-Revised: Short Form (CPRS-R:S). North Tonawanda, NY: Multi-Health Systems, Inc, 1997.
41. Achenbach TM, Ruffle TM. The Child Behavior Checklist and related forms for assessing behavioral/emotional problems and competencies. Pediatr Rev 2000;21:265–71.
42. Bruni A, Bellini F, Mietto L, Boarato E, Viola G. Phospholipid absorption and diffusion through membranes. In: Hannin I, Pepeu G, eds. Phospholipids: biochemical, pharmaceutical, and analytical considerations. New York, NY: Plenum Press, 1990:59–68.
43. Sadou H, Leger CL, Descomps B, Barjon JN, Monnier L, Crastes de Paulet A. Differential incorporation of fish-oil eicosapentaenoate and docosahexaenoate into lipids of lipoprotein fractions as related to their glyceryl esterification: a short-term (postprandial) and long-term study in healthy humans. Am J Clin Nutr 1995;62:1193–200.
44. Beblo S, Reinhardt H, Demmelmair H, Muntau AC, Koletzko B. Effect of fish oil supplementation on fatty acid status, coordination, and fine motor skills in children with phenylketonuria. J Pediatr 2007;150:479–84.
45. Wozniak J, Biederman J, Mick E, et al. Omega-3 fatty acid monotherapy for pediatric bipolar disorder: a prospective open-label trial. Eur Neuropsychopharmacol 2007;17:440–7.
46. Garg ML, Sebokova E, Thomson AB, Clandinin MT. Delta 6-desaturase

activity in liver microsomes of rats fed diets enriched with cholesterol and/or omega 3 fatty acids. Biochem J 1988;249:351–6.
47. Garg ML, Thomson AB, Clandinin MT. Effect of dietary cholesterol and/or omega 3 fatty acids on lipid composition and delta 5-desaturase activity of rat liver microsomes. J Nutr 1988;118:661–8.
48. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n−3 fatty acids in humans. Am J Clin Nutr 2006;83(suppl): 1467S–76S.
49. Grimsgaard S, Bonaa KH, Hansen JB, Nordoy A. Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. Am J Clin Nutr 1997;66:649–59.
50. Marangoni F, Angeli MT, Colli S, et al. Changes of n−3 and n−6 fatty acids in plasma and circulating cells of normal subjects, after prolonged administration of 20:5 (EPA) and 22:6 (DHA) ethyl esters and prolonged washout. Biochim Biophys Acta 1993;1210:55–62.
51. Subbaiah PV, Kaufman D, Bagdade JD. Incorporation of dietary n−3 fatty acids into molecular species of phosphatidyl choline and cholesteryl ester in normal human plasma. Am J Clin Nutr 1993;58:360–8.
52. Terano T, Hirai A, Tamura Y, Kumagai A, Yoshida S. Effect of dietary supplementation of highly purified eicosapentaenoic acid and docosahexaenoic acid on arachidonic acid metabolism in leukocytes and leukocyte function in healthy volunteers. Adv Prostaglandin Thromboxane Leukot Res 1987;17B:880–5.
53. Hamazaki K, Itomura M, Huan M, et al. Effect of omega-3 fatty acid-containing phospholipids on blood catecholamine concentrations in healthy volunteers: a randomized, placebo-controlled, double-blind trial. Nutrition 2005;21:705–10.
54. Kamada T, Yamashita T, Baba Y, et al. Dietary sardine oil increases erythrocyte membrane fluidity in diabetic patients. Diabetes 1986;35: 604–11.
55. Antalis CJ, Stevens LJ, Campbell M, Pazdro R, Ericson K, Burgess JR. Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. Prostaglandins Leukot Essent Fatty Acids 2006;75:299–308.
56. Brookes KJ, Chen W, Xu X, Taylor E, Asherson P. Association of fatty acid desaturase genes with attention-deficit/hyperactivity disorder. Biol Psychiatry 2006;60:1053–61.
57. Alhambra MA, Fowler TP, Alhambra AA. EEG biofeedback: a new treatment option for ADD/ADHD. J Neurotherapy 1995;1:39–43.
58. Rugino TA, Copley TC. Effects of modafinil in children with attention-deficit/hyperactivity disorder: an open-label study. J Am Acad Child Adolesc Psychiatry 2001;40:230–5.
59. Pepeu G, Pepeu IM, Amaducci L. A review of phosphatidylserine pharmacological and clinical effects. Is phosphatidylserine a drug for the ageing brain? Pharmacol Res 1996;33:73–80.
60. Potter AS, Newhouse PA, Bucci DJ. Central nicotinic cholinergic systems: a role in the cognitive dysfunction in attention-deficit/hyperactivity disorder? Behav Brain Res 2006;175:201–11.
61. Nemets H, Nemets B, Apter A, Bracha Z, Belmaker RH. Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. Am J Psychiatry 2006;163:1098–100.
62. Amminger GP, Berger GE, Schafer MR, Klier C, Friedrich MH, Feucht M. Omega-3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study. Biol Psychiatry 2007;61:551–3.
63. Itomura M, Hamazaki K, Sawazaki S, et al. The effect of fish oil on physical aggression in schoolchildren–a randomized, double-blind, placebo-controlled trial. J Nutr Biochem 2005;16:163–71.
64. Hoffman DR, Theuer RC, Castaneda YS, et al. Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. J Nutr 2004;134:2307–13.
65. Brossard N, Croset M, Normand S, et al. Human plasma albumin transports [13C]docosahexaenoic acid in two lipid forms to blood cells. J Lipid Res 1997;38:1571–82.
66. Lemaitre-Delaunay D, Pachiaudi C, Laville M, Pousin J, Armstrong M, Lagarde M. Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [(13)C]DHA in phosphatidylcholine. J Lipid Res 1999;40:1867–74.

The American Journal of Clinical Nutrition