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TITLE: Increased erythrocyte eicosapentaenoic acid and docosahexaenoic acid are associated with improved attention and behaviour in children with ADHD in a 12-month randomised controlled three-way crossover trial

Abstract

Objective: To investigate effects of omega-3 fatty acids (n-3 PUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on attention, literacy and behaviour in children with ADHD. **Method:** Ninety children were randomised to consume supplements high in EPA, DHA or linoleic acid (control) for four months each in a cross-over design. Erythrocyte fatty acids, attention, cognition, literacy and Conners' Parent Rating Scales were measured at 0, 4, 8, 12 months. **Results:** Fifty three children completed. Outcome measures showed no significant differences between the 3 treatments. However, in children with blood samples (n=76-46), increased erythrocyte EPA+DHA was associated with improved spelling (r=.365, p<.001) and attention (r=-.540, p<.001) and reduced oppositional behaviour (r=-.301, p.003), hyperactivity (r=-.310, p<.001), cognitive problems (r=-.326, p<.001), DSM-IV hyperactivity (r=-.270, p=.002) and DSM-IV inattention (r=-.343, p<.001). **Conclusion:** Increasing erythrocyte DHA and EPA via dietary supplementation may improve behaviour, attention and literacy in children with ADHD.

Introduction

Many children experience difficulties with learning and behaviour. When problems with attention, hyperactivity and impulsivity interfere significantly with school and home life this may result in a diagnosis of attention deficit hyperactivity disorder (ADHD). The worldwide prevalence of ADHD is estimated at 5% although there is significant variability between countries that may be attributed to differences in methodology between studies, such as diagnostic criteria (Polanczyk et al., 2007). There is often an overlap between ADHD symptoms and other disorders, such as conduct and/or mood disorders (Root & Resnick, 2003) and learning disorders (Mayes et al., 2000), with at least a quarter of children with ADHD having a comorbid learning disorder (Mayes et al., 2000).

The etiology of ADHD and comorbid disorders has both genetic and environmental components. Among these, there is evidence for a role of nutrition and diet (Sinn, 2008). In particular, omega-3 polyunsaturated fatty acids (n-3 PUFA) have received increasing attention for their role in mental health since the discovery of the high concentration of the long-chain n-3 PUFA, docosahexaenoic acid (DHA) in the mammalian brain (Crawford & Sinclair, 1971; Sinclair & Crawford, 1972), and its role in a range of important related functions in the brain including increased membrane fluidity and neurotransmission (Haag, 2003). Eicosapentaenoic acid (EPA) is a long-chain n-3 PUFA precursor to DHA and has important roles including the production of prostaglandins. However its role in brain function is unclear as its concentration in the brain is much lower than DHA.

n-3 PUFA are essential fatty acids that must be obtained in the diet, and yet their consumption, primarily due to a reduced intake of dark leafy vegetables, nuts, seeds and oily fish, has declined in modern diets. Concurrently, consumption of omega-6 (n-6) PUFA, from vegetable oils and processed foods, has increased. This has resulted in an estimated altered ratio of n-6:n-3 intake from 1:1 in traditional diets to 15-16:1 in modern diets (Simopoulos,

2002). n-3 PUFA have a variety of roles in body and brain function, including anti-inflammatory, anti-thrombotic and vasodilatory functions whereas n-6 PUFA increase inflammation, thrombosis and vasoconstriction. Hence an altered ratio in favour of n-6 is likely to result in increased inflammation and reduced blood flow which may occur not only peripherally, but also in the brain and thereby potentially impact adversely on mental health (McAfoose & Baune, 2009; Sinn & Howe, 2008).

A growing body of studies has investigated effects of n-3 PUFA on mental illness (Sinn et al., 2010), including effects on learning and behaviour problems associated with ADHD in children (Hirayama et al., 2004; Johnson et al., 2008; Milte et al., 2012; Richardson & Montgomery, 2005; Sinn & Bryan, 2007; Sinn et al., 2008; Stevens et al., 2003; Voigt et al., 2001). Overall these studies have found significant improvements in learning and behaviour with an increased intake of n-3 PUFA, and it may be concluded from the body of work that EPA is more effective than DHA (Bloch & Qawasmi, 2011). However there are methodological issues that may account for the apparent benefit of EPA over DHA. For instance, while two studies used pure DHA and showed no benefit for learning and behaviour (Hirayama et al., 2004; Voigt et al., 2001), Hirayama et al. (2004) conducted their study in Japanese children who have one of the highest intakes of fish and are therefore less likely to have low levels of n-3 PUFA; Voigt et al. (2001) recruited boys who were taking stimulant medication, and accordingly t-scores of parent ratings at baseline were already in the normal range, thereby limiting the potential for improvement to be detected. Studies that have found positive effects of n-3 PUFA used fish oil with a naturally occurring high ratio of EPA to DHA (3:1); however the positive outcomes may be attributable, at least to some degree, to the DHA component of the supplement, and also to methodological attributes in those studies such as sufficient dosage, medication-naïve samples and duration of supplementation over 12 weeks or more.

Another interesting observation from these studies is the range of diagnoses that were included. In Richardson and Montgomery (2005), children with Developmental Coordination Disorder were recruited, of which a third had ADHD symptoms in the clinical range and the whole sample were on average a year behind in reading and spelling. This study found highly significant improvements on teacher ratings of attention and behaviour on Conners' Teacher Rating Scales and reading and spelling. Johnson et al (2008) reported the strongest responders, in their sample of children and adolescents diagnosed with ADHD, in the subgroup with learning disorders.

We therefore recruited children with ADHD symptoms and parent-reported learning difficulties to investigate the relative efficacy of EPA versus DHA for improving behavioural and learning outcomes, and measured erythrocyte (red blood cell) membrane fatty acids. As reported previously, in baseline regression analyses for participants who provided blood samples (*N*=75) we found that higher n-3 PUFA predicted lower anxiety/shyness, higher DHA predicted better word reading, and higher n-6 PUFA levels predicted poorer reading, vocabulary, spelling and attention. We discovered following reading and spelling assessments that 36% were behind their age level in both, whom we classified as having learning difficulties. Comparisons between those with and without learning difficulties showed that the former had significantly lower levels of erythrocyte DHA (Milte et al., 2011a). We also reported previously that following four months of supplementation with fish oil high in EPA or DHA or safflower oil control (containing n-6 PUFA linoleic acid; LA), we found no significant differences in outcome measures between these groups; however, in those with blood samples, increased erythrocyte DHA was associated with improved word reading and lower parent ratings of oppositional behaviour. In the subgroup with learning difficulties (*N=17*), these effects were stronger: increased DHA was associated with improved word

reading, spelling, attention and lower parental ratings of oppositional behaviour, hyperactivity, restlessness and overall ADHD symptoms (Milte et al., 2012).

The latter results were reported from the first four months of the present 12-month study, which is the focus of this paper. This study aimed to investigate the effects of supplementation with EPA-rich or DHA-rich fish oil versus LA-rich safflower oil on behaviour, attention and literacy over 12 months in a randomised three-way crossover trial. Relationships between erythrocyte PUFA status and outcome measures over each phase of the trial were also investigated.

Methods

A 12-month randomised controlled 3-way crossover trial in children aged 6-13 with ADHD symptoms was conducted in Adelaide and Brisbane, Australia, approved by the Human Research Ethics Committees of the University of South Australia and Queensland University of Technology. The trial 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 >90th percentile on the Conners' parent rating scale (CPRS) (Conners, 2000) and parent-reported learning difficulties (described as literacy performance behind their year level at school). Children were excluded if they had consumed n-3 PUFA supplements during the 3 months prior to the study or were taking any ADHD medication. Required sample size, to provide >80% power to detect a medium effect size (Cohen, 1992) while allowing for a 30% drop-out rate, was estimated at 120 participants.

Procedure

All recruitment and assessments occurred between June 2007 and 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. A total of 199 children were assessed for eligibility after responses to the calls for volunteers. Information sheets and consent forms were sent to interested parents and guardians of 115 children who were deemed eligible though a brief screening interview over the phone. 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 children were rated >90th percentile (Conners, 2000). The study was explained to both children and their parent(s) or guardian and written informed consent was obtained.

Whilst ninety six children were recruited to the study, six children withdrew before completion of baseline assessment, leaving a total of 90 study volunteers. Children were independently allocated to one of three treatment conditions using the process of randomisation by minimisation (Altman & Bland, 2005) on the basis of age and gender. Each condition received the two omega-3 rich oils (EPA-rich and DHA-rich) and control oil (LA-rich) for 4 months each, so the three treatment conditions comprised EPA-DHA-LA, DHA-LA-EPA or LA-EPA-DHA. The period of 4 months was chosen for each supplementation period as erythrocyte PUFA concentration is reported to reach a steady state after 4-6 months (Arterburn et al., 2006). Furthermore, erythrocyte PUFA levels reportedly return to baseline 16 weeks following cessation of supplementation (Cao et al., 2006). Therefore, no washout period was included as it was assumed that washout of the previous treatment would have occurred during the following supplementation period. Study investigators involved in recruitment and data collection, parents and children were blinded to the randomisation until completion of 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, 8 and 12 months. Capsule containers were returned by

parents at each visit for compliance assessment by capsule counting. At each visit, fasted blood samples were collected into 6 ml EDTA tubes by venepuncture. After the blood sample, children were given a small snack (toast and fruit juice or water) and underwent 30-45 minutes 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. Between each visit, parents were contacted via phone or email at least three times (at 4, 10 and 15 weeks) by researchers to reduce dropouts, informally check compliance and report any adverse events.

Supplements

Based on previous reports of improved symptoms in children following supplementation with 750mg total long chain *n*-3 PUFAs/day (Richardson & Montgomery, 2005; Sinn & Bryan, 2007) and considering that compliance is variable in children, we chose to supplement with at least 1g long-chain *n*-3 PUFA/day to achieve an optimal response. Participants consumed 4 x 500mg capsules per day containing the following daily dose during the respective treatment conditions: EPA-rich fish oil, providing a total of 1109 mg EPA and 108 mg DHA; DHA-rich fish oil, providing 264 mg EPA and 1032 mg DHA; or safflower oil (control), providing 1467 mg LA per day. All oils were stabilised with a low concentration of Vitamin E. Capsules were provided by Novasel Australia.

Assessment tools

Primary Outcomes: Literacy and Behaviour

Literacy was assessed using the word reading and spelling subtests from the WIAT-III (Wechsler, 1992). Performance on the vocabulary subtest from the WISC-III (Wechsler, 1991) was also assessed. Raw scores were converted to age-scaled scores. Parent ratings of ADHD symptoms were assessed by completion of the CPRS – long version (Conners, 2000).

Secondary Outcomes: Attention and Inhibition

Different forms of attention were assessed as a secondary outcome measure using an abbreviated test battery from the TEA-ch (Manly et al., 1999). Focussed 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 two they are timed while they circle as many as they can without distracters. The second time is subtracted from the first time to eliminate 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, 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 *Score!* tasks.

Inhibition, or the ability to hold back a response, was assessed using a computerised *Go/No-go* task (Trommer et al., 1988). 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. Number of errors was calculated as a measure of response inhibition.

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

Assessment of fatty acid profiles

Relative proportions of individual fatty acids in erythrocyte phospholipids were assessed as described previously (Milte et al., 2011b). Erythrocytes were isolated within 2 hours of collection by centrifugation, washed in isotonic saline and stored at -80 °C. Erythrocytes were thawed and the lipids extracted and transesterified. The resultant fatty acid

methyl esters were separated and quantified using a Shimadzu 2010 gas chromatograph equipped with a 50m capillary column (0.32 mm ID) coated with BPX-70 (0.25 µm film thickness, SGE Pty Ltd. Victoria, Australia). Retention times were compared to those of authentic lipid standards (GLC-463, Nu-Chek Prep Inc. Elysian, MN, USA) for identification.

Statistical Assessment

Data analysis was conducted using SPSS Statistics (version 17.0, SPSS Inc., Chicago, IL, USA). Effects of EPA and DHA versus LA (control) supplementation on outcome variables were investigated using linear mixed modelling. Relationships between erythrocyte PUFA status over the 12 months and literacy, cognition and behaviour were assessed using within-subject regression. Erythrocyte EPA, DHA, EPA+DHA, total *n*-3 PUFA, total *n*-6 PUFA and *n*-6/*n*-3 ratio at baseline and after EPA, DHA and LA supplementation were investigated. Each PUFA was entered as a predictor variable into an individual regression analysis (e.g. DHA vs. word reading score, total n-3 PUFA vs. word reading score, etc.). Within-subject regression analysis was repeated for each PUFA and outcome variable. A modified Bonferroni adjustment was used to correct for multiple comparisons.

These two statistical techniques were selected as they allowed volunteers with missing data or dropouts to be included in the analysis. The significance level was set at $P < 0.05$. The pattern of missing data was determined using Little's MCAR test (1988).

Results

Descriptive statistics

Of the 90 children aged 6-13 years who commenced the study (52 at University of South Australia; 38 at Queensland University of Technology), three were excluded from analysis and are not reported further due to lack of ADHD diagnosis or symptom severity scores below the 90th percentile on Conners' global scale. Blood samples were obtained from

75 volunteers at baseline. There was no difference in ratings of ADHD symptom severity on the Conners' scale between those who provided blood samples and those who did not. Forty three children (50%) had an official, parent-reported diagnosis of ADHD. There were no differences in ratings of ADHD symptom severity between those with and without an official diagnosis. Seventy percent had word reading scores below their age level, 79% performed behind their age level in spelling and 91% in vocabulary.

See Table 1 for an outline of further demographic variables at baseline. Further details regarding the sample have been reported elsewhere (Milte et al., 2012).

Adverse Events

Twelve instances of minor adverse events were reported over the 12 months. During the LA (control) treatment, one volunteer reported bad breath and one reported gastrointestinal symptoms. During the EPA treatment one volunteer reported itchy skin and two each reported bad breath and gastrointestinal symptoms. Five children during the DHA treatment each reported one of the following: gastrointestinal symptoms, unpleasant taste, nose bleed, skin rash and yellow teeth.

Blinding

Parents were asked at the end of each treatment condition whether they thought children had received an active or placebo treatment during the previous four months, and the reason. Overall, around 40% of completers correctly guessed whether they were taking an active or placebo supplement (41% on EPA, 48% on DHA and 38% for LA). The primary reason volunteers reported correctly guessing which supplement they were on was they were/were not deriving benefits from the respective treatment. Whilst taking EPA and DHA, only two volunteers each correctly guessed the supplement they were taking due to a reported "fishy" taste, therefore treatment blinding was considered successful.

Completers and compliance

A total of 33 children (37%) discontinued the intervention over the 12 months (Figure 1). Data were available for fifty-six children after consuming EPA, 54 after DHA and 57 after LA. Mean (SD) compliance was 83 (17), 86 (14) and 85 (17) percent by capsule counts for EPA, DHA and LA respectively. However there was a large variation in compliance (20% - 100%) over the study. Blood samples were obtained for 50 children after EPA supplementation, 45 after DHA supplementation and 46 after LA supplementation and were analysed for erythrocyte PUFA content. Mean changes in erythrocyte n-3 and n-6 PUFAs reflected the expected changes due to the supplement in each respective phase (Table 2). However it can be seen from this table that after the LA control condition, blood n-3 PUFA levels did not go completely back to their baseline values.

Primary Analysis

There were 58 missing observations in the demographic data and 25 missing observations in the baseline data due to incomplete questionnaires or inability to complete the cognitive test. Birth weight had the most missing values (43%). In the follow up data there were 2632 missing observations; a mean of 35 missing observations per variable. The majority of missing data was due to dropouts and the remainder due to incomplete questionnaires or inability to complete cognitive tests. Analysis using Little's MCAR test showed data were likely to be missing completely at random. The application of a linear multilevel model was therefore appropriate for this missing pattern. Analysis of treatment effects on outcome variables between the three groups was conducted using unstructured linear mixed effects modelling and therefore taking all 87 cases into consideration. There were no significant treatment effects for literacy, cognition or parent-reported behaviour (Table 3).

Associations between changes in erythrocyte PUFA and changes in outcome variables

Within-subject regression analysis was used to investigate relationships between erythrocyte PUFA status and outcome measures at baseline and after supplementation with EPA, DHA and LA. The PUFAs EPA, DHA, EPA+DHA, total n-3 PUFA, total n-6 PUFA and n-6:n-3 ratio were investigated (Tables 4 and 5). Within-subject changes in erythrocyte PUFA were associated with improvements in literacy, attention and behaviour. Particularly, increases in erythrocyte EPA, DHA and total omega-3 PUFA and decreases in erythrocyte n-6 and the n-6:n-3 ratio were associated with improvements. EPA+DHA (i.e. the Omega-3 Index (Harris & von Schacky, 2004)) and n-6:n-3 PUFA ratio gave the most consistent correlations. Outcome measures most commonly improved included overall literacy (word reading and spelling) attention (Sky Search and Creature Counting) and subscales of parent-rated behaviour through the CPRS.

Discussion

This 12-month three-way crossover trial compared the effects of supplementation with the long-chain n-3 PUFAs EPA and DHA with the n-6 PUFA LA on literacy, attention and behaviour in children with ADHD with and without learning difficulties. We found no differences in treatment effects between groups. However when we investigated via regression analysis correlations between increased erythrocyte levels of n-3 and n-6 PUFAs and outcome measures we found a number of significant associations: i.e. increased levels of n-3 PUFAs (most consistently EPA+DHA together) were associated with improved literacy, attention and parent-rated behaviour while there were negative associations between increased n-6 PUFA levels and various outcome variables. Accordingly, reduction in the ratio of n-6:n-3 was a consistent predictor of improved outcomes.

When we had analysed the first 4-months as a parallel design (Milte et al., 2012), we similarly found no significant differences between groups and yet there were significant correlations between increased erythrocyte DHA and improved literacy and parent ratings of

oppositional behaviour – and correlations were stronger and more prevalent in the subgroup of children with learning difficulties. It is likely that the lack of treatment effects between groups over both the first 4 months and following each treatment after 12 months can be attributed in part to a lack of statistical power to detect outcomes as a result of considerable variability in supplement compliance. It is also possible that we did not achieve the washout effect that we had anticipated following each treatment – in particular we noted that DHA levels did not go back to baseline after supplementation with DHA was ceased. As DHA in particular is incorporated into neural membranes it is likely to be retained for some time, and any possible anti-inflammatory effects of eicosanoids from EPA in the brain similarly may have prolonged its effects. Furthermore, it is possible that if children experience improvements in learning or behaviour following optimisation of n-3 levels (and the n-6:n-3 ratio), a degree of that learning may be retained after cessation of supplementation. Another potential bias that could have arisen with the crossover design is that the order of the three treatments could have interacted with any carryover effects given that only three out of six possible orders was used. Fortunately the taking of blood samples enabled us to employ a regression design to assess relationships between blood PUFA levels and outcome measures at each time point.

This study highlights the importance of confirming intervention results with blood samples, as these can confirm and account for degree of compliance (both with treatment and with placebo) and variability in baseline nutrient levels. It is noteworthy that Schoenthaler et al. (1997), for instance, found that multivitamin/mineral supplementation in habitually violent juvenile offenders had no effect on violent acts in those in the treatment group whose blood concentration of vitamins and minerals did not change during the study, but they found highly significant reductions in violent acts in participants whose blood concentrations of vitamins and minerals were corrected during the study.

This study was limited by a small sample size because we were unable to meet our recruitment target (i.e. recruited 90 out of the 120 that the study was powered for, and had a 37% drop-out rate). Despite 12 months of active recruitment at two sites it proved difficult to find eligible children with ADHD or symptoms in the clinical range who also had learning difficulties, were not on stimulant medication and were willing to have blood samples taken. We did not end up with a whole sample of children with learning difficulties (defined as being behind their age level in reading and spelling) although this provided interesting comparisons in our baseline and four month data as outlined earlier. We were unable to make those comparisons in these 12 month analyses due to small numbers.

Although not all children had a formal diagnosis of ADHD, they all had symptoms in the clinical range and there was no difference in severity of symptoms on parent rated questionnaires between those with and without a diagnosis. These results may be generalisable to children with an ADHD diagnosis and/or with symptoms in the clinical range, although it should be noted that we did not undertake our own assessment of ADHD symptoms via DSM criteria, which could be a limitation. Our four month results suggest, in support of previous research (Johnson et al., 2008; Richardson & Montgomery, 2005), that children with comorbid learning difficulties may be most likely responders and this should be investigated further. Interestingly, a recent study (Richardson et al., 2012) reported significant improvements following DHA supplementation in reading, cognition and behaviour in children from a general school population whose reading performance was in the lowest 20th percentile so this body of work would benefit from further investigation in populations of children who have learning difficulties, whether or not they have a diagnosis of ADHD.

The meta-analysis by Bloch (2011) concluded that positive results of omega-3 fatty acid supplementation in children with ADHD were attributable to EPA. However this could

be attributed to the fact that a number of the studies that were included used a supplement with a naturally occurring higher amount of EPA than DHA – but still contained significant levels of DHA. Blood samples were not taken and therefore it is not clear from those studies whether effects were attributable to EPA or DHA. Importantly, we note that the total erythrocyte content of EPA+DHA in our study was larger after DHA than after EPA even though the total EPA+DHA capsule content of the two conditions was about the same. DHA is incorporated directly, whereas EPA undergoes conversion via DPA to DHA and some would have remained as DPA; this could account for the difference. Therefore, in studies that report effects with EPA, we cannot rule out the possibility that the effects may have been due to DHA formed from EPA. However, our regression analysis relates effects to actual levels of EPA and DHA at each time point.

Conclusion

Increasing erythrocyte DHA and EPA via increased dietary intake of *n*-3 PUFA and decreasing intake of *n*-6 PUFA (i.e. reducing the ratio of *n*-6:*n*-3 PUFA) may improve behaviour, attention and literacy in children with ADHD symptoms. This study is the first to directly compare effects of EPA and DHA in ADHD using erythrocyte PUFA analysis to correlate efficacy with incorporation of individual fatty acids. Despite previous suggestions that EPA is superior to DHA, the body of results from this study suggest that DHA is an important contributor to benefits seen.

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Table 1: Baseline descriptive statistics (n=87)

Age	8.91 (1.729)
Male, n (%)	67 (77)
Birth weight (kg)	5.30 (2.34)
Parent-rated health[a]	4.11 (0.81)
Number of weeks breastfed	21.7 (28.5)
Length of gestation (weeks)	39.2 (3.27)
Level of primary parent's education, n (%)	
Grade 10	26 (29.9)
SACE/Matriculation	17 (19.5)
TAFE certificate	19 (21.8)
University diploma/degree	20 (22.9)
Postgraduate degree	1 (1.1)
Unknown	4 (4.6)

Note: Parametric data presented as M (SD).

[a]Rated on a scale whereby 1 = poor; 5 = excellent.

Table 2. Mean (SD) change in erythrocyte PUFAs (% of total) at baseline and after treatment.

	Baseline n=75	After EPA n=50	After DHA n=45	After LA n=46
Omega-3 fatty acids				
EPA	0.60 (0.24)	2.74 (0.68)	1.46 (0.39)	0.57 (0.19)
DHA	3.50 (0.69)	4.20 (0.45)	7.33 (1.08)	4.21 (0.84)
EPA+DHA	4.09 (0.85)	6.94 (0.96)	8.78 (1.36)	4.78 (0.90)
total n-3 PUFA	6.58 (1.10)	10.9 (1.34)	10.9 (1.35)	7.14 (1.81)
Omega-6 fatty acids				
AA	11.2 (1.43)	9.26 (0.90)	9.29 (0.90)	10.7 (1.71)
DPA n-6	0.48 (0.11)	0.27 (0.06)	0.35 (0.05)	0.45 (0.09)
total n-6 PUFA	24.2 (2.47)	21.7 (1.67)	21.5 (1.49)	25.6 (2.16)
n-6/n-3	3.78 (0.75)	2.04 (0.39)	2.03 (0.40)	3.76 (0.79)

EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; n-3 = omega-3; PUFA = polyunsaturated fatty acid; AA = arachidonic acid; DPA = docosapentaenoic acid; n-6 = omega-6.

Table 3. Behaviour, cognition and literacy scores at baseline and after each treatment

	Baseline *n*=87	After EPA *n*=56	After DHA *n*=54	After LA *n*=57
Behaviour[1]	M (SD)	M (SD)	M (SD)	M (SD)
Oppositional	70.6 (11.97)	64.7 (11.87)	64.7 (13.6)	66.8 (11.0)
Cognitive problems	76.1 (8.52)	68.9 (10.0)	67.3 (9.73)	70.6 (11.8)
Hyperactivity	74.4 (12.3)	63.9 (12.6)	65.9 (13.3)	65.7 (13.9)
Anxious/shy	59.4 (12.1)	53.1 (13.6)	53.8 (12.5)	53.3 (13.9)
Social problems	68.1 (15.5)	63.3 (15.8)	61.5 (19.1)	67.6 (15.7)
ADHD Index	75.9 (7.32)	67.7 (9.73)	65.6 (12.9)	69.7 (9.45)
Conners global	74.6 (9.35)	65.5 (10.5)	66.4 (12.0)	67.4 (9.84)
DSM-IV inattention	75.3 (8.32)	67.3 (9.64)	66.3 (9.60)	68.6 (11.3)
DSM-IV hyperactivity	74.9 (12.2)	66.0 (12.4)	67.2 (13.4)	68.1 (13.6)
DSM-IV total	75.9 (11.4)	68.0 (9.84)	68.0 (10.4)	69.8 (11.4)
Attention				
Skysearch score	5.37 (2.56)	3.33 (1.45)	3.06 (1.48)	3.31 (1.42)
CC correct	3.62 (2.27)	4.68 (2.11)	4.38 (2.13)	3.83 (2.53)
Score!	7.23 (2.26)	7.15 (2.68)	8.06 (1.46)	6.70 (2.60)
Skysearch DT	4.97 (2.37)	3.55 (1.42)	3.77 (1.50)	3.70 (1.14)
Go/no-go time/errors	69.2 (52.9)	81.3 (82.4)	80.8 (77.7)	88.5 (97.5)
Literacy				
Word reading	91.7 (19.6)	92.9 (17.1)	95.4 (16.3)	94.0 (18.4)
Spelling	87.6 (18.7)	91.2 (15.7)	93.6 (14.1)	89.5 (16.5)
Vocabulary	6.42 (2.27)	6.05 (2.67)	6.15 (2.73)	6.11 (2.85)

[1]From Conners Parent Rating Scales, represented as t-scores. EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; LA = linoleic acid; CC = creature counting; DT = divided attention.

Table 4. Within subject regression analysis between erythrocyte PUFA and changes in literacy and cognition at baseline and after treatment (n=75).

	r	*B*	*p*
Word reading			
EPA	.139	0.757	.110
DHA	.167	0.575	.053
EPA+DHA	.203	0.554	.019
Total n-3	.224	0.525	.009
AA	-.286	-1.187	.001
DPA n-6	-.247	-12.969	.004
Total n-6	-.191	-0.474	.027
n-6/n-3	-.239	-1.360	.005*
Spelling			
EPA	.265	0.791	.002*
DHA	.295	0.549	.001*
EPA+DHA	.365	0.540	.000*
Total n-3	.384	0.486	.000*
AA	-.378	-0.851	.000
DPA n-6	-.262	-7.530	.002
Total n-6	-.217	-0.295	.012
n-6/n-3	-.353	-1.086	.000*
Vocabulary			
EPA	-.025	-0.071	.778
DHA	.103	0.185	.243
EPA+DHA	.069	0.099	.432
Total n-3	.029	0.036	.738
AA	-.079	-0.172	.368
DPA n-6	-.072	-1.967	.414
Total n-6	-.073	-0.094	.410
n-6/n-3	-.053	-0.156	.551
Skysearch score			
EPA	-.444	-0.748	.000*

DHA	-.424	-0.431	.000*
EPA+DHA	-.540	-0.429	.000*
Total n-3	-.506	-0.343	.000*
AA	.490	0.548	.000
DPA n-6	.523	8.388	.000
Total n-6	.340	0.254	.001*
n-6/n-3	.523	0.885	.000*
CC correct			
EPA	.159	0.222	.070
DHA	.139	0.121	.114
EPA+DHA	.189	0.132	.030
Total n-3	.201	0.120	.021
AA	-.301	-0.316	.000
DPA n-6	-.230	-3.059	.008
Total n-6	-.236	-0.149	.007
n-6/n-3	-.217	-0.312	.013
Score!			
EPA	.013	0.018	.887
DHA	-.036	-0.033	.686
EPA+DHA	-.022	-0.016	.802
Total n-3	-.015	-0.009	.870
AA	-.046	-0.051	.600
DPA n-6	-.060	-0.838	.494
Total n-6	-.046	-0.030	.604
n-6/n-3	-.014	-0.020	.878
SkysearchDT			
EPA	-.087	-0.115	.333
DHA	-.134	-0.112	.135
EPA+DHA	-.151	-0.100	.092
Total n-3	-.157	-0.089	.080
AA	.244	0.245	.006
DPA n-6	.118	1.498	.189
Total n-6	.069	0.041	.443

n-6/n-3	.148	0.197	.112
Gonogo time/errors			
EPA	.113	6.678	.204
DHA	-.088	-3.222	.322
EPA+DHA	-.014	-0.420	.872
Total n-3	.024	0.596	.789
AA	-.117	-5.214	.187
DPA n-6	-.123	-69.694	.166
Total n-6	-.049	-1.306	.581
n-6/n-3	-.051	-3.117	.566

*significant after modified Bonferroni correction.

PUFA = polyunsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; n-3 = omega-3; AA = arachidonic acid; DPA, docosapentaenoic acid; n-6 = omega-6; CC = creature counting; DT = divided attention.

Table 5. Within subject regression analysis between erythrocyte PUFA and changes in parent-reported behaviour on Conners' Parent Rating Scales at baseline and after treatment (n=75).

	r	B	p
Oppositional			
EPA	-.199	-0.684	.022
DHA	-.253	-0.545	.003*
EPA+DHA	-.301	-0.517	.000*
Total n-3	-.252	-0.370	.004
AA	.227	0.594	.009
DPA n-6	.248	8.159	.004
Total n-6	.108	0.170	.217
n-6/n-3	.233	.833	.007
Cognitive problems/inattention			
EPA	-.222	-1.006	.011
DHA	-.270	-0.766	.002*
EPA+DHA	-.326	-0.737	.000*
Total n-3	-.283	-0.549	.001*
AA	.150	0.517	.086
DPA n-6	.201	8.747	.021
Total n-6	.034	0.071	.698
n-6/n-3	.269	1.270	.002*
Hyperactivity			
EPA	-.281	-0.924	.001*
DHA	-.211	-0.435	.016
EPA+DHA	-.310	-0.509	.000*
Total n-3	-.320	-0.450	.000*
AA	.275	0.692	.002
DPA n-6	.260	8.291	.003
Total n-6	.125	0.190	.157
n-6/n-3	.290	0.999	.001*
Anxiety/shyness			

EPA	-.115	-0.240	.190
DHA	-.244	-0.319	.005
EPA+DHA	-.252	-0.262	.004
Total n-3	-.212	-0.189	.015
AA	.260	0.412	.003
DPA n-6	.170	3.399	.052
Total n-6	-.158	-4.227	.072
n-6/n-3	.224	0.486	.010
ADHD Index			
EPA	-.171	-0.965	.050
DHA	-.141	-0.502	.109
EPA+DHA	-.198	-0.561	.023
Total n-3	-.190	-0.459	.030*
AA	.053	0.227	.549
DPA n-6	.087	4.710	.326
Total n-6	-.047	-0.121	.595
n-6/n-3	.151	0.891	.085
Conners global			
EPA	-.201	-0.980	.022
DHA	-.083	-0.251	.350
EPA+DHA	-.166	-0.402	.060
Total n-3	-.193	-0.401	.028
AA	.110	0.410	.213
DPA n-6	.144	6.782	.103
Total n-6	.018	0.040	.839
n-6/n-3	.164	0.831	.063
DSM-IV Inattentive			
EPA	-.193	-0.728	.027
DHA	-.310	-0.733	.000*
EPA+DHA	-.343	-0.647	.000*
Total n-3	-.287	-0.462	.001*
AA	.146	0.421	.094
DPA n-6	.177	6.393	.043

Total n-6	.025	0.042	.779
n-6/n-3	.260	1.024	.003*
DSM-IV Hyperactive			
EPA	-.242	-0.763	.005
DHA	-.186	-0.371	.033
EPA+DHA	-.270	-0.428	.002*
Total n-3	-.285	-0.385	.001*
AA	.237	0.569	.007
DPA n-6	.209	6.346	.017
Total n-6	.101	0.145	.252
n-6/n-3	.259	0.851	.003*

**significant after modified Bonferroni correction.*

PUFA = polyunsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; n-3 = omega-3; AA = arachidonic acid; DPA, docosapentaenoic acid; n-6 = omega-6; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders 4th Edition.