Biochemical and psychological effects of omega-3/6 supplements in male adolescents with ADHD: A randomized, placebo-controlled, clinical trial

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This study was financially supported by the Mother and Child foundation, and Vifor Pharma (formerly Equazen Nutraceutical Ltd). Capsules for this trial were provided by Vifor Pharma. This study was supported by the United Kingdom Clinical Research Collaboration-registered King's Clinical Trials Unit at King's Health Partners, which is part funded by the NIHR Biomedical Research Centre for Mental Health at South London and Maudsley NHS Foundation Trust and King's College London and the NIHR Evaluation, Trials and Studies Coordinating Centre.

ISRCTN registration: http://www.controlled-trials.com/ISRCTN27741572/MAAFA

Keywords: ADHD, omega-3, omega-6, LC-PUFA, clinical trial, fatty acid

Abstract

An abnormality in long chain-polyunsaturated fatty acid (LC-PUFA) levels has been implicated in Attention Deficit Hyperactivity Disorder (ADHD). Studies evaluating LC-PUFA supplementation for therapeutic efficacy in ADHD have shown mixed and therefore inconclusive results. Seventy-six male adolescents (aged 12 -16 years, *M* =13. 7) with ADHD were assessed for the effects of 12 weeks omega-3 and omega-6 supplements on biochemical and psychological outcomes in a randomized, placebo-controlled, clinical trial. The primary outcome measure was change in the Conners' Teacher Rating Scales (CTRS) following 12 weeks of supplementation of LC-PUFA or placebo. At baseline, the placebo and treatment groups had comparable levels of LC-PUFA as measured by red blood cell phosphatidylcholine. In the treatment group, supplementation enhanced eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and total omega-3 fatty acid levels. No superiority of LC-PUFAs to placebo was observed on the primary outcome. Furthermore, there were no reliable treatment effects on aggression, impulsivity, depression and anxiety. Future studies should use larger sample sizes and longer supplementation period to detect small-modest effects for clinical recommendations in ADHD.

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) has an estimated global prevalence of approximately 5% to 13% (Faraone and Sergeant 2003). The condition is defined by the Diagnostic and Statistical Manual of Mental Disorders, version5 (DSM-5) (American Psychiatric Association 2013) and similarly by the International Classification of Diseases, version 10, with the term "hyperkinetic disorder" being reserved for more severe cases (World Health Organization 1992). The two distinct behavioral dimensions, inattentive and hyperactive-impulsive, have been recognized to exist across cultures in a variety of ethnic groups, cross-culturally (Barkley 2003). Children with ADHD will often struggle to follow instructions or abide by rules (Taylor and Dopfner 2004). They are also more likely to generally misbehave, frequently interrupt and/or intrude on others' conversations and activities (de Boo and Prins 2007). In addition, they are at risk for risk-taking, rule-violating, noisy and disruptive behavior which frequently results in negative responses from peers, teachers and parents (McQuade and Hoza 2008, Solanto and Pope-Boyd 2009). Clearly, such behaviors are debilitating and approximately 50% of adolescents with ADHD are estimated to be at risk from substance abuse, increased levels of anxiety and depression, educational failure via either exclusion or drop out (Frick and Dickens 2006, Tapert and Baratta 2002, Tercyak and Lerman 2002).

Research spanning the last 3 decades has supported the role of long-chain polyunsaturated fatty acids (LC-PUFAs) in neurodevelopment (Marszalek and Lodish 2005). Eicosapentaenoic acid (EPA, omega-3), docosahexaenoic acid (DHA, omega-3), gamma linoleic acid (GLA, omega-6), and arachidonic acid (AA, omega-6), docosapentaenoic acid (DPA, omega-6) are members of the omega-3 and omega-6 fatty acids family. Their parent compounds are alpha linoleic acid (ALA) and linoleic acid respectively.

DHA is especially concentrated in synaptic regions of the brain and constitutes approximately 30% of the ethanolamine and serine phosphoglycerides of brain tissue (Crawford and Bazinet 2009). Within brain tissues DHA preferentially accumulates in growth cones, astrocytes, synaptosomes,

myelin, microsomal and mitochondrial membranes (Bourre and Bonneil 1992). The perinatal increase in cortical DHA concentrations coincides with active periods of neurogenesis, axonal myelination and synaptogenesis (Cao and Kevala 2009, Green and Glozman 1999, Yavin and Himovichi 2009). Animal studies have provided robust evidence that changes in retinal and brain DHA can alter neuronal structure and function (Zimmer and Dalpal 2000, Zimmer and Delion-Vancassel 2000). Diets deficient in ALA lead to a lower accumulation of DHA in brain phospholipids and structural changes in membranes in the central nervous system are in turn related to behavior (Fienners and Sinclair 1973, Reisbick and Neuringer 1994, Sinclair and Crawford 1972). Depriving rats of omega-3 fatty acids results in behavior abnormalities but repletion of DHA reverses the effect (Chalon 2006, Fedorova and Salem 2006, Levant and Radel 2004, Moriguchi and Greiner 2000).

EPA is important in its role in the production of eicosanoids which have anti-inflammatory, anti-thrombotic and vasodilatory properties. EPA inhibits the manufacture of several inflammatory cytokines such as interleukins 1 β and 6 and tumor necrosis factor α, which are implicated in depression (Salem and Moriguchi 2001, Salem and Litman 2001, Song and Manku 2008).

ADHD has frequently been associated with abnormal erythrocyte and plasma levels of omega-3/6 fatty acids (Antalis and Stevens 2006, Chen and Hsu 2004, Stevens and Zentall 1996, Stevens and Zentall 1995). Improvement of symptoms following supplementation has been reported in a range of related disorders including depression (Nemets and Apter 2006, Peet and Horrobin 2002), those at risk from suicide (Sublette and Hibbeln 2006), and those with aggression/anti-social behavior (Corrigan and Gray 1994, Gesch and Hammond 2002, Zaalberg and Nijman 2010); and poor developmental outcomes have been reported if the maternal consumption of seafood (high omega-3) is less than 340 grams per week (Hibbeln and Davis 2007).

Several randomized, placebo-controlled, double-blind trials with omega-3/6 fatty acids in children/adolescents with symptoms of either ADHD, literacy/reading and writing difficulties (e.g.,

dyslexia) or developmental co-ordination disorder have reported statistically significant improvements in learning and/or behavior (Richardson and Burton 2012, Richardson and Montgomery 2005, Richardson and Puri 2002, Sinn and Bryan 2007); others have reported little (Johnson and Ostlund 2009) or no effect (Hamazaki and Hirayama 2004, Voigt and Llorente 2001). Supplementation trials using fatty acids fortified foods has thus far appeared to be the least successful (Hamazaki and Hirayama 2004).

Bloch and Qawasmi (2011) conducted a recent meta-analysis of 10 well-designed trials involving 699 children. They reported that omega-3 supplementation had a small but statistically and clinically significant effect size in reducing symptoms of ADHD (effect size SMD = 0.31, 95% CI 0.16 – 0.47, z = 4.04, p = < .001). When the different omega-3 fatty acids were considered separately, higher doses of EPA (which collectively ranged from 80 to 750 mg daily, were significantly but modestly correlated with efficacy (β=0.36 (95% CI: 0.01–0.72), t=2.30, p=0.04, R2=0.37). Doses of other omega-3 LC-PUFAs such as DHA and ALA were not significantly related to efficacy. A Cochrane review authored by Gilles and colleagues (2012) included 13 studies in their review. They reported a somewhat higher improvement in the omega-3/6 fatty acid intervention group (RR2.19, 95% CI 1.04-4.62); but that there were no statistically significant differences in parent-rated or teacher-rated ADHD symptoms when all participants receiving LC-PUFA supplements were compared to those receiving placebo. Further research was considered necessary. Sonuga-Barke and colleagues (2013) provided the most recent meta-analysis. Eleven omega-3 and/or omega-6 supplementation trials met their inclusion criteria. Five involved omega-3 supplements, two involved omega-6 supplements, and the remainder used both omega-3 and omega-6 supplements. The overall result was a small but statistically significant preference for supplementation over placebo (standardized mean difference=0.21).

Bloch and Qawasmi (2011) highlighted the valid point that available studies have small sample sizes and individually lack the statistical power to demonstrate a substantive effect .Other potential

reasons for the lack of consistent findings in the clinical trial literature are the large variation in methodological design, differences in dose, duration of supplementation (e.g., 8, 12, 16 or 34 weeks), and choice of formula of fatty acids used (e.g., EPA-rich versus DHA-rich). Biochemical results are mostly not reported, so it may be unclear whether supplementation has in fact had its intended effect. ADHD diagnosis is often neglected. Parents are usually both the deliverers of the treatment and the raters of its effect with the possibilities arising that concealment of allocation may be compromised, or that responses will be magnified subjectively because of the effort that has been expended.

Methods

In consideration of the research to date, the primary aim of the present study was to investigate whether LC-PUFA supplementation was a safe and effective treatment in the context of a 12 week, randomized, placebo-controlled, double blind clinical trial named The Maudsley Adolescent ADHD Fatty Acid (MAAFA) trial. This was a collaborative research trial with London Metropolitan University. The key objective of the trial was to assess whether LC-PUFA supplementation would improve symptoms of ADHD at 12 weeks post-randomization as measured by Conners' Teacher Rating Scales (CTRS-L, which assessed each of 59 items of child behavior on a 4-point scale) (Conners and Sitarenios 1998). The secondary objective tested whether blood levels of fatty acids increased post-supplementation and whether other beneficial effects of problems associated with ADHD could be detected.

Participants (see Figure 1.)

A total of seventy-six male adolescents were recruited for this study. At the time the study was designed, the meta-analyses of effect sizes described above were not available, and the sample size was therefore calculated using an expected effect size of 0.7, in order to be comparable with the effect sizes of established therapies such as parenting classes or stimulant medication. This resulted

in a total of 38 patients (which included an additional 10% drop out rate) in each intervention arm. Children who met eligibility criteria were randomized by the Mental Health and Neuroscience Clinical Trials Unit based at the Institute of Psychiatry, King's College London. Allocation was stratified by whether the child attended a day or boarding school and their age group (12 – 14 years and 15 – 17 years) using minimization randomization. Participants were drawn from various special educational settings (e.g., boarding schools, mainstream schools with provision for children with emotional and behavioral difficulties) in England. Children who were taking concomitant medication underwent a 48 hour wash out period for stimulant medication, prior to their assessment visit.

Criteria for inclusion were based on a mean standardized score > 65 (> 95th percentile) on both CTRS-L and the Conners' Parent Rating Scales [Conners C. , Sitarenios, Parker, Epstein, 1998], aged between 12 and 17 years old. Intelligence quotient (IQ) had to be higher than 70 and was assessed using the Kaufman Brief Intelligence Test, Second Edition (KBIT-2) (Kaufman 2004). A diagnosis of ADHD was confirmed through a semi-structured interview based on DSM-IV criteria (ChIPS) (Rooney and Weller 1999). The Barratt Impulsivity Scale was also performed to describe characteristics of the participants (Patton and Stanford 1995).

Sub-group Analysis of ADHD.

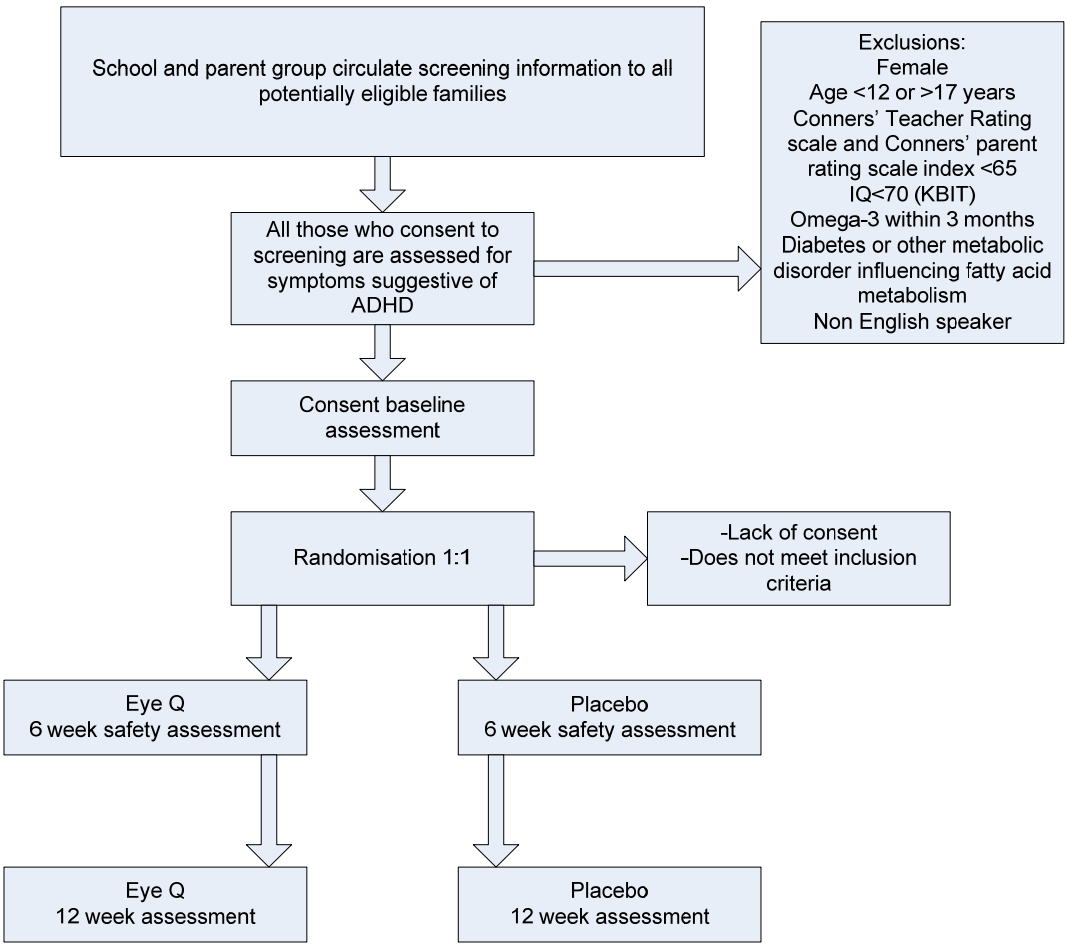
The ADHD group was assessed into sub-groups by the Children's Interview for Psychiatric Syndromes. 65.8% were found to have the combined type, 23.7% the inattentive type and 10.5% the hyperactive/impulsive type.

Ethics

This study was approved by the Research Ethics Committee in the UK (MREC: 06/Q0702/19). It was conducted as a formal "clinical trial using medication", and has obtained an International Standard Randomized Controlled Trial Number (ISRCTN27741572).

Informed consent/assent was obtained from participants and their parents, according to the National Health and Medical Research Council (UK) guidelines. Children, parents and teachers gave signed consent to take part and were fully briefed on the ethical considerations of the study. They were also advised they could withdraw at any point with no obligation.

Figure 1. Flowchart of participants



Procedure & Materials

Each child was accompanied by a caregiver to The Institute of Psychiatry at The Maudsley Hospital where approximately 15 ml of fasting blood was taken followed by the baseline assessments which included electroencephalography/event-related potential and neuropsychological assessments (not reported in this publication).. The blood was taken by a qualified phlebotomist and stored at -80 °C.

Total Red Blood Cell Lipid Analysis.

Total lipids were extracted from 1 ml of red blood according to the Folch method (Folch and Lees 1957). The red cells were homogenized in chloroform and methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene as an antioxidant, under nitrogen. Fatty acid methyl esters (FAMEs) were prepared by heating the extracted total lipid in 4ml of 15% acetyl chloride in methanol for 3 hours at 70°C, under nitrogen in a sealed vial. FAMEs were separated by a gas-lipid chromatograph (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a capillary column (30m×0.32mm i.d., 0.25u film, BP20). Hydrogen was used as a carrier gas, and the injector, oven and detector temperatures were 235°C, 250°C and 178°C, respectively. FAMEs were identified by comparison with relative retention times of authentic standards and calculation of equivalent chain length values. Peak areas were quantified by a computer chromatography data system (EZChrom Chromatography Data System, Scientific Software Inc., San Ramon, CA, USA). Results are expressed in ng/ml.

Interventions

The investigative medicinal product or active treatment was LC-PUFA capsules, Equazen Eye Q. The daily dose of 6 capsules provided a combination of omega-3 fatty acids (EPA 558 mg and DHA 174 mg) and omega-6 fatty acid γ-linoleic acid 60 mg, and vitamin E 9.6 mg (in the natural form, α-tocopherol). This, or indistinguishable placebo (medium chain triglycerides) as a placebo, was given for a 12 week period. These were provided in four identical bottles labeled with an identifying code and in compliance with *Good Manufacturing Process*. Bottles were collected at the end of the study and assessed for compliance. At six weeks parents and teachers were contacted by telephone or email to monitor adverse effects and compliance. All primary and secondary measures were evaluated at baseline and following 12 weeks of intervention.

Outcomes

Primary analysis

The primary analysis performed on the Intention to Treat (ITT) complete case sample was statistically analyzed by applying a linear regression of ADHD index adjusted for baseline CTRS ADHD index, school type (day, boarding), age (≤14, ≥15) and an indicator variable for treatment (LC-PUFA, placebo). The ADHD index *t*-value was used to test the null hypothesis that the treatment coefficient in the model is zero. Rejection of the null hypothesis at the 2-sided 5% significance level was considered to be a successful demonstration of efficacy.

Secondary analysis

The model used to complete each secondary analysis was a linear regression outcome on the ITT complete case sample adjusted for corresponding baseline score, school type (day, boarding), age (≤14, ≥15) and an indicator variable for treatment (LC-PUFA, placebo). ADHD index t-value was used to test the null hypothesis that the treatment coefficient in the model is zero. Rejection of H0 at the 2-sided 0.0083% significance level was considered to be a successful demonstration of efficacy.

Safety analysis

Summaries of incidence rates (frequencies and percentages), severity and relationship to drug of adverse events and reactions classified by body system were prepared. A participant may be counted more than once within each body system. Summaries were presented for each treatment group for those participants in the safety sample. Only events starting after randomization were included. Fisher's exact test was performed at the 5% significance level to test the association between adverse events and reactions by body system and event type.

Patients

The patient disposition across the different data sets analyzed was well balanced between the Safety data set and the ITT data set. The per protocol analysis had 4 (15%) more patients in the placebo intervention than the LC-PUFA intervention. Treatment groups were homogenous with respect to demographics.

Results

Demographics (see Table1.)

One hundred and thirty eight patients were screened for eligibility. Of those, 76 male adolescents were randomized. Six randomized patients did not receive either intervention before withdrawing from the study. 86% of participants randomized (65) were day students in school. The age range was between 12 and 16 with a mean age of 13. 7 years. The mean composite IQ score was 96.1 (*SD* = 12.8) which did not differ significantly between active and placebo groups at baseline. A total of 15 children were taking concomitant medications as confirmed by interview, of which 7 were randomized into the placebo group and 8 in the treatment group.

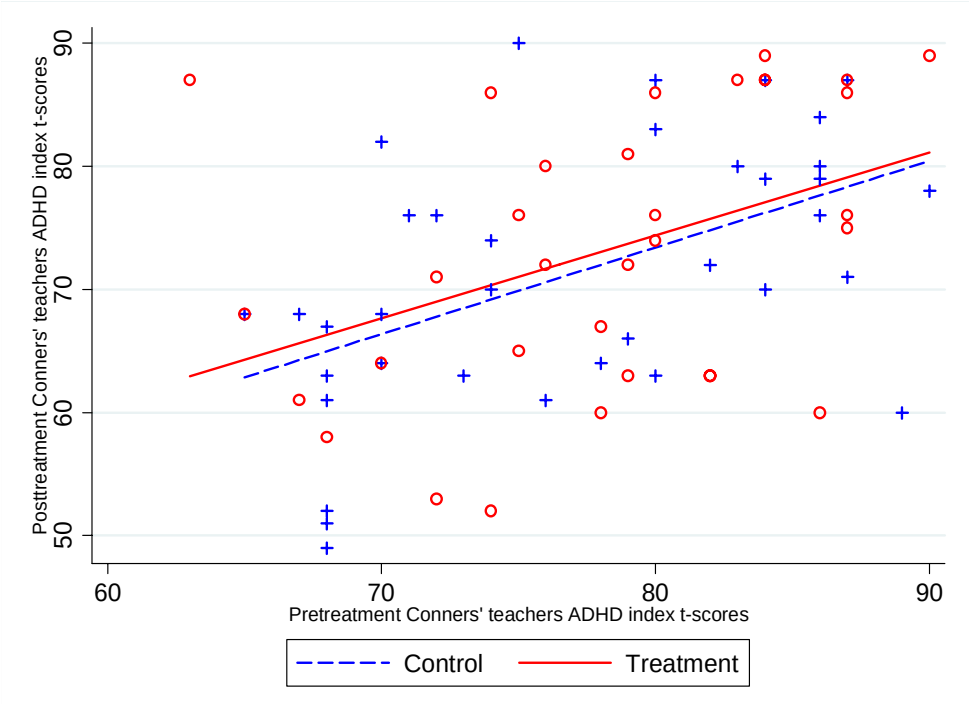
Table 1. Demographic Characteristics

	Control n=38	Active n=38	Total n=76
School Type Day Boarding	 33 (86.8%) 5 (13.2%)	 32 (84.2%) 6 (15.8%)	 65 (85.5%) 11 (14.5%)
Age at randomization (years) mean (SD)	 13.7 (1.2)	 13.7 (1.1)	 13.7 (1.1)
IQ(K-BIT2) Verbal Nonverbal Composite	 97.84 ±11.42 95.11 ±14.81 96.18 ±12.85	 99.68 ±11.69 92.63 ±14.19 95.79 ±12.89	 98.76 ±11.52 93.87 ±14.46 95.99 ±12.78
Psychostimulant medication	7 (18.4%)	8 (21%)	15 (19.7%)
Type of ADHD (ChIPS*) Inattentive Type Hyperactive/impulsive Type Combined Type	 9 (23.7%) 3 (7.9%) 26 (68.4%)	 9 (23.7%) 5 (13.2%) 24 (63.2%)	 18 (23.7%) 8 (10.5%) 50 (65.8%)
CTRS-L (T) ADHD Index	76.8 ± 7.6	78.2 ± 6.8	77.5 ± 7.2
CPRS-L (P) ADHD Index	74.7 ± 5.9	74.8 ± 5.6	74.8 ± 5.7

Primary outcome

Linear regression of the CTRS ADHD index adjusted by treatment, baseline ADHD index score, school type (day, boarding) and age (< 15 years, >15 years) failed to show a significant difference between treatment at 12 weeks (p = .671). This analysis was performed on the ITT complete case sample consisting of 69 patients (placebo = 36, LC-PUFA = 33). Sensitivity analysis on the ITT complete case sample was carried out by adjusting by the treatment and baseline ADHD index score only. This analysis failed to show a significant result between treatment at 12 weeks (p = .617). Per Protocol complete case analysis was performed on 50 participants (placebo = 27, LC-PUFA = 23) using the same method as the ITT complete case sample. The analysis failed to show a significant difference between treatment at 12 weeks (p = .164). Removing points of influence also failed to show a significant outcome at the 5% level.

Figure 2. Pre-treatment and post-treatment scores by treatment of the Conners' Rating Scale Teacher ADHD Index t-scores



Pre-treatment and post-treatment scores in each group showing fitted lines. The difference between the two groups is shown by the vertical distance between the two lines.

Secondary Outcomes: Biochemical results (see Table 2.)

There were changes in the expected direction on red blood cell phosphatidylcholine measures. Total omega-3 levels rose from a mean of 2.57 (SD 0.75) to 2.73 (SD 0.73) in the control group; and from 2.60 (SD 1.16) to 3.62 (SD 1.21) in the group receiving supplementation; $p < 0.001$. Both EPA and DHA levels rose significantly from the base line to the end of the trial in the group receiving supplementation but not in the controls. On the other hand, DPA, which shares the same enzyme for conversion with DHA, was decreased after intervention in the active group. GLA level did not increase significantly.

Table 2. Omega-3 levels in Red Blood Cell Phosphatidylcholine

	Before		After	
	Control	Active	Control	Active
c16:0	32.95±3.35	32.32±3.84	33.52±4.14	34.12±3.07
c18:0	11.86± 2.88	12.56±4.84	11.76±1.58	11.71±1.57
Total-saturated	45.38± 4.35	45.64±5.95	45.83±5.11	46.57±3.15
c18:1ω9	15.56± 1.57	14.88±3.53	15.49±1.16	16.05±1.26
c18:1ω7	1.26±0.36	1.21±0.359	1.20±0.38	1.28±0.36
Total-mono	17.57±1.60	17.14±3.42	17.45±1.34	18.04±1.31
Omega-6				
c18:2	21.67± 2.84	20.24±4.96	22.23±2.10	21.66±2.59
c18:3	0.12±0.11	0.14±0.15	0.16±0.21	0.11±0.08
c20:3	2.18±0.45	2.06±0.60	2.17±0.43	2.04±0.44
AA	6.81±2.22	6.82±2.19	6.78±1.16	6.27±1.07
c22:4	0.44±0.32	0.57±0.63	0.46±0.30	0.37±0.33
DPA	0.39±0.32	0.61±1.52	0.40±0.32	0.35±0.35*
Total ω-6	31.86±1.97	30.78±3.68	32.36±2.73	31.06±2.88
Omega-3				
c18:3	0.19±0.11	0.20±0.10	0.23±0.07	0.18±0.07
EPA	0.41±0.18	0.44±0.21	0.46±0.20	1.04±0.65**
DHA	1.45±0.46	1.42±0.76	1.54±0.45	1.93±0.59**
Total ω-3	2.57±0.75	2.60±1.16	2.73±0.73	3.62±1.21**

Mean±SD (%)
No difference between control and active group before intervention
No difference before and after intervention in control group
Compare means before and after intervention in active treatment group: *p<0.05, **p<0.01

Secondary Outcomes: Impulsiveness

To account for multiplicity an unadjusted p value = .0083 needed to be achieved to declare a significant outcome. The total impulsivity score of the Barratt impulsivity scale showed some indication of an increase in the LC-PUFA group after supplementation (Table 3). The observed results were not free from bias as there was selection of participants who did not return baseline and/or 12 week questionnaire.

Table 3. Barratt-Impulsiveness Scale before and after intervention

	Before		After	
	Control (n=23)	Active (n=25)	Control (n=16)	Active (n=19)
Attentional Impulsivity score	32.48±6.69	33.92±4.17	31.38±6.79	32.79±4.71
Motor Impulsivity score	26.09±5.84	25.04±5.07	25.81±6.24	27.32±4.52
Non-planning impulsivity score	21.91±4.40	20.08±3.25	19.56±3.33	21.32±3.9*
Total score	80.48±13.09	79.04±9.52	76.75±12.44	81.42±11.00**

Mean±SD *p=0.014 **p=0.024

The numbers for a complete case analysis were only 27, as only a subset of participants completed the baseline and week 12 outcome for this measure. Per protocol complete case analysis indicated a 3.1 point increase in the LC-PUFA group (95% CI (0.4, 5.9) *p*-value=0.028).

No significant changes were noted for the Conners' Parent Rating Scale.

Safety results

A total of five 5 patients, 7%, of the safety sample (70 patients) had an adverse event (placebo = 2, LC-PUFA = 3). Out of these 5 patients, 4 had more than 1 adverse event as 12 adverse events were reported in total (placebo=7 adverse events, LC-PUFA=5 adverse events). Ten of the

adverse events were reported as mild in severity. One was classed as moderate being a bone fracture which occurred on the LC-PUFA intervention but was thought to have no relation to the drug. Three patients withdrew from treatment due to adverse events reported. Fishers exact test failed to show a significant difference between the adverse events and treatment reported at the 5% level (p =1.000).

One patient, who had withdrawn from the trial early, returned 127 days after initial randomization and requested that the fatty acids should be resumed. This was agreed to (but not as part of the trial); but on the second day he had a seizure. This serious adverse event subsequent to the trial was deemed to be remotely related to the LC-PUFA intervention and the event was moderate in severity.

Discussion

This intervention study demonstrated that the primary outcome failed to show a significant difference between active and placebo intervention at 12 weeks. The fatty acids supplementation used in this study, which contained a higher ratio of EPA (6 capsules = 558 mg) compared to DHA (174 mg per 6 capsules) and some GLA (60 mg per 6 capsules), did not improve teachers rated ADHD symptoms at 12 weeks follow up. Similar results were reported for the teacher scores by Sinn and Bryan et al (2007).

The likely reasons for the negative outcome may include the lack of statistical power, drop out (19 participants) and/or dose of the supplement. Our results do not exclude a small effect of the size suggested by the recent meta-analyses. In this study, male adolescents were invited to the study because the symptoms can be evaluated clearer than younger age, and males are more common in ADHD than females. Different study population and larger sample size may have shown the significant effect of LU-PUFA supplementation.

The results of blood analysis showed a significant increase of DHA level while the level of GLA did not change after intervention in the active treatment group. This suggests the dose of the

supplement for intervention might not have been high enough to affect the results. Indeed, a recent meta-analysis involving 10 trials and 699 children by Bloch and Qawasmi (2011) indicated that higher doses of one omega-3 fatty acid were significantly, though modestly, correlated with supplement efficacy in the treatment of ADHD.

The relative efficacy of omega-3 fatty acid supplementation appears to be modest in contrast to currently available pharmacological interventions, e.g., psycho-stimulant medication for ADHD. However, taking into consideration its relatively mild side-effect profile and evidence of modest efficacy, it may be rational to use omega-3 fatty acids to supplement traditional pharmacologic interventions or for children who resist or are resistant to psychopharmacologic options. Other studies also lend support to the potential beneficial effects of EPA in alleviating symptoms of low mood and depression (Bloch and Qawasmi 2011, Freeman and Hibbeln 2006, Peet and Horrobin 2002)- in keeping with the notion that EPA and DHA have separate roles in the brain (Young and Conquer 2005).

Taking into consideration the significant effects of LC-PUFA supplementation reported elsewhere over a six month period (Richardson and Montgomery 2005, Sinn and Bryan 2007) the length of the present intervention may have been too short to have a measurable impact on behavior especially if correcting a deficiency. Because the red blood cell survives about 120 days in the body, 12 weeks (84 days) supplementation might not be long enough to change the LC-PUFAs compositions.

Another possibility is that there could be a need to provide AA rather than GLA (Antalis and Stevens 2006). Furthermore, the combined effects of methylphenidate and omega-3/6 fatty acids are not well reported. A recent study by Barragan, Breuer and Döpfner (2014) reported that Omega-3/6 fatty acids had similar effects to methylphenidate (MPH), whereas the MPH + Omega combination appeared to have some tolerability benefits over MPH (REF). Almost 20% of our sample (15) were

taking psychostimulant medication and the medication remained stable during the intervention trial; it could have affected their follow up scores by both teachers and parents.

It is possible that the outcome measures (i.e., the CTRS) were not sensitive enough to detect small improvements in behavior. Teacher rating scales are often problematic and raise multiple questions concerning reliability (Sinn and Bryan 2007). In our study, we experienced the occasional problem of a changeover of teachers during term: several teachers were signed off with sickness and/or left the school prior to the collection of final scores. It is likely that any change in teacher scoring would have affected the overall scores. Arguably new teachers know the child less well than teachers who have worked consistently with the child for a considerable length of time.

The results of the secondary analyses are less reliable then hoped due to large levels of missing data both at baseline and at week 12. This reflected difficulties in compliance for parents in this school-based setting, and should be reckoned with in future such studies. In some cases the model's fit may be questioned: a rule of thumb is that there should be 10 participants for each covariate fitted in the model (in our case, baseline outcome score, age and school type). Covariates were not removed from the model as they were pre-specified a priori, but in the majority of analysis the covariates age and school were not found to be significant and may not be needed in the model if a larger trial was performed. Other covariates might have explained more variation.

The study did not have sufficient power to detect an effect size less than 0.7 SD. We initially chose this size of effect to be comparable with other interventions for ADHD; but considering the ready availability and acceptability of the intervention, a smaller effect size and larger numbers should be considered for future work.

Conclusion

In this study, U.K. adolescent males with ADHD did not show the hypothesized level of benefit from 3month LC-PUFA supplementation. It is still unknown whether other specific

populations, types of LC-PUFA supplement, and alternative intervention periods can show more effects of LC-PUFA treatment. However, many parents of ADHD children still prefer to use LC-PUFA supplements particularly when their children do not respond to psycho-stimulant medication and/or experience side effects. The negative evidence reported here is not strong enough to assert the ineffectiveness of LC-PUFA supplementation for ADHD children. It may be too early to disappoint people's expectations of LC-PUFAs and further research with the methodological improvements suggested here is strongly advised.

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