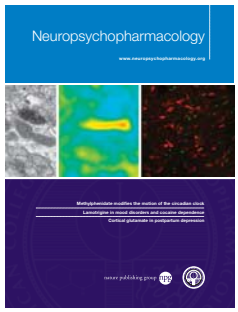

Reduced symptoms of inattention after Dietary Omega-3 Fatty Acid Supplementation in boys with and without Attention Deficit/Hyperactivity Disorder OPEN

Dienke J Bos, Bob Oranje, E Sanne Veerhoek, Rosanne M Van Diepen, Juliette MH Weusten, Hans Demmelmair, Berthold Koletzko, Monique GM de Sain-van der Velden, Ans Eilander, Marco Hoeksma, Sarah Durston

Cite this article as: Dienke J Bos, Bob Oranje, E Sanne Veerhoek, Rosanne M Van Diepen, Juliette MH Weusten, Hans Demmelmair, Berthold Koletzko, Monique GM de Sain-van der Velden, Ans Eilander, Marco Hoeksma, Sarah Durston, Reduced symptoms of inattention after Dietary Omega-3 Fatty Acid Supplementation in boys with and without Attention Deficit/Hyperactivity Disorder, *Neuropsychopharmacology* accepted article preview 19 March 2015; doi: 10.1038/npp.2015.73.

Received 5 November 2014; revised 17 December 2014; accepted 10 March 2015; Accepted article preview online 19 March 2015

Reduced symptoms of inattention after Dietary Omega-3 Fatty Acid Supplementation in boys with and without Attention Deficit/Hyperactivity Disorder

Running title

Omega-3 fatty acids in children with ADHD

Authors

Dienke J. Bos MSc[1], Bob Oranje PhD[1], E. Sanne Veerhoek MSc[1], Rosanne M. Van Diepen MSc[1], Juliette M.H. Weusten MSc[1], Hans Demmelmair PhD[2], Berthold Koletzko MD PhD[2], Monique G. M. de Sain-van der Velden PhD[3], Ans Eilander PhD[4], Marco Hoeksma PhD[4], Sarah Durston PhD[1]

Affiliations

[1]NICHE Lab, Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, The Netherlands

[2]Division of Metabolic and Nutritional Medicine, University of Munich Medical Center, Dr. von Hauner Children's Hospital, Munich, Germany

[3]Department of Medical Genetics, University Medical Center Utrecht, The Netherlands Wilhelmina Children's Hospital, University Medical Center Utrecht, The Netherlands

[4]Unilever Research & Development, Vlaardingen, The Netherlands

Corresponding author

Dienke J. Bos, NICHE Lab, Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, HP A.01.126, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands, T: +31 88 755 9840, E-mail: d.j.bos-2@umcutrecht.nl

Keywords: ADHD, Omega-3, DHA, EPA, cognitive control, dietary intervention

Word count

Abstract: 241

Manuscript body: 3.968 (including in-text references)

Figures: 3

Tables: 2

Supplemental information: 3

Abstract

Attention Deficit/ Hyperactivity Disorder (ADHD) is one of the most common child psychiatric disorders, and is often treated with stimulant medication. Non-pharmacological treatments include dietary supplementation with omega-3 fatty acids, although their effectiveness remains to be shown conclusively. In this study, we investigated the effects of dietary omega-3 fatty acid supplementation on ADHD symptoms and cognitive control in young boys with and without ADHD.

Forty boys with ADHD, aged 8-14 years, and thirty-nine matched, typically developing controls participated in a 16-week double-blind randomized placebo-controlled trial. Participants consumed 10 grams of margarine daily, enriched with either 650mg of EPA/DHA each or placebo. Baseline and follow-up assessments addressed ADHD symptoms, fMRI of cognitive control, urine homovanillic acid and cheek cell phospholipid sampling.

EPA/DHA supplementation improved parent-rated attention in both children with ADHD and typically developing children. Phospholipid DHA-level at follow-up was higher for children receiving EPA/DHA supplements than placebo. There was no effect of EPA/DHA supplementation on cognitive control or on fMRI measures of brain activity.

This study shows that dietary supplementation with omega-3 fatty acids reduces symptoms of ADHD, both for individuals with ADHD *and* typically developing children. This effect does not appear to be mediated by cognitive control systems in the brain, as no effect of supplementation was found here. Nonetheless, this study offers support that omega-3 supplementation may be an effective augmentation for pharmacological treatments of ADHD.

NCT01554462: The Effects of EPA/DHA Supplementation on Cognitive Control in Children With ADHD - http://clinicaltrials.gov/show/NCT01554462

Introduction

Attention Deficit/ Hyperactivity Disorder (ADHD) is one of the most common child psychiatric disorders and is characterized by symptoms of inattention, hyperactivity and impulsivity (American Psychiatric Association, 1994). Due to their efficacy, pharmacological treatments using stimulant medications, such as methylphenidate, have typically been the primary treatment for ADHD. However, in recent years there has been a growing interest in non-pharmacological treatments, to provide an alternative for parents and clinicians looking for alternative or additive treatments.

One popular non-pharmacological treatment for ADHD is dietary supplementation using long chain omega-3 polyunsaturated fatty acids (LC-PUFA). However, there has been a fair amount of debate on the efficacy of this treatment. Two early studies reported a significant reduction of ADHD symptoms in healthy children after supplementation with the omega-3 PUFAs Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) (Richardson and Montgomery, 2005; Sinn and Bryan, 2007). However, a later attempt to replicate these findings in a group of children diagnosed with ADHD failed to find such effects (Johnson *et al*, 2009). In fact, even though reduced plasma omega-3 PUFA levels have been reported in children and adolescents with ADHD (Antalis *et al*, 2006; Burgess *et al*, 2000; Chen *et al*, 2004; Colter *et al*, 2008; Spahis *et al*, 2008), to date most intervention-studies investigating the efficacy of omega-3 PUFAs to reduce ADHD symptoms have yielded conflicting or negative results (Bélanger *et al*, 2009; Dean *et al*, 2014; Gustafsson *et al*, 2010; Hirayama *et al*, 2004; Raz *et al*, 2009; Richardson and Puri, 2002; Stevens *et al*, 2003; Vaisman *et al*, 2008; Voigt *et al*, 2001).

Recently however, two independent meta-analyses, provided new evidence for possible beneficial effects of omega-3 PUFA supplementation on symptoms of ADHD

with small but positive effect sizes between 0.14 (Sonuga-Barke *et al*, 2013) and 0.31 (Bloch and Qawasmi, 2011). Furthermore, an open-label, exploratory study by Barragán and colleagues showed clinical benefits of treatment with omega-3 PUFA supplements in addition to methylphenidate (MPH), over treatment with MPH alone in children with ADHD (Barragán *et al*, 2014).

The neurobiological mechanism underlying an effect of omega-3 supplementation is far from clear. Omega-3 PUFAs are thought to play an important role in cell membrane elasticity and myelination and may thus affect neural signal transduction (Bazinet and Layé, 2014). Animal studies indicated that omega-3 PUFA deficiency, and specifically DHA-deficiency, results in decreased neuron size in rats that were raised on a DHA-deficient diet (Ahmad *et al*, 2002). Furthermore, both dopaminergic and serotonergic neurotransmission have been shown to be reduced in rats on an omega-3 PUFA deficient diet (Chalon, 2006; Zimmer *et al*, 2000, 2002), with marked effects in the frontal cortex. Specifically, lower frontal cortical omega-3 PUFA status correlated with hyperactive and impulsive behavior in rats (Vancassel *et al*, 2007). Interestingly a vast body of human literature has implied dopamine dysfunction and impairments in cognitive control in ADHD (Durston and Konrad, 2007; Kirley *et al*, 2002; Swanson *et al*, 2000). Taken all this together, this may suggest a potential target for omega-3 supplementation in the treatment of this disorder.

Therefore, we set out to investigate the effects of omega-3 PUFA dietary supplementation on ADHD symptoms in young boys with and without ADHD in a randomized, placebo-controlled trial. We included a typically developing reference group to investigate the specificity of treatment to subjects with ADHD. Our hypotheses were that dietary supplementation with omega-3 PUFAs would: improve symptoms of ADHD, increase phospholipid PUFA-status as assessed by cheek cell phospholipid composition and increase the rate of dopamine turnover as assessed by homovanillic acid (HVA)

excretion in urine. Further we expected that dietary supplementation with omega-3 PUFAs would improve cognitive control in ADHD and that it would increase activation in the associated prefrontal and striatal areas, as assessed with fMRI.

Materials and Methods

Participants

The study was approved by the Ethics Committee of the University Medical Centre Utrecht, The Netherlands, and took into account the ethical principles for medical research involving human subjects as stated in the declaration of Helsinki (amendment of Washington, 2002). Written and oral information was provided, after which written informed consent was obtained from all parents. All children provided written assent.

Forty boys between 8 and 14 years of age with a DSM-IV diagnosis of ADHD were recruited through the Department of Psychiatry at the University Medical Center in Utrecht, and through advertising. The clinical diagnosis was confirmed by a trained researcher using the Diagnostic Interview Schedule for Children – Parent Version (DISC-P). We chose to only include boys in this study as we wanted to minimize the number of potential confounds (such as gender) on brain activity and ADHD is more prevalent in boys than girls. The children with ADHD were either medication naïve or using psychostimulant medication (methylphenidate only). No other forms of psychoactive medication were accepted in this study (Table 1). Children with ADHD who were on stimulant medication were instructed not to take their medication for 24 hours prior to the fMRI-scan. However, children were allowed to use their medication throughout the intervention period (16 weeks). Medication continued to be managed by the outside provider (e.g. general practitioner, pediatrician, or psychiatrist). Any changes in medication status were recorded on a monthly basis by the research team.

Thirty-nine typically developing boys matched to the patients for age, hand preference and BMI (at inclusion), were recruited as a reference group (hereafter also referred to as RG) through advertising at primary and secondary schools in the wider Utrecht area, and from the pool of volunteers participating in studies by our lab.

All subjects were screened by telephone interview, to check for major neurological or psychiatric disorders, as well as psychiatric diagnoses in subjects and their first-degree relatives. Parents of the typically developing subjects participated in a DISC-P interview with trained researchers to confirm the absence of any psychiatric conditions and their first-degree relatives. None of the typically developing subjects were using any form of psychoactive medication.

IQ was assessed for all participants using a four subtest short form of the Wechsler Intelligence Scale for Children (WISC-III: (Kort *et al*, 2005)). As is often reported, children with ADHD had a slightly lower total IQ score than typically developing children (Table 1). As this is typical of the ADHD-phenotype, IQ was not entered as a covariate in any of the designs to prevent partialling out variance that is potentially relevant to the disorder (Dennis *et al*, 2009; de Zeeuw *et al*, 2012). Hand preference was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). There were no significant differences in hand preference, nor were there any differences in age, parental education (measured in years), and Body Mass-Index (BMI) (Table 1).

<Table 1> Demographic data and compliance: insert about here

<Figure 1> Insert about here

Design

The 16-week intervention followed a double-blind randomized placebo-controlled design, where investigators, parents and participants were all blind to the treatment conditions (Figure 1). The 2x2 factorial design included four groups: Children with ADHD receiving either placebo or omega-3 fortified margarine ($ADHD^{Placebo}$ and $ADHD^{Active}$, respectively) and children from the reference group receiving the same treatment

($RG^{Placebo}$ and RG^{Active}). All participants were randomly assigned to one of the treatment conditions by a member of the Unilever Center for Nutritional Intervention Trials.

Intervention

This trial was registered at clinicaltrials.gov under number NCT01554462. All participants were instructed to consume a daily dose of 10 grams of either normal or omega-3 fortified margarine. The active product was full fat (80%) margarine, containing 650mg DHA and 650mg EPA per 10 gram serving. The dose of the active ingredients DHA and EPA in the intervention product was under the US Generally Recognized As Safe (GRAS) level (FDA, 2004*).* The placebo product was a similar margarine with the same sensory properties, however with mono-unsaturated fatty acids (refined plant oils) instead of EPA and DHA; the total amount of saturated fatty acids and omega-6 fatty acid were matched in the placebo and active product (See Supplemental Materials 1 for more details).

Compliance was assessed by weighing the leftover products that parents returned to the investigators on a monthly basis. Furthermore, participants and parents kept a calendar, on which daily margarine consumption was recorded. Participants were asked to maintain their usual diet throughout the intervention period, with the following constraints: they were not allowed to use other supplements containing omega-3 or foods fortified with EPA and DHA during the intervention, nor were they to consume fatty fish more than once a week. Compliance to these requirements was measured on a monthly basis using the Diet and Lifestyle Change Questionnaire (DLCQ).

Physiological and behavioral measures

During the baseline and follow-up visits, cheek cell samples were collected using cotton swabs for analysis of phospholipid fatty acid levels. This method was chosen over

blood sampling in children, as it is not invasive. Analyses of fatty acids were performed by gas chromatography after phospholipid isolation by thin layer chromatography as described previously (Koletzko *et al*, 1999). Furthermore, urine samples were collected to measure the HVA to creatinine ratio, as a proxy for dopamine turnover. The Essential Fatty Acids Questionnaire (EFAQ) was collected to assess symptoms of FA deficiency (See Supplement Materials 1 for methodological details).

Finally, the parent-rated Child Behavior Checklist (CBCL) and Strengths and Weaknesses of ADHD symptoms and Normal behavior scale (SWAN) were collected for all participants, both of which measure the severity of ADHD-symptoms (see Figure 1). The CBCL (collected twice) was used as the primary outcome measure, while the SWAN (collected five times) was used to measure change over time. In addition to the parent-rated questionnaires, the Teacher Report Form (TRF) was sent out to classroom teachers or mentors of the participants at baseline and follow-up. However, the response rate for this instrument was very low (54 of 79 returned at baseline, 31 of 76 at follow-up), and we had to exclude these data from further analysis.

fMRI acquisition and analysis

The fMRI-study included a traditional Go-NoGo paradigm that has been described in previous work (Durston *et al*, 2003, 2006, 2009). Standard preprocessing was performed in SPM8 (Wellcome Dept. of Cognitive Neurology, www.fil.ion.ucl.ac.uk). MRI-scans were excluded if head motion exceeded 3mm or the size of one voxel at baseline and/or follow-up. Hence, 13 children with ADHD and 6 typically developing children were excluded from the SPM fMRI-analysis. For a full description of the first and second-level analysis of the fMRI study please, see Supplemental Material 2.

Statistics

All statistical analyses on the demographic, behavioral and physiological measures were performed with the SPSS statistical package 20.0 (SPSS Inc., Chicago, Illinois). Group differences on the demographic variables were analyzed using chi-square, independent samples T-tests or Mann-Whitney U-tests, as appropriate.

For the behavioral and physiological measures, independent samples T-tests or Mann-Whitney U-tests were used to analyze group differences at baseline between the diagnostic groups, and between the intervention groups. To investigate treatment effects, all variables of interest were fed into an ANCOVA (analysis of covariance) model with the baseline measurement entered as a covariate (Vickers, 2005). Intention-to-treat (ITT) analyses, including all subjects randomized into the trial, were performed using a Linear Mixed Effects (LME) model with Restricted Maximum Likelihood (REML) estimation where diagnostic status and treatment condition were included as fixed factors. Mean Adjusted Difference (MAD) and 95% Confidence Intervals (CI) were also calculated.

Initially, age included as a covariate in the analyses. However, it did not correlate with any of the measures of interest, and as such it was excluded from the final models. Significant findings were further analyzed using paired or independent samples t-tests. Pearson's correlations were performed to analyze the relationship between behavioral and physiological measures. Finally, a post-hoc power-analysis was conducted to determine the retrospective power of the study.

Results

After inclusion, 77 children successfully completed the study and participated in both fMRI sessions. One $RG^{placebo}$ participant dropped out after three months for personal reasons. One RG^{active} participant dropped out after developing a skin rash that later turned out to be unrelated to the test product. After dropping out of the study, the parents of this second participant were notified about the contents of the test product by an independent member of Unilever Center for Nutritional Intervention Trial. The researchers remained blind throughout the whole study period. Compliance was considered acceptable if two-thirds of the test-product was consumed during the intervention period, with no periods of non-consumption longer than seven days (e.g., due to illness). One participant in the $ADHD^{placebo}$ group did not meet these criteria, and was excluded from the analyses. There were no differences in compliance between the four treatment groups (X^2= 2.733, p= .435, Table 1).

Behavioral results

At baseline, subjects with ADHD scored higher than the reference group on the CBCL subscales Attention Problems (p< .001, see: Table 2), Rule Breaking Behavior (p< .001), and Aggressive Behavior (p< .001). CBCL-scores did not correlate with age in either diagnostic group. Completer analyses by means of ANCOVA showed a main effect of diagnostic status (F(1,67)=6.92, p=.011) at follow-up, where children with ADHD scored higher on CBCL attention problems. ANCOVA further showed a main effect of treatment condition, where after supplementation with omega-3 PUFAs, scores on CBCL attention problems were reduced in comparison to supplementation with placebo (Figure 2A: F(1,67)=14.99, p<.001). Although there was no interaction between diagnostic status and treatment condition, it should be noted, that the typically developing group receiving active treatment did not show a significant reduction in attention problems. The Intention-

to-treat (ITT) analysis using a Linear Mixed Effects (LME) model yielded similar results for CBCL attention problems showing a significant effect of diagnostic status (MAD = 6.37, 95% CI [8.31, 4.42], t(90)= 6.51, p < .001) and an interaction effect between treatment condition and time (MAD = -1.83, 95% CI [-2.91, -0.76], t(67)= 3.42, p = .001). There were no significant effects of treatment on the CBCL Rule Breaking and Aggressive Behavior subscales, or on the SWAN questionnaire.

Three children in the $ADHD^{Active}$ group and four children in the $ADHD^{Placebo}$ group reported that the dosage of their stimulant medication had been increased during the intervention period. The treatment effect on the Attention Problems subscale was still found in a re-analysis of the CBCL-data excluding these participants (ANCOVA: F(1,61)=14.22, p < .001, LME: MAD = -1.91, 95% CI [-3.10, -0.77], t(60)= 3.34, p = .001).

<Table 2> Behavioral and physiological measures per treatment group: insert about here

Physiological results

Scores on the Essential Fatty Acids Questionnaire (Table 2) were normalized by square root transformation. At baseline, there was a difference between children with ADHD and children in the reference group on the EFAQ (t(75)=2.72, p=.008), where children with ADHD scored higher on symptoms of FA deficiency. However, only four children met cut-off criteria for FA deficiency (3 children with ADHD, 1 RG). There was no significant effect of supplementation with omega-3 PUFAs on the EFAQ, neither for subjects with ADHD or the reference group.

Analysis of the square-root transformed cheek cell phospholipids showed no differences between the four groups at baseline (F(3,69)= 1.92, p=.135). At follow-up, there was a main effect of treatment (F(1,63)=13.03, p= .001), where %DHA in

phospholipids was higher after supplementation with omega-3 PUFA's in comparison to supplementation with the placebo product (see Figure 2B and Table 2). The ITT analysis accounted for missing data at either baseline (6 samples) or follow-up (8 samples) and similarly showed an interaction between treatment condition and time (MAD = 0.34, 95% CI [0.14, 0.53], t(72)= 3.47, p = .001). For some samples, the amount of cheek cell phospholipids was too low to assess DHA levels reliably, leading to floor values of 0 for a number of samples (24 at baseline, 21 at follow-up). Re-analysis without these samples showed the same effect (ANCOVA: F(1,29) = 8.00, p= .009), LME: MAD = 0.28, 95% CI [0.12, 0.44], t(51)= 3.48, p = .001). However, these results should be interpreted with caution, as cheek cell fatty acid data were below detection threshold for some subjects, and missing for others.

Follow-up Pearson's correlation analyses showed that at baseline CBCL attention problems correlated negatively with omega-3 LC-PUFA status in the group of children with ADHD as a whole (*r* = -.47, p= .048; see Figure 3A), but not in children in the reference group (*r* = .28, p= .166). The correlation in the ADHD-group persisted at follow-up (*r* = -.48, p= 0.042) (see Figure 3B).

The data on the HVA to creatinine ratio were normalized using a natural logarithm. At baseline, the difference in the HVA-ratio (µmol/mmol creatinine) approached significance (t(72)= 1.92, p= .059), with subjects with ADHD having slightly higher levels of this ratio than the reference group. No effects of the intervention were found on these measures, including in analyses with age or BMI included as covariates in the design.

< Figure 2 & 3 insert about here>

fMRI Task

Results typical of this task were found (Durston *et al*, 2003, 2006); see Supplemental Materials 2 and 3 for a detailed description of the fMRI results. There was no effect of dietary omega-3 supplementation on task-performance, or brain activation during the cognitive control task.

Discussion

Omega-3 PUFA dietary supplementation improved symptoms of inattention in boys with *and* without ADHD in a double blind randomized controlled trial. This effect did not appear to be mediated by dopaminergic cognitive control networks, as measures of dopamine turnover and neural activity during cognitive control were unaffected by the intervention.

At baseline, boys with ADHD had higher symptoms of inattention than typically developing boys. Furthermore, there was an effect of treatment on parent-rated symptoms of ADHD, regardless of diagnosis. This effect was driven by the measures of inattention at follow-up: subjects who had received omega-3 PUFAs had lower scores on the CBCL attention problems subscale than subjects on placebo. This ties in with earlier studies that have suggested that omega-3 PUFA supplementation improves symptoms of inattention specifically, and not symptoms of ADHD more generally (Gustafsson *et al*, 2010; Richardson and Montgomery, 2005; Sinn and Bryan, 2007). In line with recent meta-analyses (Bloch and Qawasmi, 2011; Sonuga-Barke *et al*, 2013), our results suggest that supplementation with omega-3 PUFAs may be beneficial in ADHD.

Moreover, our results indicate that typically developing children also benefit, showing the importance of omega-3 PUFA intake during development in general (Eilander *et al*, 2007; Schuchardt *et al*, 2010). Previous studies using sustained attention tasks have shown that dietary supplementation of omega-3 PUFAs leads to increases in brain activation in prefrontal areas and improved task performance in both children with ADHD and typically developing children (Bauer *et al*, 2014; Mcnamara *et al*, 2010; McNamara *et al*, 2013; Vaisman *et al*, 2008). We used a cognitive control task and found no changes in brain activity or task performance after supplementation. Furthermore, dopamine turnover did not appear to be affected by the supplementation, as HVA levels

in urine were not affected. Therefore, it appears that the neural mechanism underlying improvements in attention did not involve dopaminergic cognitive control networks.

The dietary intervention affected omega-3 PUFA levels in cheek cell phospholipids, as the level of DHA was significantly higher at follow-up for subjects who had been treated with the active product than for those who had received placebo. This observation was supported by the returned margarine tubs and diaries, and showed that the intervention was effective. Furthermore, in subjects with ADHD, higher levels of DHA were associated with lower attention problems, both at baseline and follow-up. The detected increase in DHA levels was more modest than in earlier studies where DHA was measured in plasma or erythrocytes (Muthayya *et al*, 2009; Osendarp *et al*, 2007). Cheek cell samples are more prone to contamination than blood samples and this may have contributed to this difference. An alternative explanation may be that the intervention was less effective than expected.

Most studies to date have suggested that omega-3 supplementation is effective through higher levels of EPA as opposed to DHA (Bloch and Qawasmi, 2011; Gustafsson *et al*, 2010). The biological mechanism through which DHA acts on human brain function is not clear, even though this fatty acid is abundantly present in the brain's phospholipid membranes (Bazinet and Layé, 2014; Horrocks and Farooqui, 2004). As biosynthesis of DHA from its precursors α-linolenic acid and EPA is very low, a notable increase in DHA plasma level can only be accomplished through direct DHA intake (Plourde and Cunnane, 2007). Many of the omega-3 supplements used in previous studies, with often negative results, contained a relatively low DHA to EPA ratio, possibly obscuring direct effects of DHA. However, a recent H-MRS study by McNamara and colleagues (McNamara *et al*, 2013) showed that higher erythrocyte DHA-levels were related to a better performance on a sustained attention task and ACC metabolic function in typically developing children. The results from previous work combined with

the current study suggest that the combined supplementation with EPA and DHA accounts for the treatment effect in the present study and may thus yield the greatest improvements in behavior.

Although evidence of a moderate effect of dietary omega-3 PUFA supplementation is converging, it is unlikely that it will ever achieve the same behavioral improvements as stimulant medication in ADHD, with effect sizes ranging from 0.54-0.78 (Schachter *et al*, 2001). However, it *is* noteworthy that in the present study the majority of children with ADHD received the omega-3 PUFA supplements in addition to their regular medication. The behavioral improvement persisted even when changes in medication were taken into account in the analyses. This suggests that omega-3 PUFAs may be useful as an augmentation to standard pharmacological therapies. Indeed, recent reports showed that children receiving a combination of MPH and omega-3 PUFAs needed lower doses of MPH compared to children only receiving MPH to achieve the same clinical benefits (Barragán *et al*, 2014; MTA Cooperative Group, 1999).

Obvious strengths of our study include the randomized placebo-controlled double-blind design and the inclusion of a typically developing reference group to assess whether effects were specific to ADHD. However, there are also some limitations that should be taken into consideration. The first limitation is that in some subjects the quality of the cheek cell samples did not permit a reliable detection of DHA. However, this did not affect our statistical analyses negatively, as the effect of the dietary intervention reached statistical significance even without these values included. Phospholipid values did not show the expected extent of separation between placebo and intervention groups. However, these measures did differ between the active product and placebo intervention groups, and correlated with behavioral outcomes. A second limitation is that a small number of participants with ADHD had changes made to their medication during

the intervention. However, re-analysis without these participants showed that the effect of the intervention still held without these subjects. Finally, sample sizes in the fMRI study were smaller than in the main study as a result of subject motion. However, the remaining sample was well-matched and yielded only a tiny effect size of the intervention on MR-signal. We conducted a post-hoc power-analysis to investigate what sample size would be necessary to show an effect of the intervention on brain activity, if this was indeed a real effect. The results of this power analysis indicated that only a huge sample size (N=718) would have shown a significant effect of treatment and suggests therefore that the non-significant differences in this study are most likely attributable to noise. Taken together, the absence of a treatment effect on the brain activity during a cognitive control task, on performance of the task and on dopamine turnover suggest that the effect of the intervention on ADHD symptoms is unlikely to be mediated by dopamine systems.

In conclusion, this study provides new evidence that dietary supplementation using omega-3 PUFAs may be an effective augmentation of pharmacological treatments of ADHD. This effect does not appear to be mediated by dopaminergic cognitive control networks, but may involve other systems implicated in ADHD, such as attention networks.

Funding and disclosure: This study was financially supported by Unilever Research & Development, Vlaardingen, the Netherlands. Unilever Research & Development was involved in the conception and design of the study. They provided financial support for research staff to run the study and provided the intervention product. Sarah Durston and the staff at the NICHE lab at the Department of Psychiatry of the UMC Utrecht were responsible for data collection and analysis, and for drafting and deciding upon the final content of the manuscript. Marco Hoeksma and Ans Eilander are employees of Unilever. None of the other authors declare any conflicts of interest.

Acknowledgements: The authors would like to thank all the participants and their parents for their commitment to this study. Further, we would like to thank two anonymous reviewers for their constructive input and Dr. Harry Hiemstra for his valuable advice.

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Figure Legends

Figure 1. Trial design. Schematic overview of the design of this double-blind randomized placebo-controlled trial, including all measures that were collected and the number of participants that were included at baseline. After one, two and three months interim visits took place during which compliance and behavior were measured.

ADHD = Attention Deficit/Hyperactivity Disorder, CBCL = Child Behavior Checklist, DLCQ = Diet and Lifestyle Change Questionnaire, EFAQ = Essential Fatty Acids Questionnaire, SWAN = Strengths and Weaknesses of ADHD symptoms and Normal behavior scale, RG = Reference group of typically developing children.

Figure 2. Main effect of Omega-3 PUFA supplementation. Panel A shows the mean difference between baseline and follow-up CBCL attention problems in both diagnostic groups, with main effects of diagnosis and the intervention. Panel B shows the mean difference between baseline and follow-up square-root transformed %DHA-levels as collected from cheek cell samples, with similar main effects. The asterisks denote significance at $p < .01$.

ADHD = Attention Deficit/Hyperactivity Disorder, CBCL = Child Behavior Checklist, DHA = docosahexaenoic acid, RG = Reference group of typically developing children.

Figure 3. The relation between omega-3 fatty acids and attention problems in ADHD, at baseline and at follow-up. A) Correlation at baseline between square-root transformed %DHA and CBCL attention problems in children with ADHD (r = -.47). B) The same correlation at follow-up (r = -.48). There was no correlation between %DHA and CBCL attention problems in typically developing children.

ADHD = Attention Deficit/Hyperactivity Disorder, CBCL = Child Behavior Checklist, DHA = docosahexaenoic acid

Table 1. Demographic data and compliance

		ADHD N=40	**RG** N=39	***p***
General				
Age	Mean ± SD (range)	**10.3** ± 2.0 (8.0 – 15.0)	**10.9** ± 2.0 (8.2 – 15.1)	.163
Total IQ	Mean ± SD (range)	**104.3** ± 16.2 (76 – 144)	**113.6** ± 17.4 (75 – 145)	.017*
Parental Education in years	Mean ± SD	**13.5** ± 2.0	**13.9** ± 2.5	.422
Hand preference	N Right/left/ambidextrous	39/0/1	37/0/2	.610
Medication (MPH)	N	38/40	-	
Intervention				
Body Mass Index	Mean ± SD (range)	**16.8** ± 2.8 (12.8 – 26.3)	**17.7** ± 2.1 (14.0 – 22.5)	.122
Percentage compliance	Mean ± SD	**92.2** ± 6.9	**91.4** ± 6.5	.573

Abbreviations: ADHD = Attention-Deficit/Hyperactivity Disorder, IQ = Intelligence Quotient, MPH = methylphenidate, RG = Reference group of typically developing children, SD = Standard deviation

Table 2. Behavioral and physiological measures per treatment group

	Baseline				Follow-up			
	RG		**ADHD**		**RG**		**ADHD**	
	Placebo	*Active*	*Placebo*	*Active*	*Placebo*	*Active*	*Placebo*	*Active*
Behavior – mean (SD)								
CBCL ADHD[a]	1.9 (2.1)	2.1 (2.3)	9.0 (3.1)	8.8 (2.1)	2.5 (2.3)	1.8 (1.6)	10.1 (2.2)	7.6 (3.5)
CBCL Attention Problems	2.7 (2.8)	2.5 (3.6)	8.9 (3.5)	9.1 (2.5)	**3.4 (2.8)***	**2.4 (2.6)***	**10.5 (3.3)***	**7.7 (3.0)***
CBCL Rule Breaking	1.4 (2.2)	0.7 (0.9)	3.8 (2.3)	3.1 (2.8)	1.0 (1.5)	1.2 (1.8)	4.1 (2.4)	2.4 (1.9)
CBCL Aggressive Behavior	2.2 (3.7)	2.2 (2.7)	11.0 (5.8)	9.7 (7.2)	1.8 (3.1)	2.7 (3.2)	11.8 (5.4)	7.8 (3.5)
Physiology – mean (SD)								
% DHA (C22:6 n-3)[b]	.58 (.23)	.54 (.24)	.67 (.19)	.49 (.13)	**.48 (.19)♀**	**.68 (.24)♀**	**.54 (.15)♀**	**.67 (.27)♀**
Urine Samples HVA[c]	1.4 (0.2)	1.4 (0.3)	1.5 (0.3)	1.5 (0.4)	1.3 (0.2)	1.3 (0.3)	1.4 (0.3)	1.5 (0.3)
EFAQ[d]	1.3 (2.3)	0.9 (1.8)	1.8 (1.7)	1.8 (2.1)	1.3 (2.3)	0.5 (0.9)	1.6 (2.0)	1.8 (1.7)

* Main effect of diagnosis and treatment group at follow-up (ANCOVA & LME: $p \leq .001$)

♀ Main effect of treatment group at follow-up (ANCOVA & LME: $p < .001$)

Missing data per treatment group

[a] $RG^{placebo}$: 1 baseline, 2 follow-up; RG^{active}: 2 follow-up; $ADHD^{active}$: 1 baseline, 6 follow-up

[b] $RG^{placebo}$: 1 baseline, 2 follow-up; RG^{active}: 1 baseline, 2 follow-up; $ADHD^{placebo}$: 3 baseline, 1 follow-up; $ADHD^{active}$: 1 baseline, 2 follow-up, 1 both

[c] $RG^{placebo}$: 2 follow-up; RG^{active}: 1 baseline, 2 follow-up; $ADHD^{active}$: 1 baseline, 2 follow-up

[d] $RG^{placebo}$: 2 follow-up; RG^{active}: 1 follow-up; $ADHD^{active}$: 1 baseline, 1 follow-up

Abbreviations: ADHD = Attention-Deficit/Hyperactivity Disorder, CBCL = Child Behavior Checklist, DHA = Docosahexaenoic Acid, EFAQ = Essential Fatty Acids Questionnaire, HVA = Homovanillic Acid, RG = Reference group of typically developing children, SD = Standard deviation