JOURNAL
OF THE CANADIAN ACADEMY OF CHILD AND ADOLESCENT PSYCHIATRY / DE L'ACADÉMIE CANADIENNE DE PSYCHIATRIE DE L'ENFANT ET DE L'ADOLESCENT

RESEARCH ARTICLE

Omega-6 to Omega-3 Fatty Acid Ratio in Patients with ADHD: A Meta-Analysis

Laura LaChance MD[1]; Kwame McKenzie BM, MRCPsych[1,2,3]; Valerie H. Taylor MD, PhD, FRCPC[1,4]; Simone N. Vigod MD, MSc, FRCPC[1,4]

Abstract

Objective: Omega-3 and omega-6 fatty acids have been shown to be deficient in individuals with attention deficit/hyperactivity disorder compared to controls (Hawkey & Nigg, 2014). Clinical trials of omega-3 and omega-6 supplements as treatment for ADHD have demonstrated minimal efficacy (Bloch & Qawasmi, 2011; Gillies, Sinn, Lad, Leach, & Ross, 2011; Hawkey & Nigg, 2014; Puri & Martins, 2014; Sonuga-Barke et al., 2013). Existing trials have analyzed omega-3 and omega-6 separately although the tissue ratio of these fatty acids (n6/n3) may be more important than absolute levels of either. The objective of this study was to determine the relationship between blood n6/n3 and arachidonic acid to eicosapentaenoic acid (AA/EPA), to ADHD symptoms. **Method**: A systematic literature review identified original articles measuring blood n6/n3 or AA/EPA ratio in children and youth with ADHD, compared to controls without ADHD. Three databases were searched. Blood n6/n3, and AA/EPA ratios were compared between individuals with ADHD and controls. Results were pooled across studies using quantitative synthesis. **Results**: Five articles met inclusion criteria for the meta-analysis. The pooled mean difference between patients with ADHD and controls was 1.97 (0.90-3.04) for n6/n3 (n=5 studies, I^2 83%) and 8.25 (5.94-10.56) for AA/EPA (n=3 studies, I^2 0%). **Conclusions**: Children and youth with ADHD have elevated ratios of both blood n6/n3 and AA/EPA fatty acids compared to controls. Thus an elevated n6/n3, and more specifically AA/EPA, ratio may represent the underlying disturbance in essential fatty acid levels in patients with ADHD. These findings have implications for the development of future interventions using essential fatty acids to treat ADHD, and for the use of these ratios as biomarkers for titrating and monitoring ADHD treatment with essential fatty acids.

Key Words: attention deficit/hyperactivity disorder, omega-3 fatty acids, omega-6 fatty acids

Résumé

Objectif: Les acides gras omega-3 et omega-6 se sont révélés être déficients chez les personnes souffrant du trouble de déficit de l'attention avec hyperactivité (TDAH) comparativement aux sujets témoins (Hawkey et Nigg, 2014). Les essais cliniques sur les suppléments d'omega-3 et d'omega-6 comme traitement du TDAH ont démontré une efficacité minimale (Bloch et Qawasmi, 2011; Gillies, Sinn, Lad, Leach, et Ross, 2011; Hawkey et Nigg, 2014; Puri et Martins, 2014; Sonuga-Barke et al., 2013). Les essais existants ont analysé les omega-3 et omega-6 séparément, bien que le ratio tissulaire de ces acides gras (n-6/n-3) puisse être plus important que les niveaux absolus de chacun. L'objectif de cette étude était de déterminer la relation entre le n-6/n-3 sanguin et le ratio acide arachidonique sur acide eicosapentaénoïque (AA/EPA), et les symptômes du TDAH. **Méthode:** Une revue systématique de la littérature a identifié les articles originaux mesurant le n-6/n-3 sanguin ou le ratio AA/EPA chez les enfants et les adolescents souffrant du TDAH, comparativement aux sujets témoins sans TDAH. Trois bases de données ont été recherchées. Le n-6/n-3 sanguin et les ratios AA/EPA ont été comparés entre les personnes souffrant du TDAH et les sujets témoins. Les résultats ont été totalisés entre les études à l'aide d'une synthèse quantitative. **Résultats:** Cinq articles satisfaisaient aux critères d'inclusion de la

[1]University of Toronto, Department of Psychiatry, Toronto, Ontario
[2]Centre for Addiction and Mental Health, Social Aetiology of Mental Illness, Toronto, Ontario
[3]Wellesley Institute, Toronto, Ontario
[4]Women's College Hospital and Women's College Research Institute, Toronto, Ontario

Corresponding E-Mail: laura.lachance@mail.utoronto.ca

Submitted: June 15, 2015; Accepted: January 15, 2016

méta-analyse. La différence moyenne totalisée entre les patients souffrant de TDAH et les sujets témoins était de 1,97 (0,90-3,04) pour n-6/n-3 (n = 5 études, I^2 83 %) et de 8,25 (5,94-10,56) pour AA/EPA (n = 3 études, I^2 0 %). **Conclusions:** Les enfants et les adolescents souffrant du TDAH ont des ratios élevés tant du n-6/n-3 sanguin que des acides gras AA/EPA, comparativement aux sujets témoins. Donc, un ratio n-6/n-3 élevé, et plus spécifiquement le ratio AA/EPA, peuvent représenter la perturbation sous-jacente des niveaux d'acides gras essentiels chez les patients souffrant de TDAH. Ces résultats ont des implications pour le développement de futures interventions utilisant des acides gras essentiels pour traiter le TDAH, et pour l'utilisation de ces ratios comme biomarqueurs pour titrer et surveiller le traitement du TDAH avec les acides gras essentiels.

Mots clés: *trouble de déficit de l'attention avec hyperactivité, acides gras omega-3, acides gras omega-6*

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by symptoms of inattention, hyperactivity, and impulsivity (Association, 2013; Organization, 2004). This pattern of behaviour is present in multiple settings and results in significant impairment in social, academic, and/or work function. ADHD is a common disorder with a prevalence of 5% in children and is associated with significant adverse outcomes such as poor educational functioning and increased rates of motor vehicle collisions, injury, and substance use (Association, 2013; Erskine et al., 2014). According to a recent meta-regression analysis, the prevalence of this disorder has been stable across the last three decades (Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014).

Existing treatments for ADHD include a combination of pharmacological and non-pharmacological interventions including cognitive training, neurofeedback, parent education, and behavioral change interventions (Sonuga-Barke et al., 2013). According to clinical practice guidelines, non-pharmacological interventions are considered first line treatments either alone or in combination with medications for children and adolescents with ADHD (Kendall, Taylor, Perez, & Taylor, 2008; SUBCOMMITTEE ON ATTENTION-DEFICIT/HYPERACTIVITY DISORDER & MANAGEMENT, 2011). Limitations of these non-pharmacological treatment options may include lack of established efficacy or access (SUBCOMMITTEE ON ATTENTION-DEFICIT/HYPERACTIVITY DISORDER & MANAGEMENT, 2011). Medication is highly effective for the treatment of ADHD symptoms in many individuals, with a recent meta-analysis reporting effect sizes for methylphenidate and amphetamine to be 0.72 and 0.99, respectively (S. Faraone & Buitelaar, 2010). However, concern regarding potential short and long-term adverse effects on sleep, appetite, and growth, and a desire for non-pharmacological interventions limit the use of medication for treatment (Sonuga-Barke et al., 2013). Non-stimulant psychopharmacological treatments for ADHD have different side effect profiles although lower relative efficacy (S. V. Faraone, Biederman, Spencer, & Aleadri, 2006). These factors have in part motivated the search for alternative and complimentary treatments (Graham et al., 2011).

One key area of interest is dietary supplementation using essential polyunsaturated fatty acids (PUFAs) such as omega-3 and omega-6 fatty acids. These fatty acids are required for normal neurodevelopment and neuronal functioning (M Zeman, R. Jirak, M Vecka, J Raboch, & A Zak, 2012) and cannot be synthesized by the human body. Thus, they must be taken in through the diet. Omega-3 and omega-6 fatty acids have been shown to be deficient in individuals with ADHD compared to controls (Hawkey & Nigg, 2014). However, randomized controlled trials of omega-3 and omega-6 supplementation to date have demonstrated only minimal efficacy (Bloch & Qawasmi, 2011; Gillies et al., 2011; Hawkey & Nigg, 2014; Puri & Martins, 2014; Sonuga-Barke et al., 2013). An important limitation to existing trials is that the effects of omega-3 and omega-6 are usually analyzed separately. This is problematic because the ratio of n6/n3 in blood may be more important than the absolute levels. Biological research supports this hypothesis, demonstrating that high blood levels of omega-6 relative to omega-3 fatty acids leads to an overproduction of pro-inflammatory cytokines (Simopoulos, 2011). The western dietary pattern is rich in omega-6 fatty acids and is relatively poor in omega-3 fatty acids. Notably, children and adolescents who adhere to a western dietary pattern have an increased risk of ADHD (Howard et al., 2011). It is possible that omega-3 supplementation studies have failed to consistently yield positive results because none of the studies have considered the impact of omega-6 fatty acid consumption, or the resultant blood ratios.

The main objective of this study was to conduct a meta-analysis of blood n6/n3 ratios in children and youth with ADHD, compared to controls. We also measured the ratio of arachidonic acid (AA) to eicosapenaenoic acid (EPA) (AA/EPA), because these represent biologically active forms of omega-6 and omega-3 fatty acids respectively in the brain (Adams, Lawson, Sanigorski, & Sinclair, 1996; Lotrich, Sears, & McNamara, 2013). We hypothesize that children and youth with ADHD would have an elevated n6/n3 and AA/ EPA ratio compared to controls.

Methods

Search Strategy

A computerized search of OVID Medline, PsycINFO, Embase, and Embase Classic was performed. All abstracts from 1980 to April, 2014 were retrieved using the following MeSH terms on OVID Medline: "Fatty acid, omega-3" (MeSH since 1990), "Fatty acid, omega-6" (MeSH since 2004), "Fatty acid, unsaturated" (MeSH dating back to 1966), along with their associated keywords. These results were combined with the MeSH terms: "Attention deficit disorder with hyperactivity" (MeSH since 1984) and "hyperkinetic syndrome" (MeSH prior to 1984) and their associated keywords. PsycINFO was searched using the descriptor "Fatty Acids" and several associated keywords in combination with the descriptors "Attention deficit disorder" and "attention deficit disorder with hyperactivity" and associated keywords. A similar strategy was used to search the databases, Embase and Embase Classic from 1947 to April 2014; specifically, we used the Em-Tree term "attention deficit disorder" and associated keywords in combination with the Em-Tree terms "omega-3 fatty acid, omega-6 fatty acid, unsaturated fatty acid, essential fatty acid, polyunsaturated fatty acid" and associated keywords. All abstracts generated by the systematic search were screened for possible inclusion by two raters (LL, SV) to assess whether they warranted a full-text assessment, and such papers were read in full to determine whether they met criteria for inclusion in the meta-analysis. For papers that met the inclusion criteria, all references were hand searched. Any additional citations that were of interest were retrieved. All five relevant articles were also forward tracked to search for additional articles to include. Study authors were contacted to obtain original data when it was not included in the articles. This meta-analysis was performed and reported according to Meta-Analysis of Observational Studies in Epidemiology MOOSE guidelines.

Selection criteria

We included studies of any design that were peer-reviewed and published in English where the population was restricted to children and youth (< age 25) (Dahl, 2004), with a diagnosis of ADHD confirmed by a physician or psychologist clinical interview and/or Conners' Parent or Teacher rating scale, compared to controls without ADHD. We required blood level measurements that allowed for calculation of n6/n3 fatty acid ratios (n6/n3) and/or AA/EPA ratios (AA/EPA) in those with ADHD and controls. Fatty acids were measured in plasma or RBC phospholipids or as free (non-esterifed) fatty acids in plasma or RBC membranes. They were reported as a percentage of total fatty acids with a mean and standard deviation. There is significant heterogeneity with respect to measuring and reporting tissue fatty acid levels and ratios. Therefore, we chose to limit our analysis to measures in the blood that have been studied in the existing literature and represent fatty acids that have been incorporated into adipose tissue or cell membranes. Specifically, we chose not to include measures of fatty acid ratios that are acutely impacted by dietary intake (i.e. Plasma TAGs or CEs). To limit clinical heterogeneity both ADHD and control participants were required to be free from known medical conditions that could impact fatty acids levels or systemic inflammation such as systemic metabolic, inflammatory, autoimmune or infectious diseases.

Data Extraction

The following data was extracted from relevant papers: n6/n3 fatty acid ratio (primary outcome) and AA/EPA fatty acid ratio (secondary outcome), along with demographic and clinical information pertaining to the patient and control groups. The n6/n3 ratio was chosen as the primary outcome to maximize sensitivity of the current meta-analysis by increasing our sample size and statistical power. The AA/EPA ratio was chosen as a secondary and more specific outcome because these fatty acids have been shown to be the most biologically active omega-6 and omega-3 fatty acids in the brain (Simopoulos, 2011; M Zeman, R Jirak, M Vecka, J Raboch, & A Zak, 2012). Two authors independently extracted the data (LL, SV) and data extraction forms were reviewed, with inconsistencies resolved by consensus.

Quality Assessment

A quality assessment was conducted for the papers included in the meta-analysis. Quality assessment in meta-analyses of observational studies is not commonplace (Mallen, Peat, & Croft, 2006). A systematic review published by the Canadian Agency for Drugs and Technologies in Health recommends two quality assessment tools as most appropriate for observational studies: Downs & Black (1998) and SIGN 50 (2004). The authors suggest that if a certain item does not apply to the study design that is being assessed, that this item simply be omitted (Bai, Shukla, Bak, & Wells, 2012). We adapted a quality assessment tool that two of the study authors (LL, KM) had designed for a previous publication (Lachance & McKenzie, 2014), which was a meta-analysis of cross-sectional observational studies, for use in the current study and included relevant elements of the Downs & Black 1998 checklist for measuring study quality (Downs & Black, 1998). Specifically, our tool assessed how participants and controls were recruited, and if the control and patient groups were matched with respect to socio-demographic variables. We also considered whether the control group was representative of the general population. We examined how the diagnosis was confirmed in the patient group, and whether relevant potential confounders were taken into account. We compiled a list of potential confounders based on the existing literature (Ferrucci et al., 2006; Henning, Ruud van Tuijl, Hofman, Kiliaan, & Breteler, 2003). The list of relevant potential confounders included the following: dietary omega-3 consumption,

dietary omega-6 consumption, presence of an active medical condition, smoking, blood pressure, blood cholesterol or triglyceride levels, age, and gender. Socioeconomic status is a key determinant of dietary intake and thus tissue levels of omega-3 and omega-6 fatty acids, therefore we did not consider socioeconomic status as a separate confounder (Al-Tamer & Mahmood, 2006; Cohen, Garg, Ali, Harris, & Whooley, 2008; KR, NM, KM, K, & DA, 2011). Lastly, our tool included an assessment of the statistical methods and quality of data analysis. The quality assessment yielded a score out of 16. Two authors independently conducted the quality assessments for all studies (LL, SV) and inconsistencies were resolved by consensus.

Statistical Methods

Studies were analyzed using Review Manager 5.1 software (Review Manager (RevMan) [Computer program]. Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.). For continuous outcomes, mean difference and 95% confidence intervals were calculated using a random effects model. This model assumes that the underlying true effect varies from one study to another. If there is significant heterogeneity, a random effects model will give wider confidence intervals than a fixed effects model. Therefore, when significant heterogeneity is anticipated, a random effects model is more conservative (Higgins & Thompson, 2002). The n6/n3 and AA/EPA ratios were analyzed as continuous outcomes. When studies measured the relevant ratios in both RBC phospholipids and plasma total fatty acids, they were reported separately and then combined in the analysis. Pooled mean difference for each ratio was calculated and results were compiled into Forest Plots using the Mantel-Haenszel statistical method. A chi square test was performed to measure heterogeneity. A funnel plot was performed to assess for publication bias.

Results

From the initial searches, 654 articles were retrieved after duplicates were removed. All titles and abstracts were read, and 619 papers were excluded as not relevant to the current topic at this level. The remaining 36 articles were read completely and five met inclusion criteria for the meta-analysis (Figure 1, PRISMA Flow Diagram). The five studies included 250 children and youth with ADHD and 235 control

Characteristics of included studies can be found in Table 1. Five studies measured the n6/n3 ratio in children and youth with ADHD vs. controls, and three measured the AA/EPA ratio. When a study reported a certain fatty acid ratio in multiple components of the blood using the same participants (i.e. RBC phospholipids and plasma non-esterified or free fatty acids), we chose to only analyze the more reliable marker for the general fatty acid pool. The fatty acid composition of plasma and erythrocyte (RBC) phospholipids is tightly regulated and reflects the types of fatty acids that have been incorporated into cell membranes. Plasma free fatty acids can be used as a surrogate marker of adipose tissue fatty acid composition, though this measure can be more acutely impacted by dietary intake as compared to measuring fatty acids in RBC membranes or plasma or RBC phospholipids (Hodson, Skeaff, & Fielding, 2008). All studies included in the meta-analysis scored between ten and 13 points out of a possible 16 in our quality assessment tool (Table 2, 3).

Mean fatty acid ratios for children and youth with ADHD and controls, mean differences between groups, and pooled mean differences are presented for the n6/n3 fatty acid ratio (Figure 2) and the AAEPA ratio (Figure 3). The pooled mean difference for the n6/n3 fatty acid ratio between children and youth with ADHD and controls was 1.97 (0.90-3.04). The pooled mean difference for the AA/EPA ratio between patients with ADHD and controls was 8.25 (5.94-10.56). Both were statistically significant. The funnel plot did not show evidence of publication bias (Figure 4).

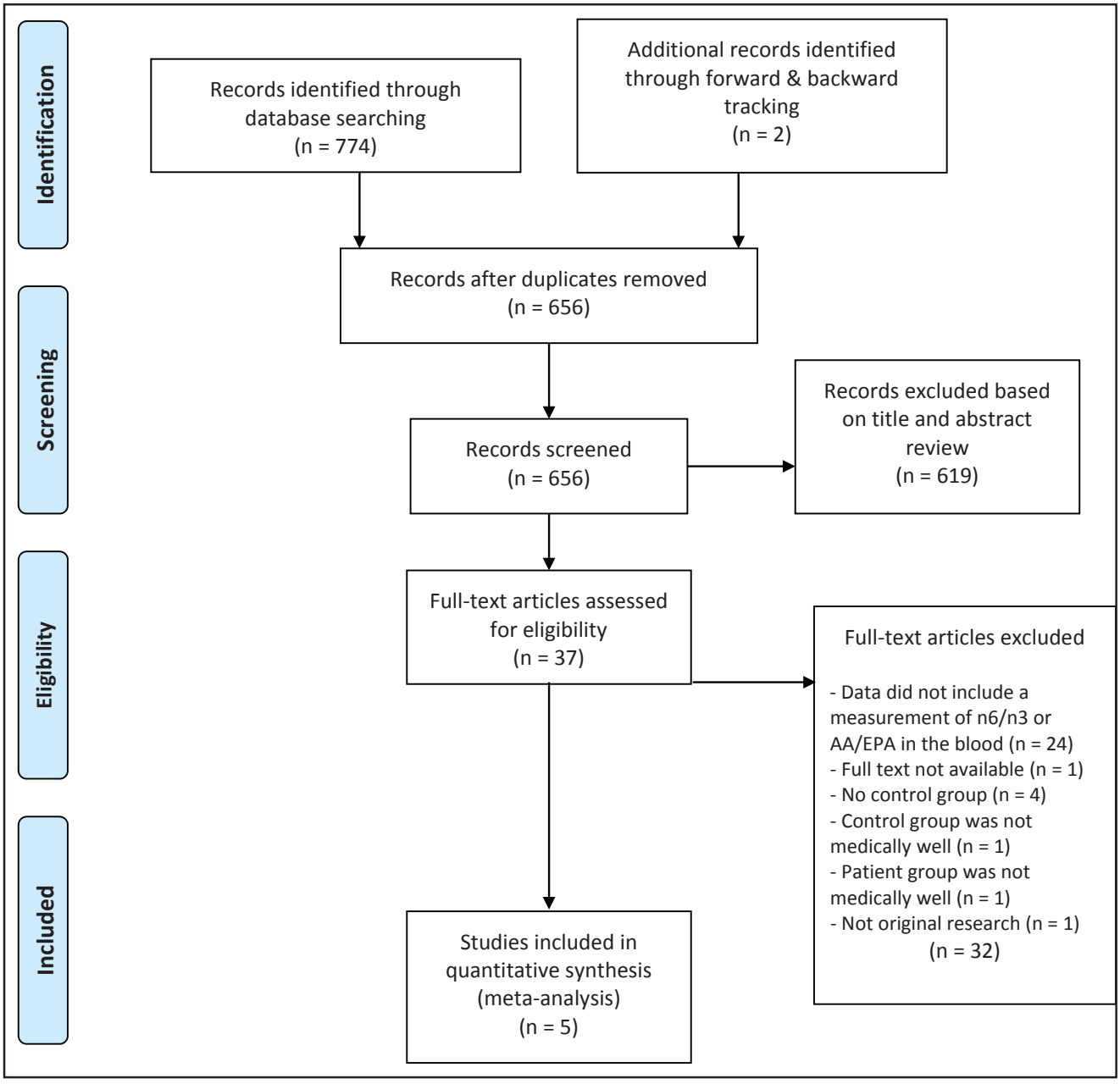
Discussion

Main Findings

The current study found that children and youth with ADHD had higher n6/n3 fatty acid ratios, and higher AA/EPA ratios compared to controls without ADHD. The effect size was largest for the AA/EPA ratio. Included studies were of high quality with minimal clinical heterogeneity, supporting the robustness of the findings.

Our results are consistent with numerous studies that support an association between ADHD and inflammation (Donev & Thome, 2010) as well as evidence that higher levels of omega-6 relative to omega-3 fatty acids in the peripheral blood can lead to an overproduction of pro-inflammatory cytokines (Simopoulos, 2011). In a recent open label study, eight weeks of EPA and DHA supplementation decreased plasma inflammatory mediators and oxidative stress in children with ADHD, although the impact of this on clinical ADHD symptoms was not assessed (Hariri et al., 2012). In addition, it is widely accepted that alterations in dopaminergic and noradrenergic pathways are associated with the pathophysiology of ADHD and that our pharmacologic treatments target these neurotransmitter systems. According to several lines of evidence from animal studies, n3 deficient animals appear to have hypofunctional dopaminergic systems at the mesocortical level, and hyperfunctional dopaminergic systems at the mesolimbic level among other abnormalities. These n3 deficient animals demonstrate impairments in working memory and conditioned learning tasks consistent with animal models of ADHD (Transler, Mitchell, & Eilander, 2013). Further research in this area is needed to clarify the mechanisms underlying these changes and whether they are reversible upon treatment with essential fatty acids.

Figure 1. Flow chart of the literature search and meta-analysis



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097 For more information, visit www.prisma-statement.org.

It is notable that in our study, there was a greater elevation in the AA/EPA ratio in children and youth with ADHD compared to controls, compared to the elevation in the n6/n3 fatty acid ratio. This is biologically consistent, because AA and EPA are the primary biologically active forms of omega-6 and omega-3 fatty acids in the brain. Total omega-6 and omega-3 fatty acids are broader measurements, including both short and long chain omega-3 and omega-6 fatty acids, and individual fatty acids within a class can have different biological activity (Perreault et al., 2014). Also consistent with our results are two small open label trials of LC-PUFAs for the treatment ADHD measured the n6/n3 fatty acid ratio in the blood in relation to clinical improvement in symptoms of ADHD. Based on these two studies, it appears that a decrease in the n6/n3 fatty acid ratio (or more specifically, AA/EPA ratio) may result in an improvement in ADHD symptoms and illness severity. One study randomized 75 patients aged 8-18 with a DSM IV diagnosis of ADHD to either placebo or an omega-3/6 supplement that contained primarily EPA and DHA, with a very

Table 1. Characteristics of Studies Included in the Meta-Analysis

First author, year	Fatty acid ratio measured	Bioassay	Patient group	Control group	Analysis
Antalis 2006	n6/n3 AA/EPA	Total plasma phospholipids, RBC phospholipids	University students with ADHD, Dx confirmed by structured interview, clinical observation, Conners' rating scale.	University student population	Explored potential mechanisms by measuring co-factors in EFA metabolism, accounted for many covariates including diet and age, Blood samples were not fasting
Chen 2004	n6/n3	Plasma free fatty acids, RBC phospholipids	Age 4-12 with ADHD, Dx confirmed by Psychiatrist based on DSM-IV criteria	Kindergarten and elementary school children	No differences in anthropometric measures, dietary intake, other nutritional biomarkers between patient and control groups.
Colter 2008	n6/n3 AA/EPA	RBC phospholipids	Age 10-16 with ADHD, Dx confirmed by physician based on DSM-IV criteria and Parent's Conners' scale	Adolescents without ADHD (confirmed by Conners' parents scale) recruited from the community	No differences in diet (7 day dietary record) and anthropometric measures between patient and control groups.
Mikirova 2013	n6/n3 AA/EPA	Plasma free fatty acids	Age 2-25 (mean=12) with ADHD, Dx confirmed by physician based on DSM-IV and parent/ teacher interview, recruited from integrative medicine clinic	Same age range with no diseases, recruited from same outpatient clinic in USA	Retrospective analysis of cases vs control samples
Stevens 1995	n6/n3	RBC total fatty acids Plasma free fatty acids	Boys mean age 9.1 with ADHD, recruited from community in Indiana by advertisement, Dx confirmed by subset of parent/teacher Conners' scale	Boys mean age 9.1 recruited from community in Indiana by advertisement, Dx of ADHD ruled out using subset of parent/teacher Conners' scale	Did not specify if blood samples were fasted or not, no difference between patient and control groups based on age, height, weight, BMI, or SES.

Dx = diagnosis, AA = arachidonic acid, EPA = eicosapentaenoic acid, n6 = omega-6 fatty acids, n3 = omega-3 fatty acids, EFA = essential fatty acids, SES = socioeconomic status, BMI = body mass index.

small amount of LA (60mg). This group found that the most "marked and long lasting changes [in blood fatty acids] in treatment responders vs. non-responders was a decrease in the n6/n3 [fatty acid] ratio"(Johnson et al., 2012). Another group titrated the dose of a PUFA supplement containing EPA and DHA to target a blood AA/EPA ratio of 1-1.5. This small, eight week long, open label study included nine patients aged 8-16 with a DSM IV diagnosis of ADHD. By the end of the study, this group found that there was a "significant correlation between the AA/EPA ratio and severity of illness" based on the clinical global impression scale (Sorgi, Hallowell, HL, & Sears, 2007).

Strengths and Limitations

This is the first study to synthesize data on the relationship between ratios of n6/n3 fatty acids and ADHD. Strengths are the systematic literature search, rigorous quality assessment, and inclusion of studies with a control group that allowed us to generate a quantitative effect size difference

Table 2. Quality assessment results

First author year	Sample of patients	Patient characteristics	Assx of confounders	Control sample	Control sample characteristics	Power	Outcome measurement	Statistical methods	Analysis	Total / 16
Antalis 2006	2	2	2	2	2	0	2	1	0	13
Chen 2004	2	2	2	1	0	0	2	1	0	10
Colter 2008	2	2	2	2	2	0	2	1	0	13
Mikirova 2013	1	2	1	2	1	0	2	1	0	10
Stevens 1995	2	2	2	2	2	0	2	1	0	13

Table 3. Quality Assessment Tool

Criteria	Score	Description
Sample of patients	2	The study demonstrates that patients are representative of the population of patients with ADHD.
	1	The study describes how patients were selected but does not demonstrate that they are representative of the population.
	0	The study does not describe how patients were selected and there could be bias in sampling.
Patient characteristics	2	The study uses a validated tool to ensure that respondents have a diagnosis of ADHD at the time the study was completed.
	1	The study uses a self-report diagnosis.
	0	The study does not explain how the diagnosis of ADHD was made.
Assessment of confounders	2	The study ensures that all known confounders that could affect results are not present in the patient sample.
	1	The study ensures that some potential confounders are not present in the sample.
	0	The study does not measure confounders.
Control sampling	2	The study recruits a control sample from the same population as the patient sample.
	1	The study recruits a control sample from a different population type than the patient sample.
	0	The study does not explain how the control sample was identified.
Control sample	2	The study ensures that the control sample is free of ADHD and other relevant potential confounders.
Characteristics	1	The study ensures that the control sample is free of ADHD.
	0	The study does not account for potential confounders in the control group.
Power	1	The study has sufficient power to detect a clinically important effect.
	0	The study does not have sufficient power to detect a clinically important effect.
	0	The study does not present a power calculation.
Outcome measurement	2	The study measures blood fatty acid levels shown to be representative of fatty acid levels incorporated into adipose tissue or cell membranes.
	1	The study measures blood fatty acids levels that have been shown to be acutely impacted by dietary consumption of fatty acids.
	0	The study does not measure fatty acid levels in the blood.
Statistical methods	1	The study provides estimates of the random variability in the data for the main outcomes.
	0	The study does not provide estimates of the random variability in the data for the main outcomes.
Analysis	2	The study uses a regression type analysis and has a clear rationale and method for identifying and deciding on which covariates to include in the analysis.
	1	The study uses a regression analysis but does not offer a clear rationale or method for choosing the covariates.
	0	The study does not use a regression analysis.

Figure 2. Blood omega-6 to omega-3 ratio in patients with ADHD vs. control subjects

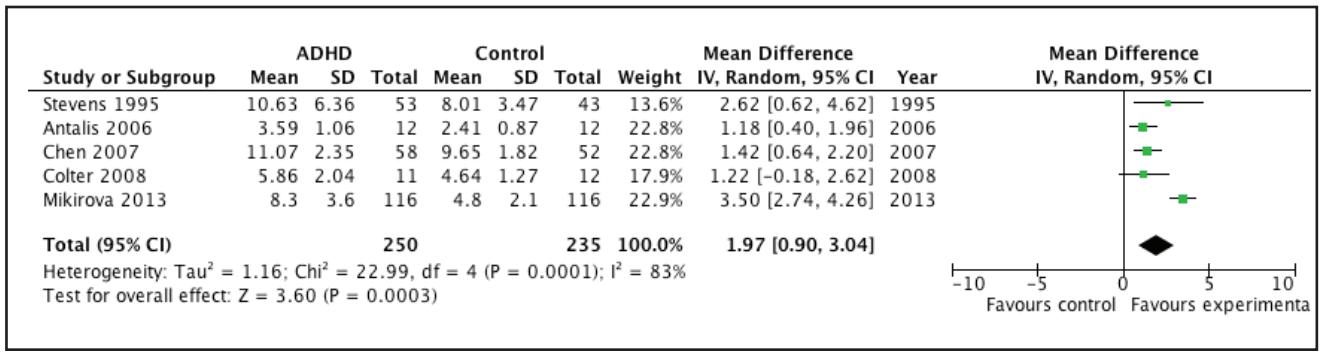


Figure 3. Blood arachidonic acid (AA) to eicosapentaenoic acid (EPA) ratio in patients with ADHD vs. control subjects

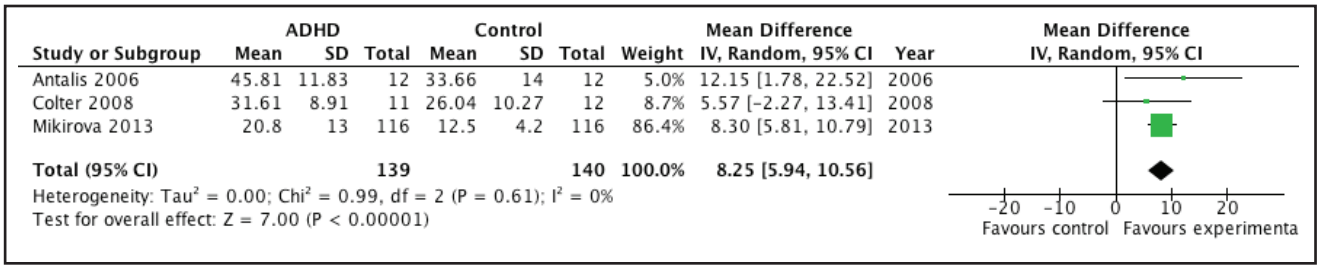
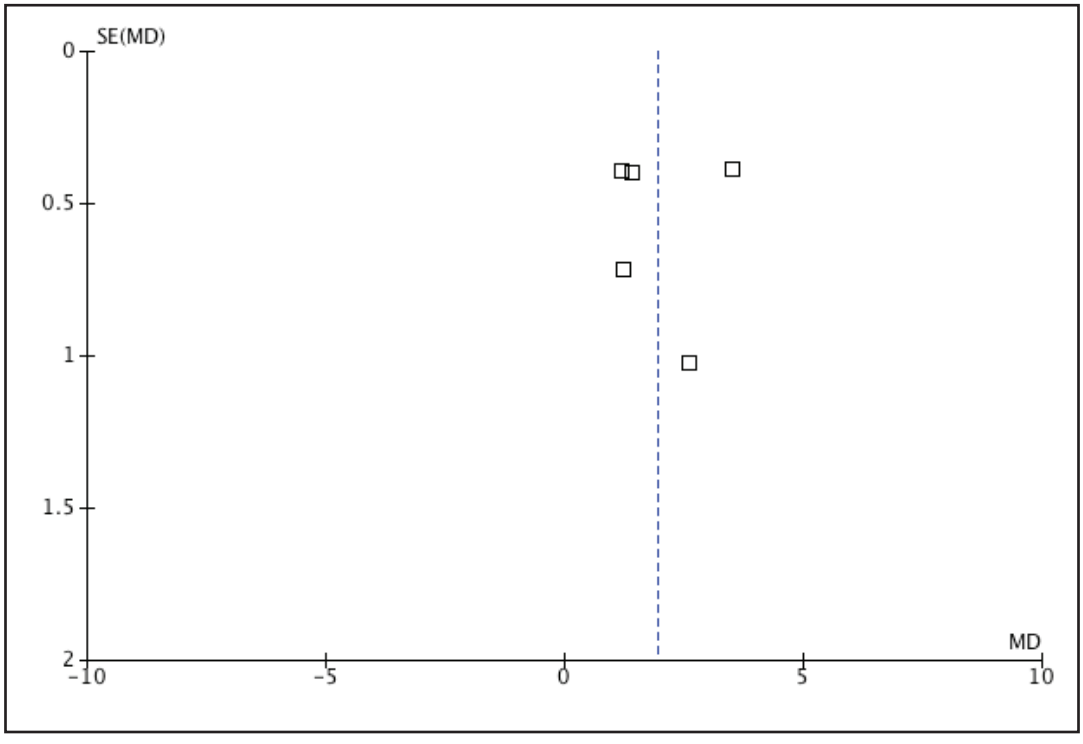


Figure 4. Funnel Plot for n6/n3



between affected children and youth and controls. Limitations are common to those of all meta-analyses in terms of the limitations of the included studies. While meta-analytic outcomes demonstrated minimal clinical heterogeneity, statistical heterogeneity in the n6/n3 ratio was significant. Across studies, there was variability in the diagnosis of ADHD with not all studies requiring diagnostic interviews and variability in the measurement of fatty acids. We attempted to mitigate this limitation by including the most reliable type of fatty acid measurement within each study. For example, the study by Antalis et al. (Antalis et al., 2006) measured the n6/n3 and AA/EPA ratio in both plasma and RBC phospholipids in the same participants. We chose to only include data regarding the ratios for RBC phospholipids in our meta-analysis since this measure is more reliable as a long-term marker of the general fatty acid pool as described above. It is also worth noting that our total number of included studies was relatively small, thus limiting the power of our findings.

Conclusions and Future Directions

The findings of this meta-analysis help explain why previous trials of omega-3 supplementation for treatment of ADHD in children and youth have been largely unsuccessful despite evidence that low omega-3 levels are associated with ADHD. The findings support future interventional research that incorporates consideration of blood essential fatty acid ratios for the treatment for ADHD. In future, blood fatty acid ratios could also be used to titrate dosages and to measure treatment compliance. Future research could assess whether fatty acid ratios could be used as biomarkers to identify children with ADHD who may specifically benefit from treatment with essential fatty acids to normalize the n6/n3 or AA/EPA ratios.

Acknowledgments/Conflicts of Interest

The authors have no financial relationships to disclose.

References

Adams, P., Lawson, S., Sanigorski, A., & Sinclair, A. (1996). Arachidonic Acid to Eicosapentaenoic Acid Ratio in Blood Correlates Positively with Clinical Symptoms of Depression. Lipids, 31(supplement).

Al-Tamer, Y. Y., & Mahmood, A. A. (2006). The influence of Iraqi mothers' socioeconomic status on their milk-lipid content. European Journal of Clinical Nutrition, 60(12), 1400-1405.

Antalis, C., Stevens, L., Campbell, M., Pazdro, R., Ericson, K., & Burgess, J. (2006). Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. Prostaglandins, Leukotrienes and Essential Fatty Acids, 75, 299-308.

Association, A. P. (Ed.). (2013). Diagnostic and statistical manual of mental disorders, (DSM 5): American Psychiatric Publishing.

Bai, A., Shukla, V., Bak, G., & Wells, G. (2012). Quality Assessment Tools Project Report. Ottawa: Canadian Agency for Drugs and Technologies in Health.

Bloch, M., & Qawasmi, A. (2011). Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: Systematic review and meta-analysis. Journal of the American Academy of Child & Adolescent Psychiatry, 50(10), 991-1000.

Chen, J. R., Hsu, S. F., Hsu, C. D., Hwang, L. H., & Yang, S. C. (2004). Dietary patterns and blood fatty acid composition in children with attention-deficit hyperactivity disorder in Taiwan. The Journal of Nutritional Biochemistry, 15(8), 467-472.

Cohen, B., Garg, S., Ali, S., Harris, W., & Whooley, M. (2008). Red blood cell docosahexaenoic acid and eicosapentaenoic acid concentrations are positively associated with socioeconomic status in patients with established coronary artery disease: data from the Heart and Soul Study. Journal of Nutrition, 138(6), 1135-1140.

Colter, A. L., Cutler, C., & Meckling, K. (2008). Fatty acid status and behavioural symptoms of attention deficit hyperactivity disorder in adolescents: A case-control study. Nutrition Journal, 7(1), 1.

Dahl, R. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. Annals of the New York Academy of Sciences, 1021(1), 1-22.

Donev, R., & Thome, J. (2010). Inflammation: good or bad for ADHD? Attention Deficit and Hyperactivity Disorders, 2(4), 257-266.

Downs, S., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. Journal of Epidemiology and Community Health, 52, 377-384.

Erskine, H. E., Ferrari, A. J., Polanczyk, G. V., Moffitt, T. E., Murray, C. J., Vos, T., & Scott, J. G. (2014). The global burden of conduct disorder and attention□deficit/hyperactivity disorder in 2010. Journal of Child Psychology and Psychiatry, 55(4), 328-336.

Faraone, S., & Buitelaar, J. (2010). Comparing the efficacy of stimulants for ADHD in children and adolescents using meta-analysis. European Child and Adolescent Psychiatry, 19, 353–364.

Faraone, S. V., Biederman, J., Spencer, T., & Aleadri, M. (2006). Comparing the efficacy of medications for ADHD using meta-analysis. Medscape General Medicine, 8(4), 4.

Ferrucci, L., Cherubini, A., Bandinelli, S., Bartali, B., Corsi, A., Lauretani, F.,...Guralnik, J. (2006). Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. The Journal of Clinical Endocrinology & Metabolism, 91(2), 439-446.

Gillies, D., Sinn, J. K., Lad, S. S., Leach, M. J., & Ross, M. J. (2011). Polyunsaturated fatty acids (PUFA) for attention deficit hyperactivity disorder (ADHD) in children and adolescents. The Cochrane database of systematic reviews, 7, CD007986-CD007986.

Graham, J., Banaschewski, T., Buitelaar, J., Coghill, D., Danckaerts, M., Dittmann, R. W., & Taylor, E. (2011). European guidelines on managing adverse effects of medication for ADHD. European Child and Adolescent Psychiatry, 20, 17-37.

Hariri, M., Djazayery, A., Djalali, M., Saedisomeolia, A., Rahimi, A., & Abdolahian, E. (2012). Effect of n-3 supplementation on hyperactivity, oxidative stress and inflammatory mediators in children with attention-deficit-hyperactivity disorder. Malaysian Journal of Nutrition, 18(3), 329-335.

Hawkey, E., & Nigg, J. (2014). Omega−3 fatty acid and ADHD: Blood level analysis and meta-analytic extension of supplementation trials. Clinical Psychology Review, 34, 496–505.

Henning, T., Ruud van Tuijl, H., Hofman, A., Kiliaan, A., & Breteler, M. (2003). Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam Study. The American journal of clinical nutrition, 78(1), 40-46.

Higgins, J., & Thompson, S. (2002). Quantifying heterogeneity in a meta□analysis. Statistics in medicine, 21(11), 1539-1558.

Hodson, L., Skeaff, C., & Fielding, B. A. (2008). Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Progress in Lipid Research, 47(5), 348-380.

Howard, A., Robinson, M., Smith, G., Ambrosini, G., Piek, J., & Oddy, W. (2011). ADHD Is Associated With a "Western" Dietary Pattern in Adolescents. Journal of Attention Disorders, 15(5), 403-411.

Johnson, M., Mansson, J., Ostlund, S., Fransson, G., Areskoug, B., Hjalmarsson, K.,...Gillberg, C. (2012). Fatty acids in ADHD: plasma profiles in a placebo-controlled study of Omega 3/6 fatty acids in children and adolescents. Attention Deficit and Hyperactivity Disorders, 4, 199–204.

Kendall, T., Taylor, E., Perez, A., & Taylor, C. (2008). Guidelines: diagnosis and management of attention-deficit/hyperactivity disorder in children, young people, and adults: summary of NICE guidance. British Medical Journal, 337(7672), 751-753.

LaChance, L. R. & McKenzie, K. (2014). Biomarkers of gluten sensitivity in patients with non-affective psychosis: A meta-analysis. Schizophrenia Research, 152(2), 521-527.

Lora, K. R., Lewis, N. M., Eskridge, K. M., Stanek-Krogstrand, K., & Travnicek, D. A. (2011). Correlation of omega-3 fatty acid intakes with acculturations and socioeconomic status in midwestern Latinas. Journal of Immigrant & Minority Health, 13(1), 111-118.

Lotrich, F., Sears, B., & McNamara, R. (2013). Elevated Ratio of Arachidonic Acid to Long-Chain Omega-3 Fatty Acids Predicts Depression Development Following Interferon-Alpha Treatment: Relationship with Interleukin-6. Brain, behavior, and immunity, 31, 48-53.

Mallen, C., Peat, G., & Croft, P. (2006). Quality assessment of observational studies is not commonplace in systematic reviews. Journal of clinical epidemiology, 59(8), 765-769.

Mikirova, N. A., Casciari, J. J., & Hunninghake, R. E. (2013). The orthomolecular correction of metabolic imbalances found in attention deficit hyperactivity disorder: A retrospective analysis in an outpatient clinic. Journal of Orthomolecular Medicine, 28(3).

Perreault, M., Roke, K., Badawi, A., Nielsen, D., Abdelmagid, S., El-Sohemy, A., . . . Mutch, D. (2014). Plasma Levels of 14:0, 16:0, 16:1n-7, and 20:3n-6 are Positively Associated, but 18:0 and 18:2n-6 are Inversely Associated with Markers of Inflammation in Young Healthy Adults. Lipids, 49, 255-263.

Polanczyk, G. V., Willcutt, E. G., Salum, G. A., Kieling, C., & Rohde, L. A. (2014). ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. International journal of epidemiology, 43(2), 434-442.

Puri, B. K., & Martins, J. G. (2014). Which polyunsaturated fatty acids are active in children with attention-deficit hyperactivity disorder receiving PUFA supplementation? A fatty acid validated meta-regression analysis of randomized controlled trials. Prostaglandins, Leukotrienes and Essential Fatty Acids, 90(5), 179-189.

Simopoulos, A. (2011). Evolutionary Aspects of Diet: The Omega-6/Omega-3 Ratio and the Brain. Molecular Neurobiology, 44, 203-215.

Sonuga-Barke, E. J., Brandeis, D., Cortese, S., Daley, D., Ferrin, M., Holtmann, M., & Sergeant, J. (2013). Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. American Journal of Psychiatry, 170(3), 275-289.

Sorgi, P., Hallowell, E., HL, H., & Sears, B. (2007). Effects of an open-label pilot study with high-dose EPA/DHA concentrates on plasma phospholipids and behavior in children with attention deficit hyperactivity disorder. Nutrition Journal, 6(16). doi: doi:10.1186/1475-2891-6-16

Stevens, L. J., Zentall, S. S., Deck, J. L., Abate, M. L., Watkins, B. A., Lipp, S. R., & Burgess, J. R. (1995). Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. The American Journal of Clinical Nutrition, 62(4), 761-768.

Subcommittee on attention-deficit/hyperactivity disorder, S. C. O. Q. I., & management. (2011). ADHD: Clinical Practice Guideline for the Diagnosis, Evaluation, and Treatment of Attention-Deficit/Hyperactivity Disorder in Children and Adolescents. Pediatrics. doi: 10.1542/peds.2011-2654

World Health Organization. (1992). The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines. Geneva: World Health Organization.