Omega-3 Polyunsaturated Fatty Acids in Youths with Attention Deficit Hyperactivity Disorder (ADHD): A Systematic Review and Meta-analysis of Clinical Trials and Biological Studies

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ABSTRACT

The role of omega-3 polyunsaturated fatty acids (omega-3 or n-3 PUFAs) in the pathogenesis and treatment of children and adolescents with attention deficit hyperactivity disorder (ADHD) is unclear. A systematic review followed by meta-analysis was conducted on: 1) randomized controlled trials (RCTs) assessing the effects of n-3 PUFAs on clinical symptoms and cognition in children and adolescent with ADHD; and 2) case-control studies assessing the levels of n-3 PUFAs in blood and buccal tissues of children and adolescents with ADHD. In seven RCTs, totalling *n*=534 randomised youth with ADHD, n-3 PUFAs supplementation improves ADHD clinical symptom scores (*g*=.38, *p*<.0001); and in three RCTs, totalling *n*=214 randomised youth with ADHD, n-3 PUFAs supplementation improves cognitive measures associated with attention (*g*=1.09, *p*=.001). Moreover, children and adolescents with ADHD have lower levels of DHA (seven studies, *n*=412, *g*=-.76, *p*=.0002), EPA (seven studies, *n*=468, *g*=-.38, *p*=.0008), and total n-3 PUFAs (six studies, *n*=396, *g*=-.58, *p*=.0001). In summary, there is evidence that n-3 PUFAs supplementation monotherapy improves clinical symptoms and cognitive performances in children and adolescents with ADHD, and that these youth have a deficiency in n-3 PUFAs levels. Our findings provide further support to the rationale for using n-3 PUFAs as a treatment option for ADHD.

Keywords: ADHD, adolescents, attention deficit hyperactivity disorder children, cognition DHA, EPA, meta-analysis, omega-3, PUFAs levels

INTRODUCTION

Deficiency in omega-3 polyunsaturated fatty acids (n-3 PUFAs) has recently been investigated as a potential pathogenetic mechanism in ADHD (Stevens *et al*, 1995). Although current pharmacotherapies, such as methylphenidate and atomoxetine, are able to improve ADHD symptoms (MTA 1999; Quintana *et al*, 2007), there is still about 20-40% of patients with ADHD who do not benefit from these medications (Pliszka *et al*, 2006). Therefore, novel treatments with clear efficacy and measurable biological mechanisms are essential. At cognitive levels, ADHD has been suggested to be a disorder involving an impaired inhibition control system (Barkley, 1997) and a disrupted feedback of the rewarding and motivational system (Barkley, 1997), and n-3 PUFAs have been associated with cognitive function and learning (Milte *et al*, 2011), including in patients with ADHD (Sinn *et al*, 2008; Vaisman *et al*, 2008; Voigt *et al*, 2001). Hence, n-3 PUFAs may be considered one of such novel treatments.

Several lines of evidence support the importance of n-3 PUFAs in brain disorders (Hibbeln *et al*, 2007; Su *et al*, 2008; Su *et al*, 2014). The n-3 PUFAs series include docosahexaenoic acid (DHA or 22:6 n-3) and eicosapentaenoic acid (EPA or 20:5 n-3), which are essential fatty acids (EFA) that cannot be efficiently synthesized by the human body and have to be obtained through dietary intake. EPA and DHA have

an anti-inflammatory action via inhibition of free radical generation and oxidant stress (Das, 2006), and have also been shown to regulate neurotransmitter and immune functions via the modulation of lipid rafts signalling platforms on the cell membrane (Chang *et al*, 2010). Moreover, n-3 PUFAs also improve symptoms of depression (Lin and Su, 2007; Su *et al*, 2003; Su *et al*, 2008; Su *et al*, 2014) and Alzheimer's Disease (Chiu *et al*, 2008).

There is promising evidence that n-3 PUFAs may be relevant to ADHD. In epidemiological studies, children of mothers who have lower seafood intake during pregnancy are at risk of suboptimal outcomes for prosocial behaviours, fine motor coordination, verbal communication and social development (Hibbeln *et al*, 2007). Moreover, we have shown that children with ADHD have greater severity of EFA deficiency, a clinical syndrome associated with insufficient fatty acid levels and comprising symptoms such as dry and scaly skin, eczema, and dry eyes (Chang *et al*, 2016). In addition, EFA dietary deficiency in children with ADHD correlates negatively with plasma DHA levels (Stevens *et al*, 1995), and we have recently shown that EFA deficiency positively correlates with ADHD symptoms (Chang *et al*, 2016). However, several case-control studies have reported no dietary differences, or even higher dietary PUFAs intake, in ADHD (Chen *et al*, 2004; Colter *et al*, 2008; Gow *et al*, 2013; Stevens *et al*, 1995). Interestingly, some clinical trials with n-3

PUFAs supplementation in ADHD have shown improvement in clinical symptoms (Manor *et al*, 2012; Perera *et al*, 2012; Richardson and Puri, 2002) and cognitive performances (Sinn *et al*, 2008; Vaisman *et al*, 2008; Voigt *et al*, 2001), but others have found no beneficial effects (Widenhorn-Muller *et al*, 2014). Hence our decision to conduct the present meta-analysis.

In terms of PUFAs levels, lower red blood cells (RBCs) PUFAs (Stevens *et al*, 1995) and a higher n-6/n-3 ratio (Stevens *et al*, 2003) have been reported in ADHD, and lower n-3 PUFAs levels are positively associated with the severity of ADHD symptoms in children (Colter *et al*, 2008; Stevens *et al*, 2003). However, some studies could not replicate the differences in n-3 PUFAs levels between children with ADHD and controls (Gow *et al*, 2013; Stevens *et al*, 2003). Again, this inconsistency in the literature has prompted us to conduct the present meta-analysis.

Although there were previous meta-analyses on this topic (Cooper *et al*, 2015; Gillies *et al*, 2012; Hawkey and Nigg, 2014; Puri and Martins, 2014; Sonuga-Barke *et al*, 2013), their findings might be confounded by heterogeneity in the clinical samples, including both children and adult subjects (Hawkey *et al*, 2014) or subjects with diagnosis other than ADHD (Cooper *et al*, 2015; Puri *et al*, 2014), as well as by the inclusion of non-parallel trials (Hawkey *et al*, 2014; Puri *et al*, 2014) as well as mixed supplementation interventions including n-3 PUFAs together with vitamins and

nutrients (Gillies *et al*, 2012; Sonuga-Barke *et al*, 2013). To address these issues, we have performed a systematic review and meta-analyses to examine both the efficacy of n-3 PUFAs supplementation *and* the levels of n-3 PUFAs, specifically in young (children and adolescents) subjects with ADHD. We have also examined the factors potentially modulating these findings, such as the EPA and DHA dosages in the supplementations trials, and the source tissue (RBCs, plasma, buccal cells) for the measurements of n-3 PUFAs levels.

MATERIALS AND METHODS

We conducted a systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher *et al*, 2009).

Literature Search

To identify eligible studies for this meta-analysis, a computerized search was performed for all publications available up to 31st March 2017 through Cochrane Central Register of Controlled Trials, Embase, Ovid Medline, PsychInfo, limited to literature in English and human studies. The search terms used are listed in Supplementary Table S1. References of eligible trials and appropriate reviews were

searched for additional citations. Unpublished or ongoing trials were searched on ClinicalTrials.gov website and authors contacted to request relevant data. Our initial search identified 4415 studies (Fig 1).

Inclusion Criteria of Studies in the Meta-analysis

The characteristics of included articles are described in Table 1, Supplementary Tables S2 and S3.

N-3 PUFAs Supplementations and Clinical Symptoms

Our criteria were: 1) studies were randomized, double-blind, placebo-controlled trials of n-3 PUFAs supplementation with DHA and EPA alone or in combination; 2) participants were school-aged children (4-12 years) and adolescents (13-17 years) who had a diagnosis of ADHD; 3) the study measured clinical symptoms of ADHD as reported by parents; 4) the data allowed to calculate an effect size; and 5) the publications were in peer-reviewed journals.

We identified 8 studies (Bos *et al*, 2015; Gustafsson *et al*, 2010; Manor *et al*, 2012; Perera *et al*, 2012; Richardson *et al*, 2002; Sinn *et al*, 2008; Vaisman *et al*, 2008; Widenhorn-Muller *et al*, 2014), totalling 628 subjects: 366 received n-3 PUFAs and 262 received placebo. Seven studies were included in the meta-analysis for total ADHD clinical symptoms totalling 534 subjects: 318 received n-3 PUFAs and 216

received placebo. Seven studies were included in the meta-analysis for inattention clinical symptoms (Bos *et al*, 2015; Gustafsson *et al*, 2010; Manor *et al*, 2012; Perera *et al*, 2012; Richardson *et al*, 2002; Sinn *et al*, 2008; Widenhorn-Muller *et al*, 2014), totalling 590 subjects: 348 received n-3 PUFAs and 242 received placebo. Six studies were included in the meta-analysis for hyperactivity clinical symptoms (Gustafsson *et al*, 2010; Manor *et al*, 2012; Perera *et al*, 2012; Richardson *et al*, 2002; Sinn *et al*, 2008; Widenhorn-Muller *et al*, 2014), totalling 551 subjects: 328 received n-3 PUFAs and 223 received placebo.

N-3 PUFAs Supplementation and Cognitive Performance

Our criteria were: 1) studies were randomized, double-blind, placebo-controlled trials of n-3 PUFAs supplementation with DHA and EPA alone or in combination; 2) participants were school-aged children (4-12 years) and adolescents (13-17 years) who had a diagnosis of ADHD; 3) the studies measured cognitive performance defined as omission errors, commission errors, forward memory, backward memory, and information processing; 4) the data allowed to calculate an effect size; and 5) the publications were in peer-reviewed journals.

We identified 4 studies (Sinn *et al*, 2008; Vaisman *et al*, 2008; Voigt *et al*, 2001; Widenhorn-Muller *et al*, 2014), totalling 309 subjects: 178 received n-3 PUFAs

and 131 received placebo. Three studies were included in the meta-analysis for omission errors (Sinn *et al*, 2008; Vaisman *et al*, 2008; Voigt *et al*, 2001), totalling 214 subjects: 134 received n-3 PUFAs and 80 received placebo. Two studies were included in the meta-analysis for commission errors totalling 85 subjects: 43 received n-3 PUFAs and 42 received placebo. Two studies were included in the meta-analysis for memory (Sinn *et al*, 2008; Widenhorn-Muller *et al*, 2014), totalling 224 subjects: 137 received n-3 PUFAs and 87 received placebo. Four studies were included in the meta-analysis for information processing (Sinn *et al*, 2008; Vaisman *et al*, 2008; Voigt *et al*, 2001; Widenhorn-Muller *et al*, 2014), totalling 309 subjects: 178 received n-3 PUFAs and 131 received placebo.

N-3 PUFAs Levels

Our criteria were: the studies 1) measured levels of DHA, EPA, AA, total n-3 or total n-6; and 2) used samples from RBCs membrane, blood phospholipids and cholesteryl esters, or buccal cells; 3) participants were school-aged children (4-12 years) and adolescents (13-17 years) who had a diagnosis of ADHD; 4) the data allowed to calculate an effect size; and 5) the publications were in peer-reviewed journals.

Nine studies were included in the meta-analysis on n-3 PUFAs levels (Bos *et al*, 2015; Chen *et al*, 2004; Colter *et al*, 2008; Germano *et al*, 2007; Gow *et al*, 2009; Gow *et al*, 2013; Mitchell *et al*, 1987; Stevens *et al*, 2003; Stevens *et al*, 1995), totalling 558 subjects: 297 youths with ADHD and 261 controls. Eight studies were included in the meta-analysis for DHA level (Bos *et al*, 2015; Chen *et al*, 2004; Colter *et al*, 2008; Germano *et al*, 2007; Gow *et al*, 2009; Mitchell *et al*, 1987; Stevens *et al*, 2003; Stevens *et al*, 1995), totalling 486 subjects: 268 youth with ADHD and 218 controls. Eight studies were included in the meta-analysis for EPA levels (Chen *et al*, 2004; Colter *et al*, 2008; Germano *et al*, 2007; Gow *et al*, 2009; Gow *et al*, 2013; Mitchell *et al*, 1987; Stevens *et al*, 2003; Stevens *et al*, 1995), totalling 542 subjects: 285 youth with ADHD and 257 controls. Seven studies were included in the meta-analysis for total n-3 PUFAs levels (Bos *et al*, 2015; Chen *et al*, 2004; Colter *et al*, 2008; Germano *et al*, 2007; Gow *et al*, 2013; Stevens *et al*, 2003; Stevens *et al*, 1995), totalling 470 subjects: 250 youth with ADHD and 220 controls. Eight studies were included in the meta-analysis for AA levels (Bos *et al*, 2015; Chen *et al*, 2004; Colter *et al*, 2008; Germano *et al*, 2007; Gow *et al*, 2013; Mitchell *et al*, 1987; Stevens *et al*, 2003; Stevens *et al*, 1995), totalling 567 subjects: 298 youth with ADHD and 269 controls. Eight studies were included in the meta-analysis for total n-6 PUFAs levels (Bos *et al*, 2015; Chen *et al*, 2004; Colter *et al*, 2008; Germano *et*

al, 2007; Gow *et al*, 2009; Gow *et al*, 2013; Stevens *et al*, 2003; Stevens *et al*, 1995), totalling 499 subjects: 270 youth with ADHD and 229 controls. If a study had measurements of both RBCs and plasma levels for PUFAs (DHA, EPA, AA, n-3, n-6), only measurement of RBCs level were included in the meta-analysis, since plasma levels reflect more recent fluctuations (e.g., days) in phospholipids while RBCs levels reflect more long term changes (e.g., months).

Studies that included and reanalysed the same data set as previously published studies were not regarded as independent, and only the study with the highest number of participants was included. See Fig 1 for the flow chart showing the selection of included studies.

Meta-Analytic Methods

In our analysis, the primary outcomes were comparisons of 1) clinical symptoms and cognitive performance in RCTs (omission errors, commission errors, memory and information processing) between n-3 and placebo groups; and 2) levels of DHA, EPA, AA, total n-3 PUFAs and total n-6 PUFAs, between ADHD and controls.

For each identified study, the effect size (*ES*) expressing the difference in clinical symptoms and cognitive performance between n-3 and placebo group, or the

difference in the PUFAs levels between ADHD and controls, were described as standardized mean difference (SMD) on the basis of Hedge's adjusted *g*, in which a value greater than 0 indicated n-3 PUFAs were superior than placebo, or levels were higher in ADHD subjects. When these data could not be retrieved from the publications, we contacted the authors to acquire the data of derived *ES* from other measures of variability. The results of individual studies were synthesized by the random effects model (Shadish, 1994), but which ESs were pooled and 95% confidence intervals (CIs) were calculated. The significance of the pooled effect size was determined by the z test. Sensitivity analyses were performed to determine whether any individual study was responsible for the significant results. Moreover, each study was individually removed and the significance was retested. The I^2 statistic assessed heterogeneity between studies. Publication bias was assessed using the Egger regression asymmetry tests (and inspection of the regression asymmetry plot) and the Begg adjusted rank correlation test. Meta-analyses were conducted by applying STATA (Stata Corp, 2009) and Forest Plots were created by using Review Manager 5.3 (Cochrane Collaboration, 2014). Two-sided *p* values <.05 were considered statistically significant.

RESULTS

N-3 PUFAs Improves Clinical Symptoms in ADHD

The major finding of our study is that n-3 PUFAs supplementation significantly improves parental reports of total symptom scores (7 studies, *n*=534, *g*=.38, *p*<.0001), inattention (7 studies, *n*=590, *g*=.42, *p*<.0001), and hyperactivity (6 studies, *n*=551, *g*=.48, *p*=.04) (see Fig 2). We also did a subanalysis looking at effects of n-3 PUFAs using two specific measurements, the Conner's cognition subscale (Richardson *et al*, 2002; Sinn *et al*, 2008) and the Conner's DSM-IV inattention subscale (Manor *et al*, 2012; Richardson *et al*, 2002; Sinn *et al*, 2008): n-3 PUFAs have a significant effect on both scores (2 studies, *n*=159, *g*= .49, *p*=.01; 3 studies, *n*=306, *g*= .36, *p*=.007, respectively). For both inattention (Figure 2A) and total ADHD score (Figure 2C), these effects are significant also in the subgroup analyses testing separately studies with EPA dosage of 500mg/day or greater, and studies with EPA dosage less than 500mg. However, for hyperactivity (Figure 2B), only studies with EPA dosage of 500mg/day or greater (Gustafsson *et al*, 2010; Perera *et al*, 2012; Widenhorn-Muller *et al*, 2014) show a significant effect, but not those with smaller dosages (Manor *et al*, 2012; Richardson *et al*, 2002; Sinn *et al*, 2008). Interestingly, only one study (Perera *et al*, 2012) in our meta-analysis used EPA as the sole source for omega-3 supplementation, and showed a significant effect for both inattention (*n*=93, *g*=.69, *p*=.001,) and hyperactivity symptoms (*n*=93, *g*=1.22, p<.00001).

Of note, n-3 PUFAs have no significant effect on teacher's reports of inattention, hyperactivity or total scores (Gustafsson *et al*, 2010; Manor *et al*, 2012; Widenhorn-Muller *et al*, 2014) (3 studies, n=334, p=.20).

N-3 PUFAs Improves Cognitive Performance in ADHD

The second main finding of our study is that n-3 PUFAs supplementation is superior to placebo in terms of cognitive performance for omission errors (3 studies, n= 214, g=1.09, p=.001) and commission errors (2 studies, n=85, g=2.14, p<.00001) (Fig 3), but not forward memory (2 studies, n=224, p=.66), backward memory (2 studies, n=224, p=0.08) or information processing (4 studies, n=309, p=.23) (Supplementary Fig S1).

Youth with ADHD Have Lower Levels of N-3 PUFAs

In the overall meta-analysis, irrespective of tissue source, youth with ADHD have lower levels of DHA (8 studies, n=486, g=-.56, p=.05), but no group differences are present for EPA, AA, n-3 PUFAs and n-6 PUFAs levels (Figure 4).

We also performed a secondary analysis by excluding the study by Stevens et al. (2003), which was different from all other studies in their participants' inclusion criteria (see Figure 4 and Discussion). In this analysis, we found that youth with

ADHD indeed have lower levels not only of DHA (7 studies, *n*=412, *g*=-.76, *p*=.0002), but also of EPA (7 studies, *n*=468, *g*=-.38, *p*= .0008), total n-3 (6 studies, *n*=396, *g*=-.58, *p*=.0001) and AA (7 studies, *n*=493 , *g*=-.41, *p*<.0001), but not of n-6 PUFAs (7 studies, *n*=425, *p*=.80) (Figure 4).

We also performed subanalyses looking at levels of the RBCs and plasma PUFAs separately. Youth with ADHD have lower RBCs DHA (5 studies, *n*=277, *g*=-.77, *p*<.0001), EPA (4 studies, *n*=196, *g*=-.55, *p*=.01) and n-3 PUFAs (4 studies, *n*=245, *g*=-.70, *p*=.0002) (Supplementary Fig S2). However, the subanalysis showed that there is no difference in plasma PUFAs levels (Supplementary Fig S3).

DISCUSSION

This is the first meta-analysis to examine the roles of n-3 PUFAs as both interventions and biomarkers in youth with ADHD, and to separately analyse RBCs and plasma levels of n-3 PUFAs in these individuals. We show that n-3 PUFAs supplementation improves total ADHD symptoms compared with placebo, with a modest effect size (*g*= .38). Moreover, n-3 PUFAs also improve omission and commission errors compared with placebo, with a large effect size (*g*= 1.09 to 2.14). Lastly, youth with ADHD have lower levels of DHA, EPA, n-3 PUFAs, and AA than control youth, with moderate to large effect size (*g*=-.38 to -.76).

N-3 PUFAs Improve Clinical Symptoms

N-3 PUFAs supplementation improves clinical symptoms in youth with ADHD in this meta-analysis, measured as parental reports of total ADHD, inattention and hyperactivity symptom scores. In contrast, we found no effects of PUFAs on the teacher-reported ADHD severity (Gustafsson *et al*, 2010; Manor *et al*, 2012; Widenhorn-Muller *et al*, 2014). Parental and teachers' ratings provide unique clinical information regarding ADHD symptoms in different settings, and in general show only weak to moderate correlations (Narad *et al*, 2015). For example, parents are more likely to detect changes in the child's daily activities, such as getting ready for school, getting dressed, getting ready for bed, eating meals and completing their homework. In contrast, teachers' reports are more representative of the child's behavior at school, such as peer interactions and talking in class. This could explain why only symptoms measured by parental reports seem to improve following treatment with PUFAs. However it is also important to highlight that the sample size is smaller for studies using teacher reports (n= 344 with teacher reports vs. n= 534-590 with parental reports), which could also contribute to the negative findings.

The dosage of n-3 PUFAs supplementation included in our meta-analysis ranges from 2.7mg to 640mg of DHA and 80mg to 650mg of EPA, with one study using

EPA (560mg) as the sole source of n-3 PUFAs supplementation. Our paper demonstrates that all trials included in the meta-analysis improve inattention and total ADHD symptoms scores, regardless of the EPA supplementation dosage. However, only studies with EPA doses of ≥500mg improve hyperactivity symptoms. Thus, our paper shows that EPA supplementation dosage ≥500mg should be considered when treating youth with ADHD, especially those with predominantly hyperactivity/impulsivity presentation.

N-3 PUFAs Improve Cognitive Performance

The second finding of this meta-analysis is that n-3 PUFAs supplementation shows efficacy in improving omission and commission errors, but not memory and information processing, in children with ADHD. This is consistent with epidemiological studies, where EFA deficiency correlates with cognitive impairment and increased impulsivity (associated with commission errors) (Chang *et al*, 2016). N-3 PUFAs are crucial for optimal neurotransmitter function: for example, incorporating more EPA and DHA in the cell membrane can increase cholesterol efflux (Chang *et al*, 2010), modulate lipid raft clustering and disruption (Chang *et al*, 2010), and affect the function of the dopamine transporter (DAT) (Foster *et al*, 2008),

which in turn may affect attention and executive function by regulating synaptic dopamine levels (Foster *et al*, 2008).

Youth with ADHD Have Lower Levels of N-3 PUFAs

Our overall meta-analysis, including all studies and irrespective of tissue source, shows that youth with ADHD have lower levels of DHA. DHA has been implicated in the brain development of infants and children, since lower maternal intake of n-3 PUFAs during pregnancy is associated with worse developmental outcomes in the offspring, including lower fine motor and communication scores and lower social development scores (Hibbeln *et al*, 2007). Children with developmental disorders also have lower levels of DHA (Milte *et al*, 2011).

When we excluded the study by Stevens et al. (2003) from the meta-analysis, we found that youth with ADHD also have lower EPA, n-3 PUFAs and AA levels. In the study by Stevens et al. (2003), children with ADHD had higher RBCs levels of AA and DHA when compared with healthy children, which is different from all other studies. We would argue that we are justified to exclude Stevens et al. (2003), as in this study the diagnosis of ADHD was not strictly defined, and the subjects self-referred and enrolled in the ADHD group if they reported to have been given a diagnosis of ADHD from a paediatrician, psychologist or psychiatrist. In contrast, in

all the other studies the diagnosis was confirmed by standardised clinical interviews, and/or subjects had an ADHD symptoms rating scale score of moderate severity. In fact, the enrolment criteria in Stevens's study (2003) are also different from a previous study from the same authors (Stevens *et al*, 1995), where the subjects had a clinical diagnosis of ADHD and severity confirmed by the Parent/Teacher Conner's Rating Questionnaire; and indeed, in this first study they found that ADHD children do have lower levels of plasma and/or RBCs DHA, EPA, n-3 PUFAs and AA. Taken together, these lines of evidence justify our decision to present the findings with and without this study (Stevens *et al*, 2003).

Furthermore, in our meta-analysis youth with ADHD also have lower levels of AA, while no difference in n-6 PUFAs levels were present. AA, derived from linolenic acid, is the precursor of a wide range of biologically and clinically important eicosanoids, incuding prostaglandins, thromboxanes and leukotrienes; it is also one of the most abundant fatty acids, after DHA, in the brain. Indeed, lower levels of DHA and AA have been associated with more anxiety, impulsivity and hyperactivity symptoms in ADHD (Stevens *et al*, 1995), while low dose dietary supplementation of AA had been shown to possible improve cognition (Okaichi *et al*, 2005; Ishikura *et al*, 2009). The deficiency of AA in ADHD, may be due to a reduced ability to convert linolenic acid to AA (Kinsella *et al*, 1990; Burgess *et al*, 2000). Possible steps

associated with inefficient conversion include desaturase steps, the manlonyl-CoA-dependent elongation steps, and the peroxisomal β -oxidation steps (Burgess *et al*, 2000). Moreover, ratio of linelonic acid to AA was greater in a subgroup of youth with ADHD with a greater severity of EFA deficiency (Burgess *et al*, 2000). Another explanation for the low AA levels may be an increased metabolism of AA to the eicosanoids via nonenzymatic oxidation, due to impaired cellular defense mechanism (Burgess *et al,* 2000).

It is also of note that a subanalysis of RBCs levels of n-3 PUFAs shows that youth with ADHD have lower levels of RBCs, but not plasma, DHA, EPA, n-3 and AA. Both RBCs and plasma PUFAs are common biomarkers used to reflect fatty acid intake/status in clinical studies (Chang *et al*, 2015; Chang *et al*, 2017; Lin *et al*, 2010; Su *et al*, 2014). Of note, RBCs and plasma PUFAs levels are measured with standard gas chromatography in the studies included in the meta-analysis. The units are presented as percentage, which is more reliable in cross-study comparison (Lin *et al,* 2010; Lin *et al*, 2017). Although the PUFAs levels from the meta-analysis were not directly from brain tissues, thus the results can not be directly applied to brain tissue PUFAs levels, peripheral RBCs and plasma DHA and EPA levels do highly correlate with brain DHA and EPA levels in animal studies (Connor *et al*, 1990; Lapillonne *et al*, 2002; Stark *et al*, 2008). In addition, RBCs PUFAs are more strongly correlated

with dietary intake (Sun *et al*, 2007), and reflect longer-term fatty acid consumption (e.g., months) (Sun *et al*, 2007), while plasma PUFAs reflect recent fluctuations of fatty acid consumptions (e.g., days). We also included buccal cells PUFAs measurements, a non-invasive measurement that correlates significantly with RBCs, plasma and brain PUFAs (Lapillonne *et al*, 2002). However, since only one study (Bos *et al*, 2015) in the meta-analysis used buccal cells PUFAs measurement, more studies using this method will be needed to support its role as a biomarker.

Biological Mechanisms and Clinical Impact

EPA is the most common form of fatty acids stored in our body, and will convert to DHA when needed, thus the low EPA level identified in the meta-analysis may indicate an attempt of the body to compensate for the low DHA levels. DHA is crucial for neurodevelopment, and its supplementation has been associated with learning (Milte *et al*, 2011). In contrast, EPA have been associated with mood regulation (Lin *et al*, 2010), and EPA supplementation has stronger antidepressant effects than DHA (Su *et al*, 2014), although higher DHA and EPA levels are both associated with lower anxiety and shyness (Milte *et al*, 2011).

In the context of 'personalised medicine', it is tempting to speculate that a subpopulation of youth with ADHD and with low levels of n-3 PUFAs may respond better to n-3 PUFAs supplementation, but there are no studies to date attempting this stratification approach. However, we have shown that individuals at genetic risk of developing depression in the context of the immune challenge, interferon-alpha (IFN-α), have lower levels of RBCs n3-PUFAs (Su *et al*, 2010), and that n-3 PUFAs supplementation prevents the onset of IFN-α -induced depression, arguably by replenishing the endogenously low anti-inflammatory PUFAs in the 'at risk' individuals (Su *et al*, 2014). Moreover, a recent study by Rapaport has stratified patients with major depressive disorder into a 'high' and a 'low' inflammation group, and shown that the 'high inflammation' group has a better responses to EPA (Rapaport *et al*, 2016). Indeed, some studies have found inflammatory abnormalities in ADHD, and this would support the theoretical model that PUFAs affect ADHD symptoms via an anti-inflammatory action (Su *et al*, 2014). For example, one study has shown that ADHD children have higher IL-6 and IL-10 levels (Donfrancesco *et al*, 2016), while another study has shown that n-3 supplementation in ADHD children reduces IL-6 and C-reactive protein (CRP) levels (Hariri *et al*, 2012). Therefore, stratification of ADHD children by n-3 PUFAs levels or by immune biomarkers could be one approach to optimise the therapeutic effects of n-3 PUFAs supplementation.

Limitations and Conclusions

This meta-analysis is limited by paucity of original data in some of the investigated comparisons. For example, all studies examining efficacy in clinical symptoms had parental, but only some had teacher, ratings of ADHD symptoms. Similarly, fewer studies measured memory function and information processing, which again may have contributed to the negative findings. The other limitation is that there are no actual data linking DHA/EPA baseline levels and EPA/DHA concentrations after treatment and response. Another limitation is that some of our analyses have been conducted only on 2-3 studies, which is not ideal for meta-analysis. Nevertheless, the conclusions remain reliable in that we have conducted the systematic review and meta-analyses in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISM) guidelines. Moreover, supporting literature has suggested that two studies are adequate to perform a meta-analysis (Valentine *et al*, 2010). Finally, our decision to exclude Stevens's study (2003), extensively discussed above, is scientifically justified, but does partly contravene the meta-analysis model. Notwithstanding these limitations, however, we provide strong evidence supporting a role for n3-PUFAs deficiency in ADHD, and for advocating n-3 PUFAs supplementation as a clinically relevant

intervention in this group, especially if guided by a biomarker-based personalisation approach.

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FIGURE LEGENDS

Figure 1. PRISMA Flow Diagram.

Figure 2. Forest plots showing effect sizes (Hedges's g) and 95% confidence intervals (CIs) from individual studies and pooled results comparing ADHD clinical symptoms, (A) inattention symptom scores, (B) hyperactivity symptom scores, (C) total ADHD symptom scores, between n-3 and placebo group.

Figure 3. Forest plots showing effect sizes (Hedges's g) and 95% confidence intervals (CIs) from individual studies and pooled results comparing cognitive function, (A) Omission and (B) Commission, between n-3 group and placebo group.

Figure 4. Forest plots showing effect sizes (Hedges's g) and 95% confidence intervals (CIs) from individual studies and pooled results comparing n-3 PUFAs Levels, (A) DHA, (B) EPA, (C) n-3, (D) AA, (E) n-6, between ADHD population and control group.