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Polyunsaturated Fatty Acids Are Associated With Behavior But Not With Cognition in Children With and Without ADHD: An Italian study

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Abstract

Objective: This study aimed to investigate the relationship between polyunsaturated fatty acids (PUFAs) status, cognitive, and behavioral traits of ADHD in school-aged children. **Method:** Seventy-three children with and without ADHD were assessed with cognitive tasks and behavioral rating scales including quality of life and global functioning at baseline of an intervention trial (clinicaltrials.gov NCT01796262). Correlation analyses were performed between the cognitive tasks/behavioral ratings and blood PUFA levels. **Results:** Children with ADHD had lower levels of DHA, omega-3 index, and total PUFA. PUFAs were positively associated with behavior but not consistently related to cognitive domains. **Conclusion:** The present study confirms that children with ADHD display abnormal fatty acid profiles within an Italian setting. Furthermore, PUFAs were associated with behavior but not with cognition. Accordingly, for the first time, lower blood levels of PUFA were associated not only with symptoms of ADHD but also with a poorer quality of life. *(J. of Att. Dis. XXXX; XX(X) XX-XX)*

Keywords

ADHD, fatty acids, cognition, behavior, quality of life

Introduction

ADHD is a heterogeneous neurodevelopmental disorder that is characterized by a lack of attention, excessive motor activity, and high levels of impulsivity. These difficulties have a significant impact on familial, relational, and school functioning in more than one setting (*Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* [*DSM*-5]; American Psychiatric Association [APA], 2013). ADHD is a complex condition that affects 7.2% of children worldwide (Thomas, Sanders, Doust, Beller, & Glasziou, 2015; although the Italian average prevalence is about 1% according to the National Institute of Health, 2014), and its etiology is generally considered multifactorial. Although high heritability estimates suggest a critical role of genetic factors (Faraone & Mick, 2010), environmental variables are also important to understand the pathogenesis of the disorder. In the last decades, a growing body of research has drawn attention to the role of diet in symptoms of children with ADHD as playing a potential role in the pathophysiology of the disorder and, therefore, as a possible coadjutant approach to pharmacological treatment. Several links between ADHD and nutritional factors have been reported (Stevenson et al., 2014). Among these, the most intensively investigated issue is the role of polyunsaturated fatty acids (PUFAs).

Within long-chain PUFAs, docosahexaenoic acid (DHA; omega-3), its precursor eicosapentaenoic acid (EPA; omega-3), and arachidonic acid (AA; omega-6) are relevant components of all cell membranes, phospholipids, and precursors of eicosanoids, the key mediators of biologic processes, thus influencing the quality of growth and development (Janssen & Kiliaan, 2014). DHA, in particular, has a critical role in maintaining membrane integrity and fluidity, influencing inter-cell signal processes and the release of neurotransmitters (Schuchardt &

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Hahn, 2011). With specific regard to the central nervous system, DHA and AA are the most abundant PUFAs in the brain. Although both DHA and AA appear to be essential to neuronal development and function (Uauy, Hoffman, Peirano, Birch, & Birch, 2001), they have divergent functions in respect to the inflammatory processes. More specifically, AA produces eicosanoids with inflammatory and predominantly vasoconstrictor properties, whereas EPA—the DHA precursor—has more anti-inflammatory properties (see, for example, van Elst et al., 2014). Omega-6 fatty acids are the predominant PUFA in all diets, especially in Western countries. The excessive intake of omega-6 fatty acids produces an imbalance of omega-6 to omega-3 PUFAs, which potentially leads to an overproduction of the proinflammatory prostaglandins of the omega-6 series (Simopoulos, 2011). When diets are supplemented with omega-3, the latter partially replace the omega-6 fatty acids in the membranes of almost all cells.

Colquhoun and Bunday (1981) were the first to propose an association between blood PUFA levels and hyperactivity in children on the basis of a systematic observation of symptoms of essential fatty acid deficiency in a large pediatric population. Since then, many clinical studies have investigated the efficacy of PUFA supplementation on ADHD symptoms and five meta-analyses have been carried out in the last few years. Three of them (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013) have suggested a small but significant effect of omega-3 supplementation, whereas Gillies, Sinn, Lad, Leach, and Ross (2012) did not report any relevant effect. Interestingly, another meta-analysis (Cooper, Tye, Kuntsi, Vassos, & Asherson, 2015) has indicated a small evidence of benefit limited to patients with low levels of omega-3 at baseline. Despite the amount of research on the effect of PUFA supplementation, only a few observational studies have considered the baseline PUFA status in children with ADHD. Although the results are too heterogeneous to allow firm conclusions, some differences have been replicated in independent studies and seem to be constant in research. The more reliable finding indicates lower blood levels of omega-3 in ADHD children versus healthy, normally developing, controls, as also confirmed by a recent meta-analysis (Hawkey & Nigg, 2014). Moreover, DHA levels are responsible for a major percentage of the difference in omega-3 profiles. Finally, case-control studies have indicated an increase in the omega-6/omega-3 ratio (Antalis et al., 2006; Stevens et al., 1995), or, inversely, a lower omega-3/omega-6 ratio in children with ADHD (Colter, Cutler, & Meckling, 2008). Furthermore, few observational studies have reported an inverse association between baseline omega-3 PUFA status, in particular DHA, and the rates of ADHD symptoms (Colter et al., 2008; Stevens, Zentall, Abate, Kuczek, & Burges, 1996; Stevens et al., 1995) in a general population that includes children with ADHD and typically developing controls. A positive correlation between omega-6 PUFA, omega-6/omega-3 ratio, and behavioral problems has been described in ADHD (Colter et al., 2008), and, according to a more recent study (Gow et al., 2013), a significant association between anti-social traits in adolescent boys with ADHD and lower omega-3 levels has also been described.

To date, studies that investigate differences in PUFA levels by comparing school-aged children with ADHD with typically developing controls are lacking within an Italian setting. As mentioned, constant observations in different contexts may reinforce the biological plausibility of the hypothesis of the role of PUFA status. Furthermore, to the best of our knowledge, no studies have examined the association between PUFA status and both cognition and behavior in children with and without ADHD, respectively. The aim of the present study is to check the PUFA status in ADHD patients and controls in Italian school-aged children by examining different cognitive domains (focused and sustained attention, inhibition, flexibility, reading) and behavioral symptoms in both study groups.

Method

The present work is a cross-sectional, observational study that reports baseline data from an ongoing placebo-controlled double-blind intervention trial investigating the efficacy of supplementation with DHA in children aged 7 to 14 with ADHD (the 'The Effects of DHA on Attention Deficit and Hyperactivity Disorder (DADA)' Study). The intervention trial was registered at clinicaltrials.gov as NCT01796262. A comparison group of typically developing children was included in this cross-sectional study and matched by gender, age, and full-scale IQ (the comparison group was not subsequently enrolled in the intervention trial). The study was explained to both children and their parent(s) or caregivers, and all of the participants' legal guardians gave their informed written consent prior to the children's participation. The research was approved by the ethics committee of our institute, in accordance with the Declaration of Helsinki. Data collection for the observational study began in June 2012 and ended in October 2014.

Participants

Children aged 7 to 14 with ADHD were recruited from the Child Psychopathology Unit at our institute over a 22-month period. The study coordinator contacted approximately 120 families by phone to invite children and parents to participate in the study protocol. Of these, 51 participants with ADHD and their parents agreed to participate and gave informed consent. The main reason for declining to participate was the child's refusal to have his or her blood sampled. All participants in the ADHD group had been previously

diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders* criteria (4th ed., text rev.; *DSM-IV-TR*; APA, 2000) by a child neuropsychiatrist with expertise in ADHD. The diagnoses were then confirmed independently by a child psychologist through direct observation and the administration of the semi-structured interview Development and Well-Being Assessment (DAWBA; Goodman, Ford, Richards, Gatward, & Meltzer, 2000). According to clinical diagnoses and interviews, 15.7% of children with ADHD had the inattentive subtype, 33.3% had the hyperactive–impulsive subtype, and 51% had the combined subtype.

Twenty-two healthy developing children were recruited as a control group by local pediatricians and from schools in the same areas of ADHD children, gender, age, and IQ matched to the clinical sample. Diagnoses according to *DSM-IV TR* were excluded in these children through the DAWBA parent diagnostic interview. Two subtests of the Wechsler Adult Intelligence Scale–III (WISC-III; vocabulary, block design; Wechsler, 2006) were administered to the children in the control group. The estimated Full Scale Intelligence Quotient (FSIQ) was used to match the two groups. The WISC subtests have a correlation of .93 to .95 with the FSIQ (Groth-Marnat, 1997). All participants were required to have FSIQ or estimated FSIQ scores of higher than 80 on the WISC-III or Wechsler Adult Intelligence Scale–IV (WISC-IV) scales (Wechsler, 2006, 2012). Exclusion criteria were the presence of suspected signs of social/communicative disorders, and major medical or neurological disorders. All participants were Caucasian, had normal or corrected-to-normal vision, and were drug-naïve. Moreover, no child had consumed omega-3/omega-6 supplements during the 3 months prior to the recruitment.

Procedure

All participants were assessed at our institute's Child Psychopathology Unit after a minimum 1-hr fast. Measurement of clinical parameters included height without shoes, weight in light clothing, and blood pressure (systolic and diastolic). Blood samples were obtained by collecting drops of blood from a fingertip.

Materials

The participants filled out the Pubertal Developmental Scale (Petersen, Crockett, Richards, & Boxer, 1988). Weekly frequency of fish consumption was then investigated. Last, data on parental employment were used as a measure of socioeconomic status (SES) and coded according to the Hollingshead 9-point scale for parental occupation (Hollingshead, 1975).

Cognitive profile. An abbreviated battery of cognitive tests from the Amsterdam Neuropsychological Tasks (ANT; de Sonneville, 2000) program was used to assess executive function domain. All participants completed four computerized tasks that were always administered in the same order; that is, Baseline speed, Focused attention 4 letters, Shifting attentional set–visual, and Sustained Attention. Baseline speed measures simple response times to stimulus presence; the dependent variables of this task were (a) median reaction time (RT) and (b) standard deviation (*SD*) of RTs. In the Focused attention test, participants had to respond (pressing the "yes" key) to one target letter among four letters presented on the screen at the same time, only when it was displayed in the relevant diagonal positions. Children were instructed to reject any other stimulus by using a different response key. The dependent variables were as follows: (a) RT for correct responses, (b) *SD* of RTs for correct responses, (c) misses, and (d) false alarms (Günther, Herpertz-Dahlmann, & Konrad, 2010). The Visual set-shifting task is composed of three conditions and investigates three basic cognitive variables: vigilance, inhibition, and cognitive flexibility. The stimulus is a bar with a colored square, which may skip quickly from left to right or vice versa. Depending on the color of the square, participants had to press a key that corresponded with the side where the square jumped (Condition 1) or press opposite keys (Condition 2). Condition 3 requires the children to adjust their response sets according to the color of the square, which changed throughout the task. The dependent measures of this task were (a) mean response time inhibition (difference in RTs between Conditions 2 and 1), (b) mean response time flexibility (difference in RTs between Conditions 3 and 1), (c) number of errors on inhibition, and (d) number of errors on flexibility (Daams et al., 2012). Finally, the Sustained attention assessed the fluctuation of attention over time. Children were shown 50 series of 12 different dot patterns. In each series, participants had to press a key whenever the target dot pattern appeared (4-dot pattern, 33% of the trials) or a different response key when a non-target (3- or 5-dot) pattern was presented. As dependent measure, (a) the sum of the 12 latencies per series (TEMPO × series), (b) *SD* of this sum across series, (c) misses, and (d) false alarms (Günther et al., 2010) were used. Moreover, (e) the coefficient of variation (i.e., *SD*/*M* RT; Klein, Wendling, Huettner, Ruder, & Peper, 2006) was also registered. Reading skills were also assessed using word and non-word reading subtests from the Italian standardized "Battery for the Assessment of Developmental Reading and Spelling Disorders" (Sartori, Job, & Tressoldi, 1995); both reading speed (syllables/seconds) and reading accuracy (number of errors) were registered.

Behavior profile. The parents completed Conners' Parent Rating Scale–R (CPRS-R, 1997) and ADHD rating scale IV Parent Version–Investigator completed (ADHD-RS; DuPaul, Power, Anastopoulos, & Reid, 1998) to assess ADHD behaviors. For CPRS-R, the dependent measures

included Hyperactivity as well Inattention scales, and were ADHD index, Conners' Global Index restless–impulsive, Conners' Global Index emotional lability, Conners' Global Index total, *DSM-IV* inattentive, *DSM-IV* hyperactive–impulsive, and *DSM-IV* total. The Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) was then filled out by the parents to measure the emotional and behavioral difficulties frequently associated with the disorder.

Functioning profile. To investigate the impact of ADHD on the quality of life, the Child Health Questionnaire–Parent Form 28 item (CHQ-PF28; Landgraf, Abetz, & Ware, 1996) was completed by the parents. CHQ is a well-validated measure of quality of life, comprising an overall summary score for psychosocial functioning, as well as subscales that assess self-esteem, impact of the disorder on the parents, and participation in family activities. The children's global functioning was evaluated by a clinician using the Clinical Global Impression–severity (CGI; Busner & Targum, 2007) and the Children' Global Assessment Scale (C-GAS; Shaffer et al., 1983).

Blood Collection and Fatty Acid Profiles Analysis

Whole blood samples were obtained from all participants to evaluate the fatty acid (FA) profile. The fatty acid analysis in whole blood has several advantages compared with the evaluation in other blood components. Whole blood is more easily obtainable than other components such as plasma and red blood cells; this represents a major benefit in observational and in supplementation studies where a large number of participants is needed. Moreover, although the examination of PUFA in red blood cells may provide a more reliable measurement of long-term accumulation of long-chain fatty acids, the whole blood fatty acid composition offers a more balanced picture about the status of circulating PUFA (both long- and short-chain fatty acids) in relation to fat dietary intakes (Agostoni et al., 2011; Risè et al., 2007). All the blood samples were collected on a strip of paper for chromatography after a minimum 1-hr fast, through a puncture of the fingertip that was performed with an automatic lancing device equipped with lancets. All samples were preserved at 4°C until analyzed. The strips of paper were directly transmethylated using a well-validated protocol (Marangoni, Colombo, & Galli, 2004). Fatty acid methyl esters were analyzed after injection into a gas chromatograph (GC-2014 Gas Chromatograph, Shimadzu, Japan) equipped with an SGE capillary column (30 m × 0.25mm, ID-BPX70 0.25 µm, SGE, Melbourne, Australia), Programmable Temperature Vaporizer (PTV) injector, Flame Ionization Detector (FID), and a dedicated data system. The analysis temperature started from 110, increased to 250°C at 5°C/min, and after 5 min, decreased to 220°C at the same rate. Peaks were identified by separated analysis of pure standards. Fatty acids from 14 to 24 carbons were detected. Fatty acid values were then expressed as a percentage of total fatty acids. We report single fatty acid data only for main omega-3 and omega-6, as these PUFAs are the focus of the present work. The AA/EPA and AA/DHA ratios are calculated as reliable indexes of the functional effects of long-chain PUFAs (Simopoulos, 2002). Last, the sum of EPA and DHA (the "omega-3 index"; Montgomery et al., 2013), and the sum of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) are also reported. SFA is known to be positively associated with concentrations of inflammation markers in blood, whereas MUFA can lead to a decrease of inflammatory molecules (see, for example, van Dijk et al., 2009). In the present study, SFA included palmitic acid and stearic acid; MUFA, palmitoleic and oleic acid; and PUFA included linoleic acid, linolenic acid, eicosatrienoic acid, arachidonic acid, EPA, DHA, and docosapentaenoic acid.

Statistical Analysis

First, a visual and statistical assessment of data was carried out to check the assumptions of normality, linearity, independence of observations, and homogeneity of error variance. These analyses revealed an outlier in terms of arachidonic acid (AA): EPA ratio (3 *SD*s from the next highest score) in the ADHD group. Although the reliability of this datum was verified, the participant's data were excluded from all further analysis of the fatty acid profile. A chi-square analysis was then performed to examine group differences in gender distribution. Independent-samples *t* test was used to individually examine group differences in age and IQ. Next, comparisons between children with ADHD and typically developing controls were then conducted on clinical questionnaires, cognitive measures, and blood fatty acid levels using Mann–Whitney or independent-samples *t* test according to the distributional nature of the data. Differences across groups in fatty acid concentrations for fish consumption were assessed using the Kruskal–Wallis test. Last, bivariate Spearman's rho correlations were conducted to determine associations between fatty acid levels, cognition, and behavior. Because this study was exploratory, no correction was applied for family-wise error rate; however, 95% confidence intervals (CIs) for rho were calculated using bootstrapping methodology (based on 1,000 bootstrap resamples) to appropriately indicate the likely size of the population effect (Field, 2013).

Results

Demographic, Behavioral, and Cognitive Assessment

Data on the demographic, cognitive, and behavioral measures of the participants are summarized in Tables 1, 2, and 3.

Table 1. Demographics of the Participants.

	ADHD	Controls		*p*
N	51	22		
Females:males	4:47	1:22	0.262[a]	.609
Age	11.0 ± 1.6	11.4 ± 1.9	1.024[b]	.309
IQ	103.4 ± 13.4	109.6 ± 19.5	1.580[b]	.119
SES	53.8 ± 18.6	56.1 ± 18.3	0.490[b]	.626

Note. ADHD = children with ADHD; SES = socioeconomic status.
[a]Chi-square test.
[b]Student's *t* test.

The validity of gender, age, and full-scale IQ matching was confirmed (all $p > 0.05$); SES was also balanced between groups ($p > .05$). Fish consumption was different between groups, $\chi^2(3) = 11.944$, $p = .008$, with children who had ADHD consuming larger amounts of fish weekly, as reported by parents (not consuming fish at all: ADHD = 7.8%, controls = 4.5%; less than once a week: ADHD = 2%, controls = 27.3%; once a week: ADHD = 62.7%, controls = 54.5%; more than once a week: ADHD = 27.5%, controls = 13.7%). Several significant group differences in ADHD symptoms were found, based on both clinical scores and questionnaires (CGI, C-GAS, CPRS, ADHD-RS; see Table 2), consistent with the diagnosis-based expectations. Moreover, children in the clinical group showed significantly more difficulties that often co-occurring with ADHD, such as emotional or conduct problems or difficulties with peers (SDQ). There were also significant group differences in terms of quality of life, with children with ADHD having globally lower well-being indexes (see Table 2). As for reading abilities, children with ADHD performed significantly worse than healthy controls in word and non-word reading, in both speed and accuracy. For cognitive measures, the ADHD group showed more variability in simple response time to stimulus presence (Baseline speed) and more false alarms for irrelevant target (Focused attention task) than did the group with typical development. Children with ADHD made also more inhibition and flexibility errors (Set-shifting). Finally, the ADHD group showed difficulties in sustaining attention over time, as indicated by more variability in reaction times and misses of target (Sustained attention; see Table 3).

Fatty Acid Analysis

Data on the fatty acid profile of the participants are shown in Table 4. Two children (both in the ADHD group) among the 73 participants recruited could not be analyzed due to insufficiency of the absorbed blood sample.

When compared with typically developing participants, children with ADHD had significantly lower levels of DHA, omega-3 index, and total polyunsaturated fatty acids. No other difference was found. There were no significant differences across groups in fatty acid concentrations for fish consumption, as reported by parents ($p > .05$).

Correlations Between Cognitive Measures and Fatty Acid Levels

The Spearman coefficients revealed a weak positive correlation between DHA and number of misses in the Focused attention task ($\rho = .247$, $p < .05$, 95% CI = [0.02, 0.48]), and a modest correlation between AA and mean response time of inhibition in Set-shifting ($\rho = .301$, $p < .05$, 95% CI = [0.06, 0.51]). No other significant correlations were found between cognition and fatty acid recorded.

Correlations Between Behavioral Data and Fatty Acid Levels

Significant correlations between behavioral scores and fatty acid levels of all participants ($n = 70$) are depicted in Table 5. No significant correlations were found between any CPRS scale and fatty acid levels (data of CPRS are not shown in the table).

ADHD symptoms as rated by parents (ADHD-RS) and clinical impression of severity (CGI) were negatively correlated with DHA, omega-3 index, and PUFA, and were positively associated with AA/DHA ratio. Significant negative associations were found between AA, EPA, DHA, PUFA, and many SDQ scores, while SFA correlated positively with SDQ impact. C-GAS correlated positively with omega-3 index and PUFA, and negatively with MUFA. Several positive correlations were found also between the parent's scores concerning the participants' quality of life (CHQ) and AA, EPA, DHA, omega-3 index, and PUFA (see Table 5, Figures 1 and 2). In turn, AA/DHA ratio and MUFA were negatively correlated with measures of the children's functioning at CHQ (see Table 5).

Discussion

The purpose of the present study was twofold. First, we aimed to examine, in an Italian school-aged sample, the blood PUFA profile of children with ADHD compared with their typically developing peers. To date, this is the first Italian study to compare the blood PUFA profile of children with and without ADHD. Second, we intended to describe the possible relationship between the PUFA percentages and both cognitive, behavioral traits and functioning that characterize ADHD, and represent the quality of life. To achieve these goals, we recruited two groups—children with a clinical diagnosis of ADHD between the ages of 7 and 14, and gender, age, and IQ-matched healthy controls.

With regard to the first goal of our study, we found significantly lower levels of DHA, omega-3 index (i.e., the sum of DHA and EPA; Montgomery et al., 2013), and total

Table 2. Group Means and Standard Deviations of Cognitive Measures of Participants.

	ADHD (n = 51)		Controls (n = 22)		Mann–Whitney Test	
	M	*SD*	*M*	*SD*	*U*	*p*
Reading abilities						
Word reading speed (syll/s)	2.85	1.08	3.93	1.09	276.5	**.001**
Word reading accuracy (errors)	4.98	4.46	1.68	1.55	287.5	**.001**
Non-word reading speed (syll/s)	1.70	0.64	2.30	0.79	302.0	**.002**
Non-word reading accuracy (errors)	6.63	5.46	3.82	3.20	373.5	**.024**
ANT—Baseline speed						
RT (ms)	346.46	71.38	314.59	46.36	431.5	.147
SD of RT	132.9	81.74	88.59	23.44	355.5	**.017**
ANT—Focused attention 4 letters						
RT correct responses (ms)	1,008.39	335.94	959.36	347.96	473.5	.293
SD of correct responses RT	436.44	241.38	343.38	186.72	441.0	.149
Misses	2.98	2.69	2.09	2.09	442.5	.148
False alarms relevant non-target	0.96	1.17	0.50	0.74	445.0	.128
False alarms irrelevant target	1.75	2.75	0.82	1.26	378.0	**.022**
ANT—Visual set-shifting						
RT inhibition (ms)	350.61	258.42	405.73	279.98	491	.400
RT flexibility (ms)	639.14	294.30	789.64	365.40	441	.149
Number of errors inhibition	8.12	6.70	3.45	4.23	289	**.001**
Number of errors flexibility	18.61	12.42	14.45	14.40	398.5	**.050**
ANT—Sustained attention date						
Tempo × Series	14.88	3.66	14.09	3.47	496.5	.438
SD	3.60	1.51	2.46	1.18	302.5	**.002**
Misses	35.98	25.22	19.27	18.35	282.5	**.001**
False alarms	23.59	17.15	18.86	18.46	418.5	.086
Coefficient of variation	0.24	0.07	0.17	0.05	244.0	**<.001**

Note. ADHD = children with ADHD; RT = reaction time; *SD* = standard deviation; ANT = Amsterdam Neuropsychological Task; contrasts in bold are significant at alpha = .05.

PUFA in children with ADHD compared with children with typical development. The whole blood PUFA analysis we report here therefore extends the previous findings of abnormal fatty acid percentages in ADHD children (Hawkey & Nigg, 2014) also to an Italian school-aged sample. Our findings do not yet support other results from case-control studies, indicating an increased omega-6/omega-3 ratio (Antalis et al., 2006; Stevens et al., 1995), or a lower omega-3/omega-6 ratio (Colter et al., 2008) in ADHD. However, we observed a numerical, although not significant, difference in AA/EPA ratio in children with ADHD. Because the standard deviation for this estimate was quite large, a larger sample size could have been able to detect this further significant difference. Indeed, a limitation of the present study is related to the small sample sizes of participant groups, in particular with respect to the healthy control group. A group difference emerged in weekly fish intake, with ADHD children consuming more fish than typically developing peers, as reported by parents. Nevertheless, we did not find any significant relationship between the amount of fish consumed and levels of PUFA circulating in blood, as opposed to previous findings (e.g., Montgomery et al., 2013). Unfortunately, the quantity and quality of fish consumed were not collected with daily dietary recordings and other possible dietary sources of n-3 fatty acids (e.g., nuts or vegetable oils, rich in alpha-linolenic acid) were not investigated. Future extensions of this work should include more objective measures of fish consumption and dietary nutrients on the whole to verify the well-documented relation between fish intakes and blood PUFA percentages. Besides these considerations, the role of the children's genotype should be also taken into account as a significant factor in determining the PUFA status. Caspi and colleagues (2007), for instance, indicated an association between the child's genotype for single nucleotide polymorphisms (SNP) rs174575 on FA desaturase (FADs) and his or her AA and DHA levels. In the last years, studies investigating the efficacy of FAs supplementation started to weigh these gene–diet interaction effects due to FADs polymorphism to explain the inter-individual variability in blood FA levels after supplementation (see, for example, Bouchard-Mercier et al., 2014).

Table 3. Group Means and Standard Deviations of Behavioral Measures of Participants.

	ADHD (n = 51)		Controls (n = 22)		Mann–Whitney Test	
	M	*SD*	*M*	*SD*	*U*	*p*
Strengths and Difficulties Questionnaire						
Emotional problem scale	2.90	2.20	1.27	1.12	285.5	**.001**
Conduct problem scale	3.65	1.67	0.91	1.15	91.5	**<.001**
Hyperactivity scale	7.88	2.53	1.27	1.49	7.5	**<.001**
Peer problems scale	2.75	3.45	1.05	1.46	337.0	**.006**
Prosocial scale	7.45	2.27	7.82	1.50	540.0	.802
Impact	2.94	2.55	0.09	0.29	163.0	**<.001**
Total difficulties scores	17.18	6.57	4.5	3.67	39.0	**<.001**
ADHD rating scale						
Hyperactivity–Impulsivity scale	14.69	5.51	1.91	1.90	18.5	**<.001**
Inattention scale	15.53	5.86	2.68	3.00	24.0	**<.001**
Total	30.22	10.00	4.59	4.46	10.5	**<.001**
Conners' parents rating scales						
ADHD index	73.33	10.29	43.32	6.90	11.5	**<.001**
CGI: restless–impulsive	69.33	10.63	42.45	7.04	28.5	**<.001**
CGI: emotional lability	59.16	14.13	43.68	4.74	158.0	**<.001**
CGI: total	67.27	13.30	41.95	6.32	44.5	**<.001**
DSM-IV: inattentive	70.75	14.54	44.86	7.82	64.0	**<.001**
DSM-IV: hyperactive–impulsive	68.29	13.28	41.45	4.25	33.0	**<.001**
DSM-IV: total	72.06	12.51	42.50	6.27	34.5	**<.001**
Child Health Questionnaire						
Physical functioning	0.25	0.77	0.40	0.51	504.0	.288
Role—Physical	−0.93	1.84	0.29	0.52	355.0	**.004**
General health	0.83	0.78	1.20	0.52	396.5	**.046**
Bodily pain	−0.01	1.05	0.24	0.82	497.0	.416
Role—Emotional/behavioral	−0.88	1.84	0.49	0.00	319.0	**<.001**
Parental impact—Time	−0.06	1.16	0.76	0.18	314.0	**<.001**
Parental impact—Emotional	−0.59	0.92	0.47	0.83	199.0	**<.001**
Self-esteem	−0.64	0.92	0.04	0.59	290.0	**.001**
Mental health	−1.81	1.32	0.11	1.04	153.5	**<.001**
Behavior	−1.31	0.91	0.48	0.82	88.0	**<.001**
Physical summary	0.36	0.75	0.72	0.46	401.0	.054
Psychosocial summary	−1.30	1.00	0.35	0.54	74.0	**<.001**
Children' Global Assessment Scale	68.27	8.79	96.45	4.28	3.0	**<.001**
Clinical Global Impression–Severity	3.92	0.89	1.14	0.35	1.5	**<.001**

Note. ADHD = children with ADHD; CGI = Conners' Global Index; contrasts in bold are significant at alpha = .05.

With regard to the second aim of this study, we have concurrently investigated the relationship between PUFA and both cognition, behavior, and functioning in a sample of children with and without a medical diagnosis of ADHD. Regardless of diagnosis, we found in the present work a few puzzling findings when assessing PUFA percentages and performance on a cognitive test battery. Furthermore, we did not find any significant correlation between PUFA and reading. These results deviate from previous findings on children in mainstream primary schools (Montgomery et al., 2013), where an association between low omega-3 PUFA, and poor reading and working memory skills was described. However, our data are in line with other negative findings on healthy children from the general population (Kirby et al., 2010). More interestingly, the present findings are also in agreement with recent suggestions from supplementation studies that PUFA could be not related to cognition in either mixed samples of children with and without ADHD, or in children with ADHD and reading difficulties, or in healthy children (Cooper et al., 2015).

We found yet a greater number of weak, but significant, associations between PUFA status and both ADHD symptoms and other difficulties often associated with the disorder. Higher levels of PUFA, in particular EPA, DHA, and

Table 4. Whole Blood Fatty Acid Analysis Data.

	ADHD (n = 48)		Controls (n = 22)		
	M	*SD*	*M*	*SD*	*p*
% 18:2*n*-6 (LA)	22.29	2.38	22.54	2.45	.686[a]
% 20:4*n*-6 (AA)	9.34	2.03	10.10	0.92	.056[b]
% 20:5*n*-3 (EPA)	1.04	0.78	1.13	0.45	.094[b]
% 20:6*n*-3 (DHA)	1.71	0.46	1.93	0.53	**.044**[b]
Ω-3 INDEX (DHA + EPA)	2.85	1.25	3.06	0.63	**.031**[b]
AA/EPA	13.21	8.91	11.72	5.44	.909[b]
AA/DHA	5.69	1.39	5.60	1.72	.821[a]
SFA	33.92	3.93	34.05	1.88	.878[a]
MUFA	27.53	3.83	25.68	2.30	.051[b]
PUFA	37.64	4.09	39.96	3.19	**.022**[a]

Note. LA = linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ADHD = children with ADHD; contrasts in bold are significant at alpha = .05.
[a]Student's *t* test.
[b]Mann–Whitney test.

their sum—the omega-3 index—were associated with lower parental rates of ADHD symptoms, lower clinical scores of severity, and a better global functioning. However, elevated AA/DHA ratio, SFA, and MUFA levels corresponded to more severe symptomatology. These findings confirm previous results from other observational studies (Colter et al., 2008; Stevens et al., 1996; Stevens et al., 1995), suggesting, in addition, a significant relationship exists between PUFA levels and clinical ratings of behavior. Furthermore, we described here a clear pattern of positive correlation between several PUFA levels (AA, EPA, DHA, omega-3 index, total PUFA) and children's quality of life as judged by parents on CHQ, whereas indices of omega-6/omega-3 ratio (i.e., AA/DHA) and MUFA were conversely associated with lower quality of life. Finally, five out of 10 PUFA levels we analyzed showed significant correlations with mental health score and behavior CHQ scale. Our overall results suggest, therefore, that lower PUFA may be associated not only with ADHD symptoms but also with lower functioning, and with a worse quality of life for children. An opposite trend was observed for measures of omega-6/omega-3 ratio, saturated and monounsaturated fatty acids. These concurrent investigations do not seem to have been assessed together previously.

As already mentioned for the fatty acid analysis, some limitations should also be considered for this second part of the study. The present report represents a cross-sectional, observational study; as a consequence, although the findings provide some evidence of an association between PUFA and ADHD behaviors, this correlational analysis's results should be interpreted with caution and cannot indicate any causal relationship. Although we do not consider appropriate to apply corrections to multiple comparisons because this study was exploratory and many of the clinical measures used are intercorrelated, the present results require replication on a larger scale to verify the generalizability of the novel findings we documented. Indeed, it is opportune to consider that the biologic plausibility of a hypothesis is accepted when trials with sufficient sample size, calculated on the basis of the variability of the study parameters in previous studies, include a sample size that is adequately powered on a statistical standpoint.

We feel that the present data also confirm, in an Italian school-aged setting, that children with ADHD display an abnormal essential fatty acid profile, suggesting that PUFA deficiency could be one of the multiple etiological factors of ADHD (Transler et al., 2010). Of the functions explored, cognition seems poorly associated with PUFA status in both ADHD and control children, as opposed to significant associations between PUFA and behavior. Different mechanisms could be responsible for changes in cognitive performance and changes in behavioral responses (Coghill et al., 2007). Finally, these results preliminarily indicate that lower blood levels of PUFA could be associated, in ADHD, not only with core symptoms but also with a poorer quality of life. Future observational and intervention studies, if undertaken to confirm the present findings, should include larger sample sizes that are adequately powered and that also consider the children's quality of life. Furthermore, future studies in which the samples are large enough to study possible differences in fatty acid composition related to ADHD subtypes could also be fruitful.

Table 5. Spearman Coefficients Values for Significant Correlations Between Fatty Acid Measures and Behavioral Measures of All Participants (n = 70).

	% 18:2*n*-6 (LA)	% 20:4*n*-6 (AA)	% 20:5*n*-3 (EPA)	% 20:6*n*-3 (DHA)	Ω-3 INDEX (DHA + EPA)	AA/EPA	AA/DHA	SFA	MUFA	PUFA
Strength and Difficulties Questionnaire										
Conduct problem scale		−0.293* [−0.491, −0.091]		−0.272* [−0.469, −0.064]	−0.252* [−0.472, −0.01]					−0.304* [−0.485, −0.096]
Hyperactivity scale				−0.267* [−0.483, −0.035]	−0.260* [−0.476, −0.027]					
Impact			−0.275* [−0.468, −0.059]					0.281* [0.06, 0.486]		
Total difficulties scores				−0.278* [−0.464, −0.058]	−0.242* [−0.45, −0.036]					
ADHD rating scale										
Inattention scale				−.0248* [−0.456, −0.027]			0.239* [0.003, 0.459]			
Total score				−0.242* [−0.469, −0.007]			0.247* [0.003, 0.476]			
Children' Global Assessment Scale					0.247* [−0.025, −0.467]				−0.248* [−0.432, −0.024]	0.297* [0.084, 0.486]
Clinical Global Impression–Severity				−0.258* [−0.473, −0.035]	−0.285* [−0.491, −0.059]					−0.295* [−0.506, −0.077]
Child Health Questionnaire										
Role—Emotional/behavioral		0.280* [0.028, 0.496]		0.274* [0.002, 0.506]					−0.273* [−0.471, −0.044]	−0.263* [0.029, 0.474]
Parental impact—Emotional				0.265* [0.029, 0.483]			−0.258* [−0.492, −0.02]			
Parental impact—Time									−0.286* [−0.509, −0.047]	
Mental health		0.353** [0.115, 0.557]	0.274* [0.028, 0.484]	0.353** [0.106, 0.582]	0.311** [0.083, 0.515]					0.300* [0.053, 0.513]
Behavior		0.291* [0.079, 0.475]	0.246* [0.002, 0.471]	0.349** [0.111, 0.582]	0.322** [0.075, 0.532]					0.322** [0.101, 0.494]
Psychosocial summary		0.327** [0.092, 0.525]		0.335** [0.085, 0.557]	0.287* [0.045, 0.509]					0.313* [0.07, 0.524]

Note. LA = linoleic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. Lower and upper bounds of 95% confidence intervals for Spearman's rho coefficients are provided in square brackets.
*p < .05. **p < .01.

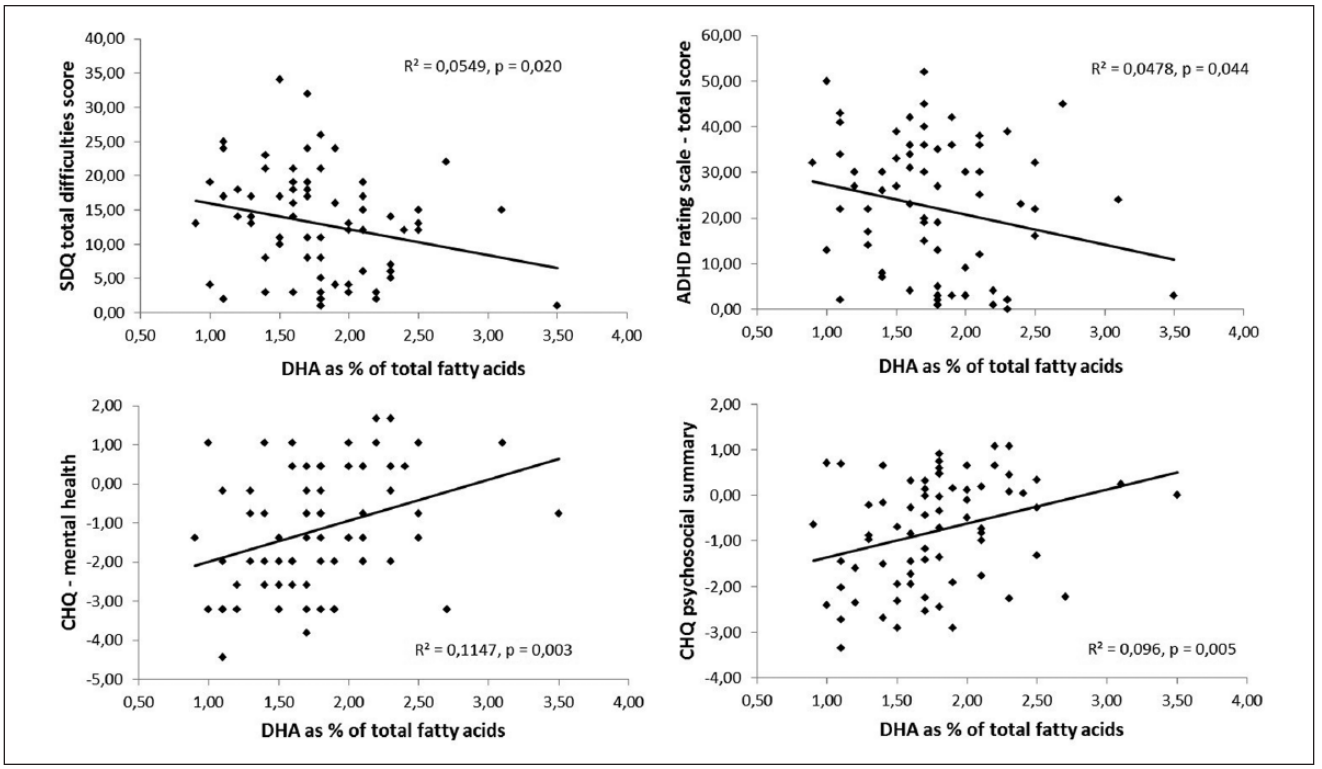

Figure 1. The relationship between DHA shown as percentage of total fatty acids in the whole blood and Strength and Difficulties Questionnaire–total difficulties score (upper left panel), ADHD rating scale–total score (upper right panel), Child Health Questionnaire–mental health score (lower left panel), and Child Health Questionnaire–psychosocial summary (lower right panel).

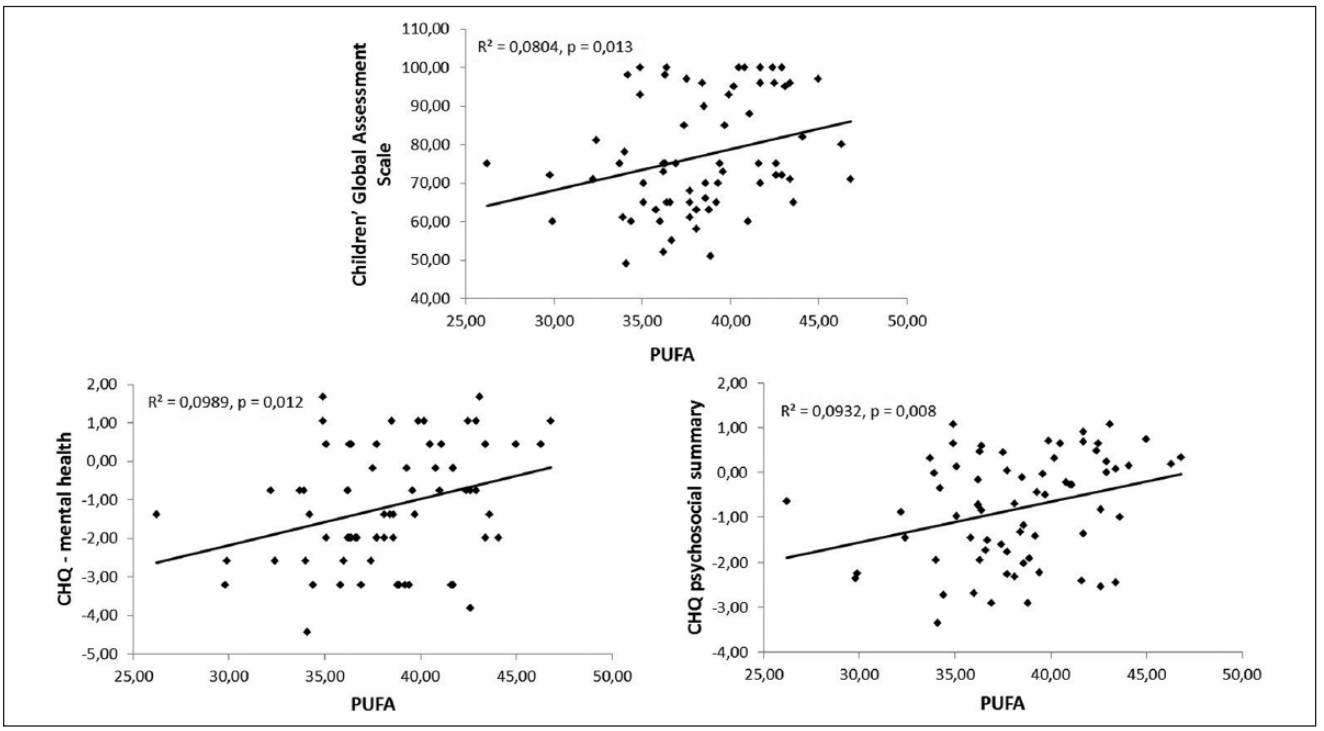

Figure 2. The relationship between PUFA and Children's Global Assessment scale (upper panel), Child Health Questionnaire–mental health score (lower left panel), and Child Health Questionnaire–psychosocial summary (lower right panel).

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