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Omega-3 Fatty Acids Are Related to Abnormal Emotion Processing in Adolescent Boys with Attention Deficit Hyperactivity Disorder

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Abstract

Background—Attention Deficit Hyperactivity Disorder (ADHD) is associated with poor emotion regulation in addition to the core symptoms. There is evidence that children with ADHD have lower omega-3 levels and that supplementation with omega-3 can alleviate both ADHD and affective symptoms. We therefore investigated differences between ADHD and non-ADHD children in omega-3/6 fatty acid plasma levels and the relationship between those indices and emotion-elicited event-related potentials (ERPs).

Methods—Children/adolescents with ($n = 31$) and without ADHD ($n = 32$) were compared in their plasma omega-3/6 indices and corresponding ERPs during an emotion processing task.

Results—ADHD children had lower mean omega-3/6 and ERP abnormalities in emotion processing, independent of emotional valence, relative to controls. Some ERP abnormalities were significantly associated with lower omega-3 levels in the ADHD group.

Conclusions—The findings reveal for the first time that lower omega-3 fatty acids are associated with impaired emotion processing in ADHD children.

Keywords

Omega-3 fatty acids; EPA; emotional processing; ADHD

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a debilitating neurodevelopmental disorder with age-inappropriate symptoms of hyperactivity, impulsivity and inattention [1]. Neurobiological evidence has shown that ADHD is persistently associated with abnormalities in fronto-striatal and fronto-cerebellar networks, in addition to the thalamus and parietal cortex [2, 3]. Children with ADHD are often at risk for emotional dysregulation, that is, extreme emotional reactivity and instability [4] which together with the core symptoms adversely impacts interpersonal relationships with peers, siblings, teachers and parents [5, 6]. Children/adolescents with ADHD perform poorly in facial recognition tasks as evidenced by an inability to correctly identify the facial expressions of others especially emotions of fear, anger and sadness compared to non-ADHD children [7, 8]. However, despite the evidence that emotional problems exist in ADHD, much less is known about the underlying neurobiology involved in these processes [1].

Essential fatty acids, in particular both arachidonic (AA, c20:4n6) and docosahexaenoic (DHA, c22:6n-3) acids, have been demonstrated to be critical for brain development, structure and function [9, 10]. The work of Crawford and Sinclair provided pioneering experimental evidence that specific omega-3 fatty acid deficiencies induced behavioural pathologies [11]. Since then evidence has persistently suggested a role for omega-3 fatty acids in the regulation of mood and affect [12] while eicosapentaenoic acids (EPA) have been found to modulate both serotonergic and dopaminergic systems [13, 14]. Evidence that children and young adults with ADHD have lower plasma levels of omega-3 fatty acids, is in consistent with some studies finding abnormalities [15–17] and other none [18, 19]. Contributions of differing dietary intakes and potential differences in metabolism have not been well controlled. However, a recent meta-analysis of 10 dietary omega-3 supplementation trials which included 699 children with ADHD reported that EPA rich preparations were consistently and significantly associated with clinical efficacy [20]. Most importantly, elevated omega-3 status seems to be associated with the improvement of affective processes such as child depression [21], anti-social, violent and psychopathic behaviours [22, 23], and aggression [24]. Clinically, the efficacy of EPA rich preparations as a therapeutic intervention for adult patients with major depressive disorders has also been reported [25, 26]. Although, preliminary evidence of associations between omega-3 and emotion processing [27] have not included comparisons to typically developing children. Thus, lower omega-3 status may adversely affect both ADHD symptoms and emotional processes.

Event Related Potentials and Face Processing

To test if low omega-3 PUFA levels were related to emotion processing in ADHD children, we employed event related potentials (ERPs) of facial emotion processing. ERPs are an imaging technique with a high temporal resolution and refer to those EEGs signals which directly quantify the electrical response of the cortex to cognitive, sensory or affective events, time-locked to the stimulus onset or behavioural response (e.g., a button press). They are advantageous for the study of emotion processing as they permit the investigation of automaticity during different, temporally separate, stages of emotion processing [28]. There

are some neurobiological and imaging data suggesting impaired face processing in ADHD [29, 30] and to a greater extent, in clinical groups related to child psychopathy [31]. However, the ERP literature on emotion processing in child ADHD is limited [27, 32, 33]. We adopted the Halgren and Marinkovic (1994) ERP model for the appraisal and response to emotional stimuli. In this model, there are 2 stages, “orienting” and “event integration” in distinguishing conscious and non-conscious emotion perception. Orienting can be described as the automatic interruption of continuing processing [34, 35], free from conscious consideration and captured by the N2/P3a/slow wave [34]. N2 deflections are also particularly sensitive by facial emotional expressions [34]. The event integration aspect is captured by N4/P3b waves and reflects the cognitive integration of overt emotional experiences [35]. The N4 deflection is a marker also of semantic processing [36]. Aside from studies of facial expressions, there is consistent evidence that children with ADHD are impaired in cognitive processes which involve response expectation and preparation, selective attention, response inhibition and conflict monitoring (e.g., the capability to interrupt an activated response and to actively suppress responses) [37]. These impairments are characterised by abnormal waveforms which are the most prominent during tasks of motor response inhibition and selective attention in frontal and parietal brain regions relative to controls [38–46]. In contrast to the fairly sizeable neuroimaging literature on cognitive functions in ADHD, however, the neurophysiological correlates of emotional dysfunction in children/adolescents with ADHD are fairly unexplored. Singhal and colleagues (2012) employed ERPs to investigate both emotion and attention processing using an altered version of the emotional oddball task in adolescents with either ADHD or affective disorders compared to a non-clinical control group. They reported differences between groups in both early (P1) and late positive potentials (LPP) with the clinical groups demonstrating augmented amplitudes to fearful facial expressions compared to sad and neutral faces. In addition, they reported an emotion-induced in P3 amplitudes reflecting attention processing as well as a sustained effect on emotion on target processing in the clinical sample only [33]. In relation, to, to date we could find only 1 previous publication reporting preliminary data a small sample size comparing omega-3 fatty acids and emotion processing in children with ADHD [27].

The aims of the present study were threefold; determine whether (1) children with ADHD had reduced fatty acid plasma levels compared to controls, (2) whether ERPs in relation to the emotion processing model by Halgren (1994) were abnormal in children with ADHD and (3) whether these ERP abnormalities were associated with plasma essential fatty acids indices. For the ERP analyses, we tested neuronal responses to facial stimuli depicting five emotions (fear, sad, happy and anger) contrasted with a neutral face (i.e., with no expression) between ADHD and controls. We hypothesised that relative to controls the ADHD group would be impaired in the two stages of emotion processing according to the model by Halgren (1994) characterised by both N2 and N4 waves. We further postulated, that levels of omega-3 long chain polyunsaturated fatty acid 9 omega-3 LC-PUFAs in plasma choline phosphoglycerides would be lower in ADHD compared to controls in line with the fatty acid literature in ADHD [15–17]. With respect to the relationship between LC-PUFA and ERP responses to facial expressions of emotion, we postulated that (1) omega-3 LC-PUFA levels would be negatively correlated with brain function as indexed by

the N4 and N4/P3b complex to both positive and negative emotions; (2) building on our previous research [27], that serum omega-3 PUFA, (in particular EPA) levels would be positively correlated with P3 responses to happy faces (i.e., the higher the omega-3 LC-PUFA - the greater the activation to happy faces); and finally (3) that omega-6 LC-PUFA would be positively associated with N4 responses to negative stimuli, based on the literature reporting links between omega-6 fats and greater negative affect [47].

Method

Participants

The MAAFA trial enrolled 78 male children of whom EEG data were obtained from 63 subjects comprising 31 children who met the criteria for ADHD and 32 control children. The EEG/ERP data from the ADHD participants were collected during baseline assessments during the Maudsley Adolescence ADHD Fatty Acid (MAAFA) trial. Children were recruited for this trial from various special educational settings with a provision for children with emotional and behavioural difficulties from the London area. They were screened to ensure they met criteria for ADHD by way of a semi-structured interview (Children's Interview for Psychiatric Syndromes, *ChIPS*) based on DSM-IV criteria (see Rooney, Fristad, Weller & Weller, 1999). For inclusion, both Parent and Teacher Connor Rating Scales (CPRS/CTRS) had to be equal to or above 65 (> 95th percentile). The MAAFA trial ERP data was age and sex matched to a healthy control group who were screened under the same research criteria to ensure they did not meet research criteria for ADHD. Intelligence quotient (IQ) for all participants had to be higher than 70 on the prorated IQ as assessed using the Kaufman Brief Intelligence test (K-BIT). Exclusion criteria included that omega-3/6 supplements had not been taken for a period of 3 months prior to the assessments. Informed consent/assent was obtained from all participants and their parents and the study was approved by the local Ethics Research committee. Twenty two of the ADHD participants were medication naïve and the remaining nine underwent a 48 hour wash out period for stimulant medication, in line with current EEG practice.

Procedure & Materials

Participants were accompanied by an appropriate adult to the Maudsley Hospital where approximately 16 ml of fasting blood was taken by a qualified phlebotomist. The participants were then given a complimentary breakfast at the Maudsley restaurant. All undertook a standardised EEG/ERP assessment. Participants were given £20.00 for their participation and all associated travel expenses were refunded to the parent/guardian.

Blood analysis—Plasma total lipids were extracted according to the modified method of Folch [51] by homogenizing 1 mL of sample with chloroform/methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) under oxygen-free nitrogen (OFN). Lipid extracts were applied onto a silica gel plate (VWR, UK) and thin layer chromatographically (TLC) separated with chloroform/methanol/methylamine (65:35:15 v/v/v). Choline phosphoglyceride (PPC) were visualized by spraying developed plates with 2,7-dichlorofluorescein (0.01% w/v in methanol). Fatty acid methyl esters (FAMES) were prepared by heating the scraped bands in 4 ml of 15% acetyl chloride/methanol for 3h at

70°C in a sealed vial under OFN. FAMES were separated by gas chromatography (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a capillary column (60 m BPX70, SGE Analytical, UK). Fatty acids were identified and quantified by Chromatography software (Agilent EZChrom Elite, San Ramon, CA, USA) as standardized by Bueno et al. (2012) [52]. This study reports data from plasma choline phosphoglyceride (PPC) fatty acids only.

ERP recording—A NeuroScan Quik cap and NuAmps amplifier (sampling rate = 500 Hz) were employed to collect EEG data from 26 electrodes using an adapted 10–20 system following an internationally standardized protocol LabNeuro™ (Brain Resource, 2010). Participants sat in a light and sound attenuated room with an ambient temperature of 24°C. Data were recorded relative to a virtual ground, but referenced offline to linked mastoids. Horizontal eye movements were recorded with electrodes placed 1.5cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3mm above the middle of the left eyebrow and 1.5cm below the middle of the left bottom eye-lid. Skin resistance was < 5 kOhms. A continuous acquisition system was employed and data was electrooculography (EOG) corrected offline [53]. A low pass filter with attenuation of 40dB per decade above 100 Hz was employed prior to digitization.

ERP area under the curve—Average ERPs were calculated for event types corresponding to a stimulus type in each paradigm. For each channel, the individual single-trial epochs were filtered with a low-pass Tukey filter function that attenuates frequencies above 25 Hz. A cosine ramp from 1 down to 0.5 between 25Hz and 35Hz is used as an envelope on the FFT data in the Tukey filter. The single trials were then averaged to form conventional ERPs. The averages of the pre-stimulus period –300 to 0 ms were subtracted from the ERP data. The signal was then down sampled by a factor of 4 (leading to 8 ms samples). The amplitude of the waveform (in microvolts) relative to the zero baseline is calculated for single time points in 8ms, then multiplied by a factor of 8 to achieve a measure of the area under the curve in a unit of microvolt-milliseconds. The AUC is the integral of the curve over a specified time range. Using the AUC measure, the space between the ERP waveform and baseline was divided into multiple 50ms time-windows that can be approximately mapped onto ERP component time-windows. P2 is mapped onto time unit 5 (200–250 ms), N2 on to time unit 6 (250–300 ms), P3a onto time unit 7 (300–350 ms) and P3b (350–400 ms) on time unit 8. Units are in microvolts multiplied by ms.

Emotion processing task—EEG data were recorded during an emotion perception task containing 48 grey scale stimuli of facial expressions. The stimuli represented 3-D facial expressions of happiness, sadness, anger, fear and disgust relative to neutral faces and were chosen from a standardised set of stimuli [54]. The stimuli were made up of eight different individuals representing each expression. The images were tailored for orientation (i.e., so that the eyes of each image were at the central horizontal in all cases) and equivalent luminance. A maximum of 192 stimuli (8 different individuals representing each expression recurred four times) were shown pseudo-randomly under both covert (to measure non-conscious, automatic processing) and overt (to measure controlled processing) conditions.

Only the data from the overt condition at mid-line sites are reported. Participants were advised to focus on each face in preparation for post-test questions to ensure attention.

Statistical methods

The ERP data were analysed using a series of mixed model 2 (group) $\times$ 5 (condition/facial expression) $\times$ 4 (electrode position) analyses of variance (ANOVA). The between subjects factor was group (ADHD, control children), while facial expressions (Fear, Neutral, Sad, Happy and Anger) and electrode position (Fz, FCz, Cz, and Pz) were within-subjects factors. The dependent measures were the AUC amplitude responses (at different time points). The time points of interest were: time point 4 (P2: 150–200 ms), 5 (N2: 200–250 ms), 6 (P3a: 250–300 ms), 7 (N4/P3b: 300–350 ms) and 8 (N400: 350–400 ms). Significant main effects of electrode site or condition were not further followed up with post-hoc tests. However, all group or interaction findings were followed up with post-hoc tests and pairwise comparisons using a Bonferroni corrected .05 rejection criterion, unless noted otherwise. All data were assessed for violation of sphericity employing the Mauchly's test and corrected using the Greenhouse-Geisser estimates of sphericity. For each time point the corrected results where necessary are reported and not individually discussed.

For all plasma PC analyses, independent-samples *t* tests were conducted to compare the plasma fatty acid levels between ADHD and control children. The key fatty acid indices chosen were from the omega-3 and omega-6 series, namely (1) c18:3n-3 (ALA), (2) c20:5n-3 (EPA), (3) c22:5n-3 (DPA), (4) c22:6n-3 (DHA) and (5) Total n-3 and (6) c18:2n6 (LA), (7) c18:3n6 (GLA), (8) c20: 4n6 (AA) and (9) total n-6 respectively. The false discovery rate (FDR) correction for multiple testing was employed for all analyses [55]. Here the term significant indicates analyses surviving multiple testing and do not imply consideration of clinical significance. Evaluation for normal distributions? Really- T tests? Not ANOVA? Regression analyses? Univariate? Multivariate? What software and what version?

There were statistically significant differences between measures of composite (overall) IQ between the control group ($M = 118.73$, $SD = 13.58$) and the ADHD group ($M = 96.32$, $SD = 13.96$), $t(67) = -6.73$, $p < .001$, with ADHD scoring lower in IQ than controls. IQ is typically lower in ADHD children [48]. For this reason we did not covary for IQ, since when the covariate is an attribute of the disorder or of its treatment, or is intrinsic to the condition, and hence differs between groups, it becomes meaningless to “adjust” group effects for differences in the covariate, and ANCOVA cannot be used to control treatment assignment independent of the covariate [49, 50]. However, to test the impact of IQ on the findings, we correlated measures that differed between groups with IQ.

Results

Population characteristics

There were no statistically significant differences in age between the ADHD group ($M = 14.00$, $SD = xxx$) and the control group ($M = 14.46$, $SD = xxx$), $t(65) = -1.72$, $p = .09$. Among the ADHD group, 28 males were right handed, one was left handed and two were

ambidextrous. Among the control group, 28 were right handed, and four were left-handed. A Chi-square test for independence indicated no significant group differences in the handedness distribution, $\chi^2 = 3.71$, $p = .15$. APA recommends that a chi square test be reported with the format: χ^2 (df, N =) = 3.71, $p = .15$

ERP Results

Time Point 4 (P2 deflection)—The mean values (μV) and standard errors for the AUC P2 amplitude responses for ADHD and control children are plotted in Figure 1. A trend was observed for P2 responses for the main effect of group (ADHD versus controls), $F(1, 61) = 3.24$, $p = .07$, with greater negative activation among healthy controls ($M = -162.47$, $SE = 30.68$) compared to ADHD ($M = -83.74$, $SE = 31.17$). There were significant main effects for electrode site, $F(1.42, 86.49) = 21.36$, $p < .001$, with greater activation at Fz ($M = -155.73$, $SE = 22.73$) and for faces, $F(4, 244) = 8.65$, $p = .001$, with happy faces producing greater positive going activation ($M = 160.93$, $SE = 26.29$).

A trend was observed for the interaction between electrode site and group, $F(3, 183) = 2.32$, $p = .07$. Post-hoc tests revealed that this was due to significantly greater differences in activation at FCz and Cz electrode sites between controls and ADHD with enhanced negativity for controls compared to ADHD.

There was a significant interaction between faces and group, $F(4, 244) = 2.84$, $p < .03$ you should report the exact probability, not smaller than .03. Post-hoc tests revealed that this was due to significant differences between ADHD and controls in P2 responses to fear, neutral and angry faces. The control group displayed greater negative going activation to facial expressions of sad compared to ADHD. There was also a significant difference in responses to neutral faces between ADHD and controls. Finally, there was a significant difference between facial expressions of anger between ADHD and controls. As per before the control group displayed greater negative going activation relative to the ADHD group.

There was a significant interaction between electrode site and faces, $F(6.47, 394.46) = 2.77$, $p < .02$ again report the exact probability – I suspect this should read like $p = .02$. Post-hoc tests revealed this was due to significant differences between all facial expressions and electrode sites, with happy faces generating the greatest negative activation at Fz, FCz, and Cz, this shifted to sad faces generating the highest negative activation at Pz electrode site.

Finally, a trend was observed for the 3 way interaction between electrode site, faces and group, $F(12, 732) = 1.56$, $p = .09$. Post-hoc analyses showed that this was due to significant differences at Fz for responses to fear and anger between ADHD and controls. There were also significant differences between FCz for responses to neutral, sad and angry faces and Cz for responses to neutral and angry faces. In all cases activation was attenuated to these facial expressions among ADHD children relative to controls.

Time Point 5 (N2 deflection)—A significant main effect of group was observed for N2 responses, $F(1, 61) = 7.12$, $p < .02$ report the exact p with greater activation to all facial expressions for the control group ($M = -130.25$, $SE = 28.52$) compared to ADHD ($M = -21.73$, $SE = 28.97$). The main effect of electrode site was significant, $F(1.34, 83.37) =$

38.26, $p < .001$ with greater activation at Fz ($M = -118.17$, $SE = 21.04$). A trend finding was observed for faces, $F(4, 244) = 2.07$, $p = .086$ with greater negative activation to angry faces, ($M = -97.55$, $SE = 24.30$; see Fig. 2).

There was also a trend for the interaction between electrode site and faces, $F(7.12, 434.23) = 1.91$, $p = .066$. There were significant differences comparing facial expressions of fear ($M = -54.56$, $SE = 25.34$) and neutral ($M = -143.24$, $SE = 31.21$, fear ($M = -54.56$, $SE = 25.34$) and happy ($M = -142.29$, $SE = 27.03$) and fear ($M = -54.56$, $SE = 25.34$) and anger ($M = -138.08$, $SE = 26.47$). Facial expressions of neutral elicited the greatest negative responses, while responses for fear produced the smallest negative responses.

Time Point 6 (P3a deflection)—Group was not a significant main effect, but electrode site was a significant main effect, $F(1.40, 85.48) = 74.83$, $p < .001$, again driven by greater negative activation at Fz, ($M = -171.66$, $SE = 23.75$). The main effect for faces was also significant, $F(3.29, 200.90) = 3.84$, $p < .01$ with greater negative activation to neutral faces, ($M = -162.84$, $SE = 32.24$).

A trend was observed for the interaction between electrode site and faces, $F(7.25, 442.06) = 1.99$, $p = .053$. Post-hoc tests showed that this was due to a significant difference between facial expressions of fear ($M = -98.98$, $SE = 30.38$) and neutral ($M = -238.84$, $SE = 34.71$) at Fz electrode site. There was also a significant difference comparing facial expressions of fear ($M = -95.76$, $SE = 35.53$) and neutral ($M = -232.63$, $SE = 35.88$) at Cz. On both occasions, neutral faces produced an increase in negative activity across frontal-central scalp regions. No other interactions reached significance.

Time Point 7 (N4/P3b deflection)—The main effect of group was not significant; however the main effect of electrode site was significant, $F(1.54, 93.75) = 96.43$, $p < .001$ which was due to greater activation at FCz, ($M = -209.64$, $SE = 25.93$). A trend finding was observed for faces, $F(3.27, 199.59) = 2.18$, $p = .086$ with greater mean activation to sad faces, ($M = -172.51$, $SE = 29.00$). None of the interactions reached significance.

Time Point 8 (N4 deflection)—The mean values (μV) and standard errors for the AUC N4 amplitude responses for ADHD and control children are plotted in Figure 3. The main effect of group was significant, $F(1, 61) = 5.42$, $p = .03$ with significantly lower activation in N4 responses in ADHD ($M = -146.32$, $SE = 31.93$) relative to controls ($M = -250.67$, $SE = 31.43$). The main effect of electrode site was also significant, $F(1.58, 96.13) = 65.72$, $p < .001$, driven by higher negative activation at Fz ($M = -250.33$, $SE = 23.70$). The main effect for faces was non-significant, $F(3.45, 210.52) = 2.26$, $p = .073$, reflecting the greater mean activation to angry faces, ($M = -233.44$, $SE = 26.10$). None of the interactions reached significance.

PPC fatty acid levels comparing Children/Adolescents to ADHD and Healthy Control Children

There were significant differences between the ADHD and control groups for ten out of the thirteen fatty acids indices (see table 1). For the omega-6 series there were significant differences for c20:2n6, $t(65) = -3.48$, $p = .01$, c20:3n6, $t(66) = -4.28$, $p = .001$, c20:4n6

(AA), $t(66) = -9.23$, $p = .001$, c22:4n6 (a metabolite of AA), $t(66) = -5.65$, $p = .001$, and total n-6, $t(66) = -5.61$, $p = .002$. For the omega-3 series, there were significant differences between ADHD and control children for c18:3n3 (ALA), $t(66) = -3.51$, $p = .002$, c20:5n3 (EPA), $t(66) = -5.88$, $p = .003$, c22:5n3 (DPA), $t(66) = -7.53$, $p = .004$, c22:6n3 (DHA) $t(69) = -11.08$, $p = .006$, c22:6n3, $t(66) = -11.32$, $p = .006$ and total omega-3, $t(66) = -11.32$, $p = .01$. In all instances, LC-PUFAs fractions in both omega-3 and 6 series were significantly higher in controls compared to ADHD (see Table 1).

Assessing the Impact of IQ—To test for potential confounds of IQ, the significant between-group findings were correlated with IQ. There were no significant relationships between N2 or N4 responses at Fz to angry faces and composite IQ in the ADHD or HC group.

PUFA Fractions and ERP Measures for the Emotion Processing Task

Relationships between P3a, N4/P3b and N4 ERP amplitude responses to Fearful, Sad, Happy and Angry faces and 7 key fatty acid indices (omega-6: LA, c18:3n6, AA, c20:4n6 and Total n-6 and omega-3: ALA: c18:3n3, EPA: c20:5n3, DHA: c22:6n3 and Total n-3) were at four electrode sites (Fz, FCz, Cz, Pz). The FDR correction for multiple tests was employed for all correlations.

P3a (time point 6) and PUFA levels—None of the relationships at this time point survived the FDR correction for multiple testing in either the ADHD or healthy control group. Of note, however, are some relationships which were significant at an alpha criterion of at or smaller than .01 prior to correction ($df = 29$ unless noted otherwise). In the ADHD group, the largest correlation was observed between LA (c18:2n6) and amplitude responses to Fear at Fz electrode site, $r = .539$. Furthermore, at Fz electrode site, DHA was negatively correlated with AUC amplitude responses to Fear, $r = -.542$. A negative correlation was also observed at Fz between total n-3 and responses to Fear, $r = -.464$. Also at Fz, there was a positive relationship between EPA and AUC amplitude responses to Sad faces, $r = .513$. Finally, at Pz, ALA was negatively correlated with facial expressions of Fear, $r = -.478$.

In the healthy control group, a significant negative correlation was observed at FCz between LA and AUC amplitude responses to Happy faces, $r(30) = -.403$. At FCz, there was a negative correlation between LA and AUC amplitude responses to Happy faces, $r(30) = -.419$. At Cz, a significant negative correlation was observed between LA and AUC amplitude responses to Happy faces, $r(30) = -.532$.

N4/P3b (time point 7) and PUFA fractions—None of the relationships at this time point survived the FDR correction in either the ADHD or healthy control group. Correlations which were reached an alpha criterion of at least .01 (prior to the FDR correction) warrant reporting. For example, for the ADHD group, there was a positive relationship between LA and N4/P3b responses to Fearful faces at Fz, $r = .503$. Also at Fz was an association between total n-6 and N4/P3b responses to Fear, $r = .451$. LA was also positively correlated with N4/P3b waves to Sad faces at Cz, $r = .440$. Finally, there was a negative relationship between ALA and N4/P3b responses to Happy faces, $r = -.514$. For the

control group, the largest correlation was observed at Fz between total n-6 and N4/P3b responses to Fear, $r(30) = -.450$, $p = .01$.

N4 (time point 8) and PUFA fractions—Only 2 relationships remained significant following the FDR correction, and 3 relationships reached trend level only, $p = .056$ in the ADHD group only. The first significant relationship was between total n-3 and N4 responses to Happy faces, $r = -.551$. The second was a negative relationship between ALA and N4 responses to Happy faces at Cz, $r = -.577$. The first trend finding was a negative correlation at FCz, ALA was significantly negatively correlated with N4 responses to Happy faces, $r = -.585$. The second trend finding, was observed at Pz; ALA was negatively correlated with N4 responses to Happy faces, $r = -.518$. Finally, at Cz, N4 responses to Happy faces were significantly correlated with total omega-3, $r = -.543$.

Of note, are some relationships, again in the ADHD group, which were significant at an alpha criterion of at least .05 prior to the FDR correction. The first a correlation between EPA and N4 responses to Sad faces at Fz, $r = .393$. The second at Fz, between LA and N4 responses to Fear, $r = .433$. The third, observed between LA and N4 responses to Anger, $r = .380$. Finally, an association at Fz between total n-6 and N4 responses to Anger, $r = .443$.

Discussion

Here we found that children and adolescents with ADHD had deficits in emotional processing and lower plasma LC-PUFAs in choline phosphoglycerides compared to healthy controls. Greater impairments in emotional processing were correlated with lower levels of omega-3 fats including ALA, EPA and DHA among the ADHD group. Children with ADHD had significant differences in both N2 and N4 amplitude responses to the processing of emotional stimuli with a trend finding for group at the early P2 waves compared to controls. ADHA children had significantly less negative activation to facial expressions of emotion as well as neutral faces in ADHD compared to controls, suggesting a generic deficit in the early and late stages of face processing in ADHD, which was not specific to emotional valence or content. Some of the trend findings in N2 and N4 ERP waves, however, showed emotion processing deficits in ADHD patients, which were specific to negative emotions. Importantly, there were also significant negative associations between omega-3 fatty acid indices and N4 in the ADHD group; higher omega-3 were associate with the N4 amplitude responses that were similar to healthy controls. There were also some trend associations between ERP measures and omega-3/6 in line with the study's predictions. The findings thus show for the first time that children and adolescents with ADHD have abnormal ERP amplitude responses in the orienting and event integration stages of emotional and non-emotional face processing and furthermore that some of these were associated with omega-3 plasma levels, which were lower in ADHD relative to controls. Overall the findings are consistent with our hypothesis that emotion processing is abnormal in ADHD and furthermore that this abnormality is associated with lower LC-PUFA levels.

ERP Deficits in ADHD

Relative to controls, children with ADHD differed in their ERP responses to facial stimuli as captured by N2 deflections, reflecting the orienting response, and the later time point N4,

reflecting the event integration aspect of face processing. In line with the model by Halgren (1994), both the N2 and N4 wave are modulated specifically by facial expressions of emotion and the results of this study are consistent with their proposed involvement during affect processing [34, 56]. Both the N4 and the late P3b are known to be abnormal during face processing in both maltreated children and those with psychopathy [57, 58]. The present study confirms for the first time that both N2 and later N4 are also abnormal in children with ADHD relative to typically developing control children. Similar abnormalities were observed for P2 components where the healthy control group in all cases displayed greater negative going activation to sad, neutral and happy faces at frontal and central scalp regions (FCz and Cz) relative to the ADHD group. P2 are related to the basic structural encoding/configural recognition of faces, modulated to emotion as early as 120–160 milliseconds and our results further lend support to this notion [59–61]. These findings thus suggest that ADHD children are impaired in the early and late phases of emotion processing as well as in neutral face processing. No differences were observed between ADHD and control groups for the P3 family (i.e., P3a and P3b deflections).

For all ERPs, the deficits in ADHD patients relative to controls were unspecific to either negative or positive facial expressions as responses were observed to all emotions as well as during neutral faces over the different time points. This suggests that ADHD children have generic deficits in early information processing [62] rather than a specific emotion recognition deficit. The ERP literature has provided consistent evidence for a cognitive-electrophysiological deficit in children with ADHD as characterised by insufficient resource allocation and covert attention orienting [44, 63] and alterations in preparatory processing related to posterior attention networks [64]. This study extends this evidence for early and late information processing deficits in ADHD in the context of cognitive and attention tasks by showing for the first time that these are also impaired in ADHD during emotion processing. The key findings here demonstrate for the first time that both the orienting and contextual evaluation stages of processing independent of emotional valence are abnormal in children with ADHD compared to controls.

N2, N4/P3b and N4 Trend Findings

Trend findings were observed for comparing different facial expressions for N2, N4/P3b and N4 deflections. For N2 deflections, a trend finding was observed for the different facial conditions with angry faces at frontal (Fz) electrodes eliciting the greatest negative-going activity in the controls compared to ADHD. For both N4/P3b and N4 deflections which were all maximal at frontal scalp regions (FCz and Fz respectively), a similar response to negative stimuli was observed with greater activation to anger and sad respectively. These findings suggest that ADHD children show attenuation in activation towards negative emotional stimuli at these time points and provide novel ERP evidence of emotion dysfunction in ADHD, in particular responses to negative emotions. These findings also complement the work of Singhal and colleagues (2012) who reported augmented amplitudes to fearful faces relative to sad and neutral expressions in clinical adolescents with ADHD and affective disorders. Williams and colleagues (2006) have provided evidence in healthy adults that signals of fear are given precedence over both positive (e.g., happy faces) and neutral signals. The diminished responses to negative facial stimuli in the ADHD children of

this study also lends support to the behavioural literature suggesting that children with ADHD have greater difficulty correctly identifying and evaluating the emotional status of others, in particular of fearful and angry faces [7].

Blood Measures of LC-PUFAs and Associations with ERPs

As predicted, there was an overall pattern of persistently higher levels of both omega-3/6 LC-PUFA in the healthy controls compared to ADHD. These findings are consistent with prior reports of levels of fatty acids in ADHD [15, 17], but not with those studies reporting no differences [18]. Given that the sample size is larger than that of previous publications and given that we have applied a correction for multiple testing to both the ERP and blood data and the findings are more robust than those of previous studies many of which did not correct for multiple testing., It is not clear why LC-PUFA levels were lower in this group of children with ADHD, but four primary processes merit consideration. First, differences in dietary intakes of LC PUFAs or their precursors, second differences in absorption or incorporation into tissues third, the metabolism of LC PUFAs from their respective precursors and forth differences in LC PUFA degradation.

Seafood is a rich source of omega-3 LC PUFAs but also a source of omega-6 LC PUFAs so lower intakes of seafood may partially explain this finding. Differences in omega-6 LC PUFA levels may reflect differences in intakes of meat, which is also a source of omega-3 LC PUFAs To our knowledge there are unfortunately no studies of dietary intakes of ADHD children to assess these possibilities. It is unlikely that differences in absorption or incorporation are explanatory as both omega-6 and -3 compete for incorporation into the phospholipid pool and an elevated level of one or the other, would normally result in the suppression of the other [65, 66]. Differences in the metabolism of LC PUFA from their precursors is possible; subjects and controls did not differ in levels of the precursors LA and ALA but did have lower levels of both omega-3 and omega-6 LC PUFA after the delta 6 desaturase, which is a fatty acid patten characteristic of the suboptimally functional FADS 1–2 variants (Polyunsaturated fatty acid levels in blood during pregnancy, at birth and at 7 years: their associations with two common FADS2 polymorphisms. **Steer CD, Hibbeln JR, Golding J, Davey Smith G.** Hum Mol Genet. 2012 Apr 1;21(7):1504–12)

However only one study by Brookes and colleagues (2006) has examined ADHD populations for SNP variants in the FADS 1–2 genes encoding the desaturase enzymes they reported a significant relationship between ADHD and *SNP rs498793* in the fatty acid desaturase 2 (*FADS2*) [67]. While differential degradation of LC PUFAs has been reported in psychiatric population and is often linked to differential rates of smoking, to our knowledge there were no smokers in this study and there are nor reports of preferential degradation in ADHD subjects. Studies which both rigorously control dietary intakes, use stable isotope method to evaluate differential metabolism and fully characterize FADS 1–2 variants would be needed to definitively assess whether the differences in LC PUFA levels reflect differences in dietary intakes of fatty acids or alterations in fatty acid metabolism.

The correlational analyses between plasma levels of LC-PUFA and ERP measures found only two relationships in the ADHD group which survived correction for multiple testing. Both were in line with the study's prediction, namely that omega-3 would be negatively

related to N4 AUC amplitudes. Both showed a negative relationship between N4 responses to happy faces and total omega-3 and ALA. In other words, the higher the omega-3 and ALA, the more negative the response to happy faces, that is, the more similar the activation to that of the controls. Given that N4 was found to be abnormal in ADHD children compared to healthy controls, these relationships suggest that the deficits in N4 may be associated with the lower plasma fatty acid levels in the patient group. The findings thus show for the first time that lower levels of omega-3 PUFAs in ADHD are associated with abnormal brain activation in relation to face processing. The effects of omega-3 deficiency in animal models are widely reported and characterised by both alterations in neuronal migration and faulty neurotransmitter systems in animal models [13, 68, 69]. EPA and DHA in particular have a modulating effects on both serotonergic and dopaminergic brain systems [14]. There is consistent evidence that neurotransmitter functions, in particular dopamine and noradrenaline are abnormal in ADHD [70]. In this study, the ADHD group compared to controls had both lower omega-3 and attenuated neural activity at two stages of the emotion processing model which were more normal in those with higher omega-3 levels, suggesting that omega-3 has a potentially causative or modulating effect. Future randomised, placebo controlled omega-3 LC PUFA supplementation trials would be needed, however, to fully address the hypothesis that omega-3 LC PUFA deficits may cause ERP abnormalities in ADHD and that omega-3 LC PUFA supplementation may normalise these deficits.

Some of the relationships observed between ERP and EFA in ADHD prior to the correction for multiple testing were also in line with the study's hypotheses, that is, that omega-6 fats would be positively associated with N4/P3b responses to negative stimuli (i.e., responses to fear, angry and sad faces). Both measures of LA and total omega-6 ratios were positively associated with fear, anger and sad faces implying that as omega-6 fats increased, N4/P3b responses to negative stimuli also increased, that is, they became more positive in activation and hence more deviant to that of controls.

Overall, the findings thus show a dissociated effect of LC-PUFA on emotional face processing in ADHD; that is, omega-3 LC PUFA appears to be associated with less severe deficits during happy emotions, while omega-6 is associated with more severe deficits during negative emotion processing in ADHD. The findings are in line with evidence of a modulating effect of omega-3 in positive affect [21, 25] while higher omega-6 has been associated with negative outcome in relation to both physical and mental ill-health [47, 71]. This finding of an association between omega-6 and ERP deficits to negative facial emotion processing may possibly be associated with evidence that increased levels of omega-6 (and simultaneous low levels of omega-3) are positively associated with symptoms of negative affect such as homicide rates, neuroticism, suicidal and depressive behaviour [47, 72, 73].

Conclusion

This study demonstrated for the first time that children with ADHD display decreased activation in the early orienting as well as in the later deflection phases associated with stimulus updating and event integration during facial emotion processing. In addition, we observed a generic deficit in affect processing, independent of valence. The results also demonstrated for the first time that some of these deficits are associated with abnormal

plasma levels of omega-3 and omega-6, which were lower in the ADHD group relative to controls. Above all, the findings extend previous evidence for lower omega 3/6 levels in ADHD, by demonstrating that lower LC-PUFA levels in ADHD are associated with abnormal emotion processing. There was also interesting emotional valence dissociation with ADHD patients, with higher omega-3 being associated with better processing of positive emotions and higher omega-6 with poorer negative emotion processing, consistent with proposition that an higher omega-3 to 6 ratios may be necessary for optimal affective processing which may in turn be abnormal in ADHD.

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Abbreviations

HUFA	Highly unsaturated fatty acids
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
AA	arachidonic acid
ADHD	Attention Deficit Hyperactivity Disorder

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Figure 1.

Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 4.

Figure 2.

Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 5.

Figure 3.

Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 8.

Table 1

Mean LC-PUFA fractions in plasma choline phosphoglycerides (PPC) in ADHD and HC groups for the emotion processing task.

Emotion Processing Task (ADHD: <i>n</i> = 29, HC: <i>n</i> = 38)				
	ADHD		Healthy Control	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Omega 6				
c18: 2n-6 (LA)	24.45	3.94	24.76	3.44
c18: 3n6	0.12	0.10	0.09	0.06
c20: 2n6	0.25	0.05	0.31	0.07 **
c20: 3n6	2.80	0.62	3.66	0.96 **
c20: 4n6 (AA)	7.65	1.25	11.63	2.25 **
c22: 4n6	0.29	0.05	0.39	0.08 **
c22: 5n6	0.27	0.15	0.25	0.08
Total n6	35.83	4.37	41.11	3.39 **
Omega 3				
c18: 3n3 (ALA)	0.24	0.10	0.34	0.13 **
c20: 3n3	-	-	0.13	0.04
c20: 5n3 (EPA)	0.53	0.16	0.99	0.45 **
c22: 5n3	0.61	0.18	1.06	0.31 **
c22: 6n3 (DHA)	1.91	0.50	3.71	0.92 **
Total n3	3.29	0.66	6.23	1.42 **

Note:

* $p < .05$,

** $p < .01$