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# Prostaglandins, Leukotrienes and Essential Fatty Acids

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## Omega-3 fatty acids are related to abnormal emotion processing in adolescent boys with attention deficit hyperactivity disorder<sup>☆</sup>

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### ABSTRACT

**Background:** In addition to the core symptoms, attention deficit hyperactivity disorder (ADHD) is associated with poor emotion regulation. There is some evidence that children and young adults with ADHD have lower omega-3 levels and that supplementation with omega-3 can improve 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:** Children with ADHD had lower mean omega-3/6 and ERP abnormalities in emotion processing, independent of emotional valence relative to control children. 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.

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### 1. 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 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 [11] provided pioneering experimental evidence that specific omega-3 fatty acid deficiencies induced behavioural pathologies. Since then evidence has persistently suggested a role for omega-3 fatty acids, in particular for EPA, in the regulation of mood and affect [12] and modulation of implicated neurotransmitter (e.g., serotonergic and dopaminergic) systems [13,14]. In contrast, there is some

**Abbreviations:** ERPs, event-related potentials; LC-PUFA, long-chain polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; ADHD, attention deficit hyperactivity disorder.

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suggestion that excessive omega-6 is associated with disorders of emotional dysregulation (e.g., neuroticism and depression) [15]. Evidence that children and young adults with ADHD have lower plasma levels of omega-3 fatty acids is inconsistent with some studies finding abnormalities [16–18] and others none [19,20]. Conversely, contributions of differing dietary intakes and potential alterations 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 significantly associated with clinical efficacy [21]. Moreover, elevated omega-3 status seems to be associated with the improvement of affective processes such as child depression [22] and anti-social, violent and aggressive behaviours [23,24]. 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.

### 1.1. Event-related potentials and face processing

To test if low omega-3 LC-PUFA levels were related to emotion processing in children with ADHD, we employed event-related potentials (ERPs) of facial emotional recognition processing. ERPs are an imaging technique with a high temporal resolution and refer to those electroencephalographic (EEG) 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). ERP components are traditionally named based on their polarity and their average time of occurrence in milliseconds post-stimulus. For example, the ERP waveform consists of either positive (P) or negative (N) voltage deflections called components or peaks. The letter P, indicates positive going waves, and is followed by the time represented in terms of milliseconds after the onset of audio, visual or somatosensory stimuli [28]. For example, P2 and P3 are both positive peaks occurring at 200 and 300 ms respectively [28]. ERPs are advantageous for the study of emotion processing as they permit the investigation of automaticity during different, temporally separate, stages of emotion processing [29]. There are some neurobiological and imaging data suggesting impaired face processing in ADHD [30,31] and to a greater extent, in clinical groups related to child psychopathy [32]. However, the ERP literature in emotion processing in child ADHD is limited [27,33,34]. We adopted the Halgren and Marinkovic (1994) ERP model for the appraisal and response to emotional stimuli. In this model, there are two stages, “orienting” and “event integration” in distinguishing conscious and non-conscious emotion perception. Orienting can be described as the automatic interruption of continuing processing [35,36], free from conscious consideration and captured by the N2/P3a/slow wave [35]. N2 deflections are also sensitive to facial emotional expressions [35]. The event integration aspect is captured by N4/P3b waves and reflects the cognitive integration of overt emotional experiences [36]. The N4 deflection is a marker also of semantic processing [37]. Aside from studies of facial expressions, there is consistent evidence that children with ADHD have impairments 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) [38]. These impairments are characterised by abnormal waveforms which are most prominent during tasks of motor response inhibition and selective attention in frontal and parietal brain regions relative to controls [39–47]. In contrast to the fairly

sizeable neuroimaging literature on cognitive functions in ADHD, the neurophysiological correlates of emotional dysfunction in children/adolescents with ADHD are fairly unknown. Singhal and colleagues [34] 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 increase in P3 amplitudes reflecting both attention processing, as well as a sustained effect on emotion on target processing, in the clinical sample only [34]. In relation, to date we could only find one previous publication reporting preliminary data comparing omega-3 fatty acids and emotion processing in a small sample size of children with ADHD [27].

The aims of the present study were threefold. We wanted to 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 four 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 theorised that omega-3 LC-PUFA in plasma choline phosphoglycerides would be lower in ADHD compared to controls in line with the fatty acid literature [16–18]. With respect to the relationship between LC-PUFA and ERP responses to facial expressions of emotion, it was predicted that (1) if omega-3 plays a role in emotion regulation, they would be associated with an attenuation of the N4 response to affect, irrespective of valence; (2) building on our previous research [27], that omega-3 PUFA (in particular EPA), levels would be positively correlated with P3 responses to happy faces (i.e., the higher the omega-3—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 suggesting a link between omega-6 and negative affect [15].

## 2. Method

### 2.1. Participants

The Maudsley adolescent ADHD fatty acid (MAAFA)<sup>1</sup> trial enrolled 76 male children of whom EEG data for this task were obtained from 63 participants only (due to faulty recording or missed appointment visits): comprising 31 children who met the criteria for ADHD and 32 matched control children. The EEG/ERP data from the ADHD participants were collected during baseline assessments during the 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 [48]) based on DSM-IV criteria. For inclusion, both Parent and Teacher Connor Rating Scales (CPRS/CTRS) had to be equal to or above 65 (> 95th percentile, see Table 1). The MAAFA

<sup>1</sup> <http://www.controlled-trials.com/ISRCTN27741572/MAAFA>.

**Table 1**  
Means and standard deviations for clinical characteristics between ADHD and HC.

	Controls (n=38)		ADHD (n=31)		t	p
	M	SD	M	SD		
Age	14.46	1.12	14	1.1	-1.72	0.09
KBIT composite IQ scores	118.73	13.58	96.32	13.96	-6.73	0.001***
No. of years of education	9.34	0.96	9.00	1.39	1.2	0.23
<b>Conners' Parent Rating Scales</b>						
Conners' ADHD Index	47.05	5.64	75.23	5.02	-21.67	0.001***
Conners' Global Index: restless-impulsive	48.58	6.59	79.45	8.92	-16.03	0.001***
CGI: emotional lability	47.97	9.40	73.90	12.47	-9.57	0.001***
CGI: total	48.13	7.38	80.16	9.81	-15.03	0.001***
DSM-IV inattentive	46.55	5.62	70.94	8.09	-14.2	0.001***
DSM-IV hyperactive-impulsive	48.76	6.69	82.58	8.85	-17.56	0.001***
DSM-IV: total	47.39	5.83	79.10	8.86	-17.13	0.001***
<b>Conners' Teacher Rating Scales</b>						
Conners' ADHD Index	48.00	7.72	76.16	6.23	-16.37	0.001***
Conners' Global Index: restless-impulsive	48.92	8.46	76.03	7.04	-14.25	0.001***
CGI: emotional lability	49.47	8.74	80.61	11.25	-12.94	0.001***
CGI: total	48.95	8.45	80.39	7.97	-15.77	0.001***
DSM-IV inattentive	48.97	8.02	70.65	7.23	-11.67	0.001***
DSM-IV hyperactive-impulsive	48.68	8.70	74.77	9.00	-12.2	0.001***
DSM-IV: total	49.68	9.78	75.23	7.72	-11.84	0.001***

\* $p < 0.05$ , \*\* $p < 0.01$ .

\*\*\*  $p < 0.001$ .

trial ERP data were 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 (KBIT). Exclusion criterion 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 National Research Ethics Service.<sup>2</sup> Twenty-two of the ADHD participants were medication naïve and the remaining nine underwent a 48 h wash out period for stimulant medication, in line with current EEG practice.

## 2.2. Procedure and 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 [49] by homogenising 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 visualised 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 3 h 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) [50]. This study reports data from plasma choline phosphoglyceride (PC) fatty acids which was available for 29 of the children with ADHD and 38 of the healthy control children.

**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 [51]. 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 (eight 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 with the exclusion of facial expressions of disgust. Participants were advised to focus on each face in preparation for post-test questions to ensure attention.

**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 LabNeuroTM (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 re-referenced offline to linked mastoids. Horizontal eye movements were recorded with electrodes placed 1.5 cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3 mm above the middle of the left eyebrow and 1.5 cm below the middle of the left bottom eye-lid. Skin resistance was < 5 kOhm. A continuous acquisition system was employed and data corrected for electrooculographic (EOG) artefacts offline [52]. A low-pass filter with attenuation of 40 dB per decade above 100 Hz was employed prior to digitisation.

**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 25 Hz and 35 Hz is used

<sup>2</sup> Camden & Islington Community Research Ethics Committee, 08/H0722/88.

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 8 ms, 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 50 ms time-windows that can be approximately mapped onto ERP component time-windows.

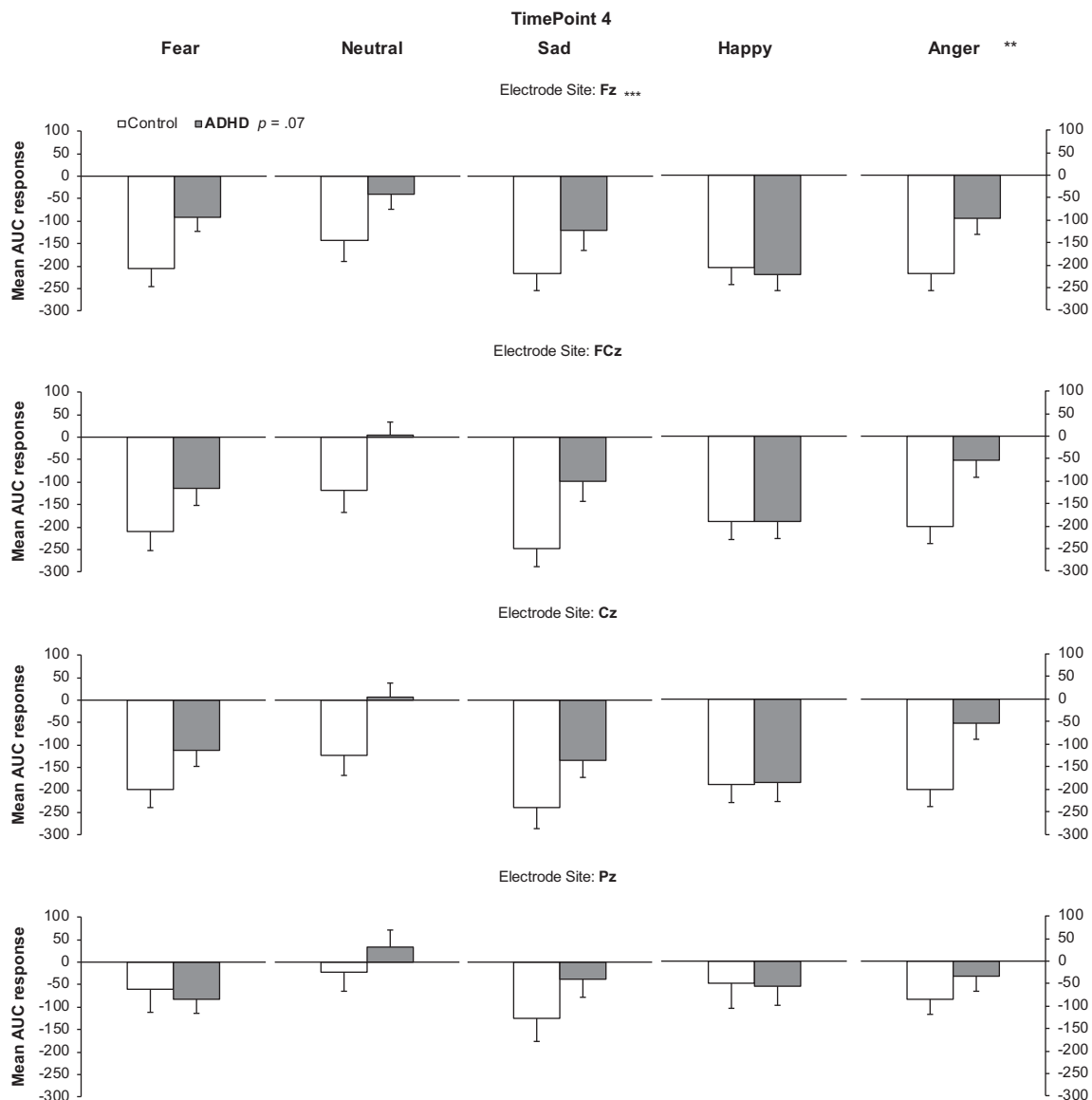
### 3. Results

#### 3.1. Statistical methods and analyses

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) using the Statistical Package for the Social Sciences (SPSS) version 15. 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). Earlier time points (i.e., 1–3 representing 0–150 ms) are not included in this study. 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 Bonferroni corrected 0.05 rejection criterion, unless noted otherwise. All data were assessed for violation of sphericity employing Mauchly's test and the  $F$  statistics corrected using the Greenhouse–Geisser correction. 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



**Fig. 1.** Mean AUC P2 amplitudes ( $\mu\text{V}$ ) at Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger in children with and without ADHD. Statistically significant main effects of group, electrode and condition (facial expression) are indicated by \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

( $n=29$ ) and control children ( $n=38$ ). 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 fatty acids analyses [53]. Here, the term significant indicates analyses surviving multiple testing and do not imply consideration of clinical significance.

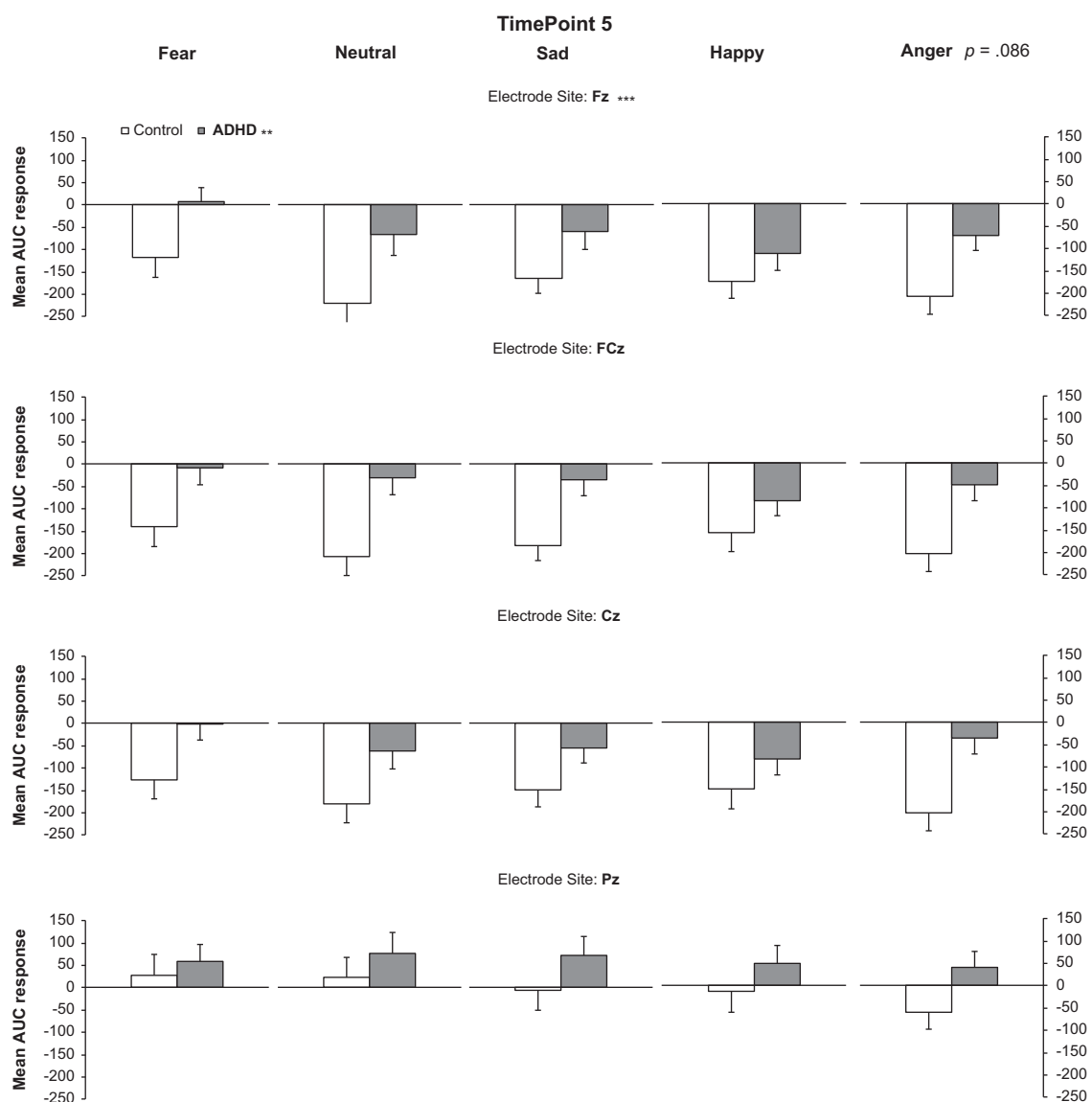
### 3.2. Age, IQ and handedness

The means and standard deviations for participant characteristics are illustrated in Table 1. There were no statistically significant differences in age between the ADHD group and the control group,  $p = .09$ . In the ADHD group, 28 males were right-handed, one was left-handed and two were ambidextrous. In 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,  $\chi^2(2, 73) = 3.71, p = 0.15$ .

There were statistically significant differences between measures of composite (overall) IQ between the control group and the ADHD group,  $p < 0.001$ , with ADHD scoring lower in IQ than controls (see Table 1). IQ is typically lower in ADHD children [54]. 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. Furthermore, ANCOVA cannot be used to control treatment assignment independent of the covariate [55,56]. However, to test the impact of IQ on the findings, we correlated measures that differed between groups with IQ.

### 3.3. ERP results

**Time point 4 (P2 deflection):** The mean values ( $\mu V$ ) and standard errors for the AUC P2 amplitude responses for ADHD and control children are plotted in Fig. 1. A trend was observed for P2 responses for the main effect of group (ADHD versus controls),  $F(1, 61) = 3.24, p = 0.07$ , with less positive going activity among the healthy controls



**Fig. 2.** Mean AUC N2 amplitudes ( $\mu V$ ) at Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger in children with and without ADHD. Statistically significant main effects of group, electrode and condition (facial expression) are indicated by \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .



( $M = -162.47$ ,  $SE = 30.68$ ) compared to ADHD ( $M = -83.74$ ,  $SE = 31.17$ ). There were significant main effects for electrode site,  $F(1.42, 86.49) = 21.36$ ,  $p < 0.001$ , with greater activation at Fz ( $M = -155.73$ ,  $SE = 22.73$ ) and for faces,  $F(4, 244) = 8.65$ ,  $p = 0.001$ , with happy faces producing greater negative-going activation ( $M = -160.93$ ,  $SE = 26.29$ ).

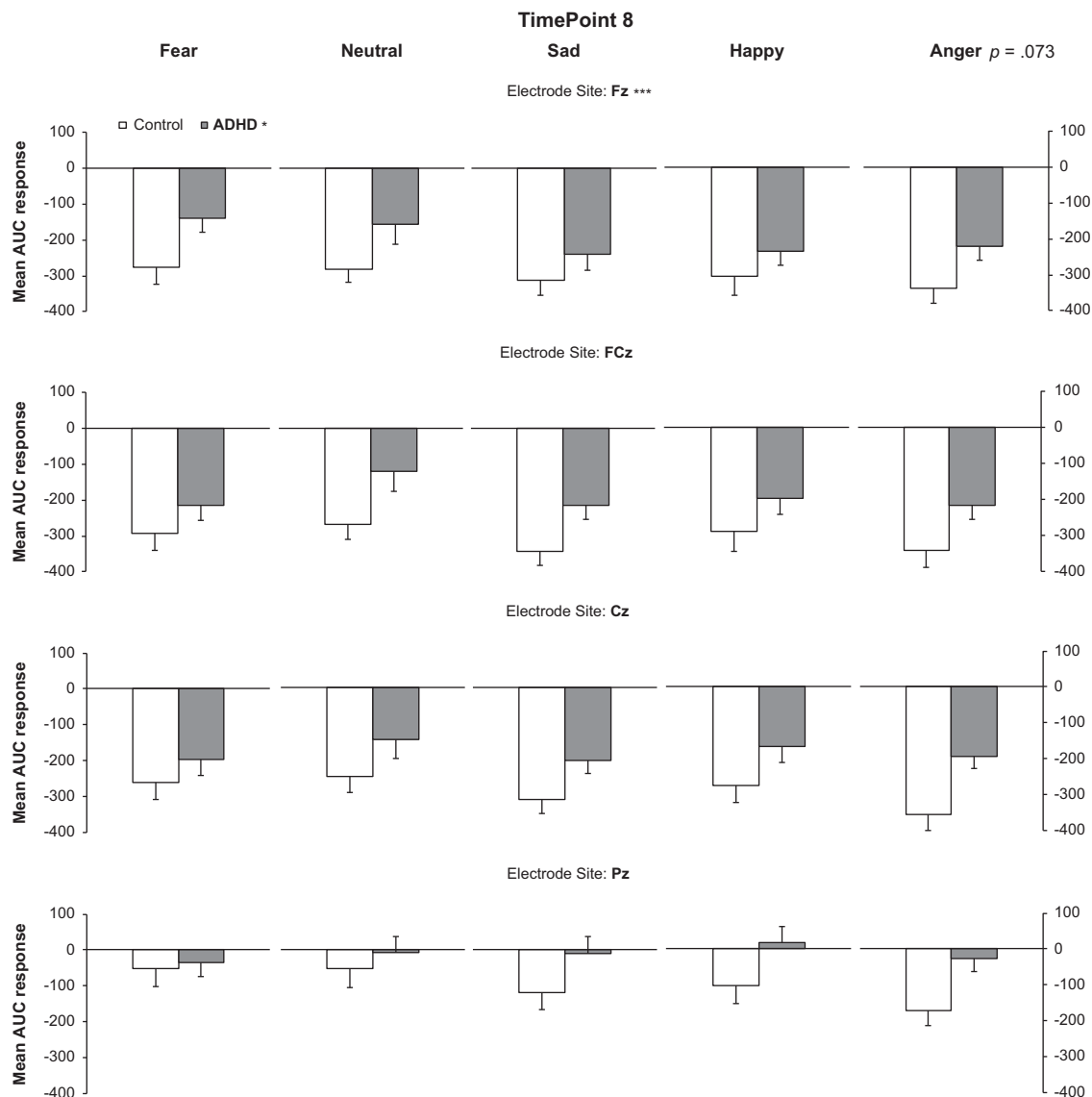
A trend was observed for the interaction between electrode site and group,  $F(3, 183) = 2.32$ ,  $p = 0.07$ . Post-hoc tests revealed that this was due to significantly greater differences in activation at FCz and Cz electrode site between ADHD and controls with enhanced negativity for controls compared to ADHD.

There was a significant interaction between faces and group,  $F(4, 244) = 2.84$ ,  $p = 0.02$ . 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 less positive going activation to facial expressions of sadness 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 less positive going activation relative to the ADHD group.

There was a significant interaction between electrode site and faces,  $F(6.47, 394.46) = 2.77$ ,  $p = 0.01$ . 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.

A trend was also observed for the 3-way interaction between electrode site, faces and group,  $F(12, 732) = 1.56$ ,  $p = 0.09$  which suggested that these group differences were present at frontocentral (Fz for responses to fearful and angry faces; FCz for responses to neutral, sad and angry faces; Cz for responses to neutral and angry faces), but not parietal sites. In all cases activation was higher to these facial expressions among ADHD children relative to controls.

*Time point 5 (N2 deflection):* The mean values ( $\mu V$ ) and standard errors for the AUC P2 amplitude responses for ADHD and control children are plotted in Fig. 2. A significant main effect of group was observed for N2 responses,  $F(1, 61) = 7.12$ ,  $p = .01$  with greater amplitudes in the control group ( $M = -130.25$ ,  $SE = 28.52$ ) compared to the ADHD group ( $M = -21.73$ ,  $SE = 28.97$ ).



**Fig. 3.** Mean AUC N4 amplitudes ( $\mu V$ ) at Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger in children with and without ADHD. Statistically significant main effects of group, electrode and condition (facial expression) are indicated by \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

The main effect of electrode site was significant,  $F(1.34, 83.37)=38.26$ ,  $p < 0.001$  with greater activation at Fz ( $M=-118.17$ ,  $SE=21.04$ ). A trend finding was observed for faces,  $F(4, 244)=2.07$ ,  $p = 0.086$  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,  $F(7.12, 434.23)=1.91$ ,  $p = 0.066$ . The N2 response to facial expressions of fear ( $M=-54.56$ ,  $SE=25.34$ ) was significantly lower to that of neutral ( $M=-143.24$ ,  $SE=31.21$ , happy ( $M=-142.29$ ,  $SE=27.03$ ) and angry faces ( $M=-138.08$ ,  $SE=26.47$ ).

**Time point 6 (P3a deflection):** There was a significant main effect of electrode site,  $F(1.40, 85.48)=74.83$ ,  $p = 0.001$ , driven by greater negative-going amplitudes (lower P3a) at Fz ( $M=-171.66$ ,  $SE=23.75$ ). The main effect for faces was also significant,  $F(3.29, 200.90)=3.84$ ,  $p = 0.01$  with greater negative-going activation to neutral faces ( $M=-162.84$ ,  $SE=32.24$ ).

A trend was observed for the interaction between electrode site and faces,  $F(7.25, 442.06)=1.99$ ,  $p = 0.053$ . Post-hoc tests showed that this was due to significantly lower P3a in response to facial expressions of fear ( $M=-98.98$ ,  $SE=30.38$ ) compared to neutral faces ( $M=-238.84$ ,  $SE=34.71$ ) at Fz electrode site. A similar effect was found at Cz to fear ( $M=-95.76$ ,  $SE=35.53$ ) and neutral ( $M=-232.63$ ,  $SE=35.88$ ) faces. No other interactions reached significance. The main effect of group was not significant.

**Time point 7 (N4/P3b deflection):** The main effect of electrode site was significant,  $F(1.54, 93.75)=96.43$ ,  $p = 0.001$  which was due to greater negative-going activity at FCz ( $M=-209.64$ ,  $SE=25.93$ ) compared to Pz ( $M=37.28$ ,  $SE=25.67$ ). A trend finding was observed for faces  $F(3.27, 199.59)=2.18$ ,  $p = 0.086$  with greater mean activation to sad faces ( $M=-172.51$ ,  $SE=29.00$ ) compared to the lowest amplitude responses which were to neutral facial expressions ( $M=-108.35$ ,  $SE=27.45$ ). Neither the main effect of group nor any interaction effects reached significance.

**Time point 8 (N4 deflection):** The mean values ( $\mu V$ ) and standard errors for the AUC N4 amplitude responses for ADHD and control children are plotted in Fig. 3. The main effect of group was significant,  $F(1, 61)=5.42$ ,  $p = 0.03$  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,  $F(1.58, 96.13)=65.72$ ,  $p < 0.001$ , driven by higher negative activation at Fz ( $M=-250.33$ ,  $SE=23.70$ ). The main effect for faces was non-significant,  $F(3.45, 210.52)=2.26$ ,  $p = 0.073$ , reflecting the greater mean activation to angry faces ( $M=-233.44$ ,  $SE = 26.10$ ). None of the interactions reached significance.

#### 3.4. PPC fatty acid levels comparing children/adolescents with and without ADHD

There were significant differences between the ADHD and control groups for 10 out of the 13 fatty acids indices (see Table 1). For the omega-6 series there were significant differences for c20:2n6,  $t(65)=-3.48$ ,  $p = 0.01$ , c20:3n6,  $t(66)=-4.28$ ,  $p = 0.001$ , c20:4n6 (AA),  $t(66)=-9.23$ ,  $p = 0.001$ , c22:4n6 (a metabolite of AA),  $t(66)=-5.65$ ,  $p = 0.001$ , and total n-6,  $t(66)=-5.61$ ,  $p = 0.002$ . For the omega-3 series, there were significant differences between ADHD and control children for c18:3n3 (ALA),  $t(66)=-3.51$ ,  $p = 0.002$ , c20:5n3 (EPA),  $t(66)=-5.88$ ,  $p = 0.003$ , c22:5n3 (DPA),  $t(66)=-7.53$ ,  $p = 0.004$ , c22:6n3 (DHA)  $t(69)=-11.08$ ,  $p = 0.006$ , c22:6n3,  $t(66)=-11.32$ ,  $p = 0.006$  and total omega-3,  $t(66)=-11.32$ ,  $p = 0.01$ . In all instances, PPC omega-3 and 6 levels were significantly higher in controls compared to ADHD (see Table 2). The FDR correction for multiple testing was employed in all analyses.

**Assessing the impact of IQ:** To test for potential confounds of IQ, the significant between-group findings were correlated with IQ.

**Table 2**

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

	Healthy control		ADHD	
	M	SD	M	SD
<b>Omega-6</b>				
c18: 2n-6 (LA)	24.8	3.44	24.5	3.94
c18: 3n6	0.09	0.06	0.12	0.10
c20: 2n6	0.31	0.07	0.25	0.05**
c20: 3n6	3.66	0.96	2.80	0.62**
c20: 4n6 (AA)	11.6	2.25	7.65	1.25**
c22: 4n6	0.39	0.08	0.29	0.05**
c22: 5n6	0.25	0.08	0.27	0.15
Total n6	41.1	3.39	35.8	4.37**
<b>Omega-3</b>				
c18: 3n3 (ALA)	0.34	0.13	0.24	0.10**
c20: 3n3	0.13	0.04	-	-
c20: 5n3 (EPA)	0.99	0.45	0.53	0.16**
c22: 5n3	1.06	0.31	0.61	0.18**
c22: 6n3 (DHA)	3.71	0.92	1.91	0.50**
Total n3	6.23	1.42	3.29	0.66**

Note: \*  $p < 0.05$ .

\*\*  $p < 0.01$ .

There were no significant relationships between N2 or N4 responses at Fz to angry faces and composite IQ in the ADHD or HC group.

#### 3.5. PPC levels and ERP measures of emotion processing

Relationships between AUC amplitudes at four electrode sites (Fz, FCz, Cz and Pz) fearful, sad, happy and angry faces and seven 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 tested using Pearson's or Spearman's correlation coefficients (as appropriate) in each group. Given previous findings supporting P3 and N4 as correlated of fatty acid content and to minimise multiple comparisons, this analyses was restricted to time windows 6-8. No significant associations were found for P3a or N4/P3b. However, correlations which reached an alpha criterion of 0.05 and at least 0.01 (prior to the FDR correction) warrant reporting and are presented as trend effects in subsequent sections. Note,  $df=29$  unless noted otherwise.

**N4 (time point 8) and PPC levels:** Following the FDR correction, only significant associations remained in the ADHD group only. There was a significant relationship between total n-3 and N4 responses to Happy faces,  $r=-0.551$ ,  $p = 0.002$  (adjusted  $p = 0.04$ ). There was also a significant negative association between ALA and N4 responses to happy faces at Cz,  $r=-0.577$ ,  $p = 0.001$  (adjusted  $p = 0.03$ ).

Trend associations were observed to Happy faces at FCz for ALA,  $r=-0.585$ ,  $p = 0.001$  (adjusted  $p = 0.056$ ) and at Pz, ALA was negatively correlated with N4 responses to Happy faces,  $r=-0.518$ ,  $p = 0.003$  (adjusted  $p = 0.056$ ) and at Cz, for total omega-3,  $r=-0.543$ ,  $p = 0.002$  (adjusted  $p = 0.056$ ).

Of note, are some relationships, again in the ADHD group, which were significant at an alpha criterion of at least 0.05 prior to the FDR correction. Association were observed at Fz between EPA and N4 responses to sad faces,  $r = 0.393$ ,  $p = 0.03$ ; LA and N4 responses to fear,  $r = 0.433$ ,  $p = 0.02$ ; LA and N4 responses to anger,  $r = 0.380$ ,  $p = 0.04$  and lastly between total n-6 and N4 responses to anger,  $r = 0.443$ ,  $p = 0.01$ .

**Trend associations for P3a (time point 6) and PPC levels:** In the ADHD group, trend associations were observed in response to

fearful faces at Fz for LA,  $r = 0.539$ ,  $p = 0.002$ , DHA,  $r = -0.542$ ,  $p = 0.002$  and total  $n-3$ ,  $r = -0.464$ ,  $p = 0.01$ . Also at Fz, there was a positive relationship between EPA and AUC amplitude responses to sad faces,  $r = 0.513$ ,  $p = 0.004$ . Finally, at Pz, ALA was negatively correlated with facial expressions of fear,  $r = -0.478$ ,  $p = 0.008$ .

In the healthy control group, negative associations were observed between AUC amplitudes responses to happy faces and LA at Fz,  $r(30) = -0.403$ ,  $p = 0.005$ , FCz,  $r(30) = -0.419$ ,  $p = 0.02$  and Cz,  $r(30) = -0.532$ ,  $p = 0.002$ .

*Trend associations for N4/P3b (time point 7) and PPC levels:* For the ADHD group, N4/P3b responses to fearful faces at Fz were associated with LA,  $r = 0.503$ ,  $p = 0.005$  and total  $n-6$ ,  $r = 0.451$ ,  $p = 0.012$ . LA was also positively correlated with N4/P3b waves to sad faces at Cz,  $r = 0.440$ ,  $p = 0.01$ . Finally, there was a negative relationship between ALA and N4/P3b responses to happy faces,  $r = -0.514$ ,  $p = 0.004$ . For the control group, the largest correlation was observed at Fz between total  $n-6$  and N4/P3b responses to fear,  $r(30) = -0.450$ ,  $p = 0.01$ .

#### 4. Discussion

This study found that children and adolescents with ADHD had deficits in emotion processing and lower fatty acid composition of plasma choline phosphoglycerides including EPA and DHA compared to healthy controls. Greater impairments in emotional processing were correlated with lower levels of omega-3 fatty acids including ALA among the ADHD group. Children with ADHD had significantly lower N2 and N4 amplitudes, irrespective of affect valence and furthermore their P2 response to neutral and negative (anger, fear, sadness) emotional expressions were exaggerated compared to healthy control children. However, at P2 there was a significant main effect of condition with greater overall activation to happy faces. The correlational analyses between ERP responses and omega-3/6 fatty acid indices found significant associations between higher omega-3 content (both total omega-3 and ALA) and greater N4 responses to happy faces in the ADHD group; that is, higher omega-3 levels were associated with the N4 amplitude responses which were more similar to healthy controls. There were also some trends and/or associations prior to the FDR correction in line with the study's prediction. For example, higher LA and total omega-6 were positively associated with N4 amplitude responses to negative emotions including fear and angry facial expressions. The findings thus show for the first time that the ERP responses of semantic processing of facial affect in children and adolescents with ADHD are impaired and that in relation to happiness this effect is associated with omega-3 plasma levels. Overall the findings were consistent with our hypothesis that emotion processing is abnormal in ADHD and furthermore that this abnormality is, in part, associated with lower LC-PUFA levels.

##### 4.1. Blood measures of LC-PUFA 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 previous reports of levels of fatty acids in ADHD [16,18], but not with those studies reporting no differences [19]. Given that the sample size is larger than some of the previous publications and that we applied a correction for multiple testing to both the ERP and blood data, the findings are more robust than those of previous studies. Especially given that many studies, in fact the majority did not report statistical corrections for multiple testing. It is not entirely clear why LC-PUFA levels were lower in this group of children with ADHD but four primary processes could be underlying these group

differences. First, group differences in dietary intakes of LC-PUFAs or their precursors; second group differences in absorption or incorporation into tissues; third, possible group differences in the metabolism of LC-PUFAs from their respective precursors, and fourth, group 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 in the ADHD group may partially explain this finding. Differences in omega-6 LC-PUFAs may reflect differences in intake of meat, which are also a source of omega-3 LC-PUFAs. However, the ADHD children in this study had higher levels of palmitic (16:0), stearic (18:0), total saturated, palmitoleic (16:1n-7), oleic (18:1n-9) and total mono-unsaturated fatty acids. These differences are unlikely to be a reflection of dietary intake. Both omega-3 and 6 levels 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 [57,58]. Differences in the metabolism of LC-PUFA from their precursors is possible; ADHD children and controls did not differ in levels of the precursors: LA and ALA but had lower levels of both omega-3 and omega-6 LC-PUFA after the delta-6 desaturase, which is a fatty acid pattern characteristic of the suboptimally functional FADS 1-2 variants [59]. However, only one study by Brookes and colleagues (2006) [60] has examined ADHD populations for SNP variants in the FADS 1-2 genes encoding the desaturase enzyme. They reported a significant relationship between ADHD and SNP rs498793 in the fatty acid desaturase 2 (FADS2) [60]. While differential degradation of LC-PUFAs has been reported in psychiatric populations and is often linked to differential rates of smoking and or oxidative stress, to our knowledge there were no smokers in this study. In order to rigorously control dietary intakes, future studies should employ stable isotope methods to evaluate differential metabolism and fully characterise FADS 1-2 variants.

##### 4.2. Group differences in ERPs

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), the N2 and N4 wave are modulated specifically by facial expressions of emotion and the results of this study confirm their involvement during affect processing [35,61]. Both the N4 and the late P3b during face processing are known to be abnormal in maltreated children and those with psychopathy [62,63]. The present study confirms for the first time that N2 and N4 waves are also abnormal in children with ADHD relative to typically developing control children. A similar pattern was observed for P2 components where the healthy control group in all cases displayed less positive going activation to sad, neutral, angry and fearful faces at frontal and central scalp regions (FCz and Cz) relative to the ADHD group. The higher P2 amplitudes to negative facial stimuli in the ADHD children supports other research studies who have reported that P2 components are emotion-sensitive and involved in the detection of threat-related stimuli in particular angry faces [64]. Barry et al. (2009) [65] reported larger visual P2 in ADHD in comparison to controls in an oddball task suggesting a potential additional load on effortful responses to facial expressions. In healthy individuals, significantly reduced P2 components have been specifically reported in response to angry faces compared to neutral facial expressions [66]. The exaggerated P2 response to negative and neutral valence observed in our ADHD group supports a recent study reporting altered P2 responses in delinquent adults with ADHD compared to non-delinquent and control adults [64]. The findings of impaired facial recognition in this study also support some of 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]. P2 is related to the basic structural encoding/configural recognition of faces, which is modulated to emotion as early as 120–160 ms. Our results further lend support to this notion [67–69]. By way of summary, our ERP findings suggest that ADHD children show atypical responses 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). This may be due to the functional specificity of P3 which is most prominent during tasks of sustained attention.

In contrast to P2 deflections which were higher in ADHD to negatively affect valence, the atypical responses in ADHD compared to controls in N2 and N4 deflections were relatively unspecific to either negative or positive facial expressions. However, a trend finding for the main effect of condition (facial expression) was observed for N2 components with greater negative-going activation to angry faces. For N4 waves, the main effect of faces was not significant. Based on these findings, there is a possibility that ADHD children may have generic deficits in early information processing [70] 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, covert attention orienting [45,71] and alterations in preparatory processing related to posterior attention networks [72]. 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 finding here demonstrates that both the orienting and contextual evaluation stages of processing independent of emotional valence are abnormal in children with ADHD compared to controls.

#### 4.3. Comparison of facial expressions

There was a significant main effect of faces for P3a responses with increased negativity to neutral faces. Trend findings were observed for the comparison of 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 affect valence was observed with greater activation to sad and angry faces respectively. Singhal et al. (2012) similarly reported augmented amplitudes to fearful faces relative to sad and neutral expressions in both non-clinical and clinical adolescents with ADHD and affective disorders. Williams and colleagues (2006) also provided evidence that signals of fear are given precedence over both positive (e.g., happy faces) and neutral signals in healthy adults.

#### 4.4. Fatty acid indices and associations with ERPs

The correlation analyses between plasma levels of LC-PUFA and ERP measures found only two significant relationships in the ADHD group which survived correction for multiple testing. These 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 total omega-3 and ALA and N4 responses to happy faces. 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. The N4

deflection has previously been associated with omega-3 and omega-6 content in schizophrenia during a word priming task [73] and also DHA during a continuous visual recognition task in Greenland Inuit, fish eating, typically developing school children [74] but to our knowledge no other studies exist in which to contrast to our findings. Given that N4 waves, which are associated with semantic processing and the contextual evaluation of emotion, were found to be abnormal in ADHD children compared to healthy controls, we postulate that lower  $n-3$  (as found in the ADHD group) may affect the ability to generate the neuronal activation required to sufficiently process the affect valence. The effects of omega-3 deficiency in animal models are widely reported and characterised by both alterations in neuronal migration and faulty neurotransmitter systems [13,75,76]. EPA and DHA, in particular, are thought to have modulating effects on both serotonergic and dopaminergic brain systems [14]. There is consistent evidence also that neurotransmitter functions, in particular dopamine and noradrenaline, are abnormal in ADHD [77]. In this study, the ADHD group compared to controls had both lower omega-3 LC-PUFAs and attenuated neural activity at two stages of the emotion processing model which were more typical in those with higher omega-3 levels, suggesting that omega-3 may have a potentially causative or modulating effect. Future randomised, placebo controlled omega-3 LC-PUFA supplementation trials would be needed to fully address the speculative notion 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. Higher levels of omega-6 have previously been negatively associated with symptoms of both depression and neuroticism [15] and our findings provide some small support for the notion that omega-6 levels are associated with negative affect.

Overall, the findings thus show a dissociated effect of LC-PUFA on emotional face processing in ADHD; that is, omega-3 appears to be associated with less severe deficits during happy emotions, while omega-6 is associated with negative face recognition in ADHD. The findings are in line with evidence of a modulating effect of omega-3 in positive affect [22,25] while higher omega-6 has been associated with negative outcome in relation to both physical and mental ill-health [15,78]. This finding of an association between omega-6 and ERP deficits to negative facial emotion processing may possibly also 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** [15,79,80].

## 5. Conclusion

This study demonstrates 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 both a generic deficit in affect processing, independent of valence and a specific exaggerated effect of P2 responses to negative facial expressions. The results also demonstrated for the first **time that some of these deficits are associated**

with abnormal plasma levels of omega-3 and total omega-6 levels, 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 propositions that a higher omega-3 to 6 ratio may be necessary for optimal affective processing which may in turn be abnormal in ADHD.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.plefa.2013.03.008>.

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