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Event Related Potential Differences in Children Supplemented with Long-chain Polyunsaturated Fatty Acids During Infancy

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Abstract

Long-chain polyunsaturated fatty acids (LCPUFA) have been shown to be necessary for early retinal and brain development, but long-term cognitive benefits of LCPUFA in infancy have not been definitively established. The present study sought to determine whether LCPUFA supplementation during the first year of life would result in group differences in behavior and event-related potentials (ERPs) while performing a task requiring response inhibition (Go/No-Go) at 5.5 years of age. As newborns, 69 children were randomly assigned to infant formulas containing either no LCPUFA (control) or formula with 0.64% of total fatty acids as arachidonic acid (ARA; 20:4n6) and various concentrations of docosahexaenoic acid (DHA; 22:6n3) (0.32%, 0.64% or 0.96%) for the first 12 months of life. At 5.5 years of age, a task designed to test the ability to inhibit a prepotent response (Go/No-Go) was administered, yielding both event-related potentials (ERPs) and behavioral data. Behavioral measures did not differentiate between groups, although reaction times of supplemented children were marginally faster. Unsupplemented children had lower P2 amplitude than supplemented children to both Go and No-Go conditions. N2 amplitude was significantly higher on No-Go trials than Go trials, but only for supplemented children, resulting in a significant Group X Condition interaction. Topographical analysis of the ERPs revealed that the LCPUFA-supplemented group developed a novel period of synchronous activation (microstate) involving wider anterior brain activation around 200 ms; this microstate was not present in controls. These findings suggest that LCPUFA supplementation during the first 12 months of life exerts a developmental programming effect that is manifest in brain electrophysiology.

Keywords

Docosahexaenoic Acid (DHA); Arachidonic Acid (ARA); Response Inhibition; Event-related potential (ERP); Microstate

Long-chain polyunsaturated fatty acids (LCPUFA) in particular, docosahexaenoic acid (DHA; 22:6n3) and arachidonic acid (ARA; 20:4n6), are found in high concentrations in the brain and have been shown to be important for cognitive and visual development (Lauritzen, Hansen, Jørgensen, & Michaelsen, 2001). DHA has been reported to affect cognitive functions such as working memory (Zhang, Hebert, & Muldoon, 2005), problem solving ability (Willatts, Forsyth, DiModugno, Varma, & Colvin, 1998), information processing speed (Henriksen et al., 2008; Willatts et al., 2013) and motor skills (Sefero lu, Erman, ahan, & Tokta , 2012). The role of LCPUFAs in cognitive development may be linked to the biological effects of LCPFUs on brain development, which are known to impact neuronal membrane structure, synaptogenesis, and myelination (Georgieff, 2007).

The class of cognitive processes that are identified as *executive function* can be characterized as those higher-order and later-developing abilities that, for example, mediate the individual's ability to endogenously maintain or sustain attention, filter information in the service of goal-directed behavior, learn and implement rules, and inhibit pre-potent options in the selection of appropriate behavioral responses (Rueda, Rothbart, McCandliss, Saccomanno, & Posner, 2005). The development of brain regions that mediate executive function emerges in early childhood, but is not fully complete until late adolescence (Diamond, 2013). Inhibitory control is a key component of executive function; it is defined as the ability to actively suppress, interrupt or delay a pre-potent response (Clark, 1996). Inhibition is typically measured via cognitive tasks that require one to ignore distracting stimuli or other task-irrelevant associations (Koch, Gade, & Philipp, 2004).

A widely used task for assessing inhibitory control is called Go/No-Go task. It usually consists of two types of stimuli: 1) a stimulus that requires a rapid response (i.e., the Go condition) that is presented so frequently that the response becomes pre-potent, and thus challenging to inhibit, and 2) a less frequent stimulus that requires inhibition of the pre-potent Go response (i.e., the No-Go condition). Inhibitory response control typically demonstrates a developmental improvements from 4 to 12 years-of-age, as indexed by mean reaction times and performance accuracy, which is largely driven by the false alarm rate to the No-Go stimuli (Durstun et al., 2002). Although the Go condition provides clear behavioral signals of response time and accuracy, correct response in the primary condition of interest—the No-Go condition—produces no overt behavior at all. Fortunately, measurement of brain activity during correct No-Go trials provides a way to assess the neuro-functional response to inhibitory control in a manner uncontaminated by any overt behavior.

Brain electrical potentials from the scalp can provide insight into consecutive temporal brain processing to a given task. Electrophysiological measures have excellent temporal (i.e., ms) resolution that can be used to characterize neuronal activity during such tasks. Thus, electrophysiological measures such as the event-related potentials (ERPs) (Bokura,

Yamaguchi, & Kobayashi, 2001; Falkenstein, Hoormann, & Hohnsbein, 1999; Pfefferbaum, Ford, Weller, & Kopell, 1985) and microstate analysis (Meier, Perrig, & Koenig, 2012; Michel, Koenig, & Brandeis., 2009; Schiller, Gianotti, Nash, & Knoch, 2013; Strik, Fallgatter, Brandeis, & Pascual-Marqui, 1998) have been widely used in studies of executive function in the Go/No-Go task.

Although electrophysiological measures have also been used extensively in studies of LCPUFA supplementation, such studies have focused mainly on low level sensory processes, such as visual acuity development (Birch et al., 2010; Colombo et al., 2011). Fewer studies have used electrophysiological measures to assess higher-level cognitive function. Henriksen et al. (2008) studied the impact of LCPUFA on newborn preterm infants, by randomizing them DHA and ARA supplementation vs. placebo groups while hospitalized at 1 week of age. Supplementation was discontinued upon discharge at an average age of 9 weeks. Follow-up assessments at 6 months-of-age to examine how infants in each treatment group differed in terms of how ERP responses habituated to frequently vs. infrequently presented stimuli. Supplemented infants showed increased habituation for frequently presented stimuli, suggesting that LCPUFA may enhance short term stimulus encoding and recognition memory processes. Another study of adolescent boys with attention-deficit hyperactivity disorder (ADHD) demonstrated that the amplitude of an ERP response (N170) was positively associated with red blood cell DHA and negatively associated with ARA/DHA ratio (Gow et al., 2009). A later study by this same group found ERP abnormalities to be associated with lower DHA levels in the ADHD group (Gow et al., 2013). In a final study, school-age children were assessed using a continuous visual recognition task and ERP components were thought to be related to recognition memory (the FN400 and a late positive component [LPC]). Higher prenatal DHA was associated with shorter FN400 latency and larger LPC amplitude, while higher childhood DHA was associated with higher FN400 amplitude. Positive associations were also observed between newborn DHA concentrations and neurobehavioral performance on memory tasks (Boucher et al., 2011).

To date, only one study has measured the effect of LCPUFA supplementation on ERP responses to a Go/No-Go task. Adult subjects were randomized to capsules containing fish oil (200 mg eicosapentaenoic acid plus 100 mg DHA) or olive oil (Control). ERPs were recorded before and 35 days after supplementation in both fish oil and control groups. Fish oil supplementation resulted in an increase in P3 amplitude to both Go and No-Go stimuli. Additionally, fish oil supplementation was reported to improve reaction time and error rate, and altered EEG frequency band distribution (Fontani et al., 2005). These results suggest that prenatal and early life LCPUFA supplementation during periods of rapid brain development may have long-term functional consequences that may be detectable using ERP method.

The aim of the current study was to determine whether the effects of LCPUFA supplementation in the first year of life would be evident on measures of executive function (i.e., inhibitory control) as indexed by behavioral and brain electrophysiologic responses in childhood. This formula-fed cohort has been followed since birth, when they were randomized to one of 4 infant formulas. The objective of the parent clinical trial

(clinicaltrials.gov, NCT00753818) was to compare the effects of different levels of LCPUFA content in infant formula on visual development, growth, cognitive outcomes, tolerance, and blood parameters. Since the trial was initiated before the addition of LCPUFA to all infant formula marketed in the United States, this was the last clinical trial to use a control formula with no LCPUFA. Results of early life visual and cognitive assessments have been reported (Birch et al., 2010; Colombo et al., 2011; Colombo et al., 2013). Briefly, LCPUFA supplementation resulted in lower heart rate and more time engaged in active stimulus processing at 4, 6 and 9 months of age in the two lower DHA doses than infants fed the control formula (Colombo et al., 2011). Supplemented infants had higher visual acuity than infants in the control group at 1 year of age irrespective of LCPUFA dose (Birch et al., 2010). From 3 to 5 years of age, significant positive effects were observed on performance of various rule-learning and inhibition tasks in children who were fed LCPUFA-supplemented formulas compared to children in the control group (Colombo et al., 2013).

The current report detailed the results of an ERPs assessment of this cohort at 5.5 years of age, at which participants responded to a Go/No-Go task. The study was planned for 6 months after the cognitive assessment at age 5 in order not to overwhelm children with tasks. Three ERP components P2, N2 and P3, which were well characterized in adults, were investigated. The P2 component is a positive deflection occurring at approximately 100–250 ms, has a fronto-central maximum (Gajewski & Falkenstein, 2013; Kranczioch, Debener, & Engel, 2003) and is thought to reflect initial visual processing, evaluation of the stimulus (Burden et al., 2009; Potts, 2004) or attention allocation (Carretié, Mercado, Tapia, & Hinojosa, 2001). The N2 component is an enhanced negativity that occurs around 200 ms and was maximal in the frontal recording site (Bruin & Wijers, 2002; Lewis, Lamm, Segalowitz, Stieben, & Zelazo, 2006) in response to No-Go stimuli. N2 is thought to represent response inhibition (Folstein & Van Petten, 2008) or the process of conflict monitoring (Donkers & van Boxtel, 2004; Smith, Johnstone, & Barry, 2008), especially when there is conflict between the prepotent Go response and the requirement to inhibit a response to the No-Go stimuli. The P3 component is a positive deflection that occurs around 300 ms, has a maximum response at fronto-central or central electrodes (Fox, Michie, Wynne, & Maybery, 2000; Jonkman, Lansbergen, & Stauder, 2003) and is believed to be associated with response inhibition (Jonkman, 2006; Spronk, Jonkman, & Kemner, 2008). Developmental differences in children result in ERP components that tend to be larger in amplitude have longer latencies and demonstrate different scalp topographies (Ciesielski, Harris, & Cofer, 2004; Friedman, Putnam, & Sutton, 1989).

The current study also featured the use of microstate analysis. ERP topography at the scalp does not change randomly and continuously over time, but instead remains temporarily stable for tens to hundreds of milliseconds. During these periods of temporary stability, similar topographies can be clustered as functional microstates (Lehmann, Ozaki, & Pal, 1987). The microstates correspond to a period of coherent synchronized activation of a quasi-stable functional neuronal network. Microstates use multichannel ERP data and consider the entire scalp potentials as a single representation of functional state. In theory, differences in scalp topographies between experimental conditions or sequential time reflect different configurations of active neuronal sources in the brain (Michel et al., 2004). Therefore each successive microstate may represent a certain information processing step

that evolves from perception of the stimulus to the behavioral response (Micah, Lucia, Brunet, & Michel, 2009). With respect to the ERPs, the ERP topography can be segmented into several microstate maps from epoch beginning to the end.

Longitudinal follow-up studies of the same cohort suggested that early life LCPUFA supplementation was involved in brain development and executive function. Therefore, our *a priori* hypothesis for the current study was that early life LCPUFA supplementation would have a long-term effect on cognitive development, which would be reflected by behavioral and/or brain electrophysiological ERP measures associated with stimulus evaluation, conflict monitoring, and response inhibition, particularly in brain regions responsible for executive function. Specifically, we expected to see differences in ERP components between control and LCPUFA-supplemented groups and within group differences between the Go and No-Go conditions. Additionally, we anticipated that there would be topographic differences between the two groups when processing different stimulus conditions.

Subjects and Methods

Subjects

Study participants were enrolled as infants in the DIAMOND (DHA Intake and Measurement of Neural Development) clinical trial (NCT00753818). The parent study and all follow up studies of the same cohort were approved by the Human Subjects Committee at The University of Kansas Medical Center. The trial enrolled healthy, full-term, formula-fed, singleton-birth infants (37–42 weeks gestation; 2490–4200 gram birth weight) between 3 September 2003 and 25 September 2005. Exclusionary criteria included conditions known to interfere with normal growth such as low birth weight, metabolic anomalies, intolerance to cow's milk, intolerance to infant formula or reports of poor formula intake. At delivery, 159 infants were randomly assigned to one of four cow's milk-based term infant formulas that varied in LCPUFA content. Following random assignment, any infant exposed to human milk within 24 hours of assignment was excluded from the study group. Infants in the control group were fed formula without ARA or DHA. The three LCPUFA-supplemented groups received formulas containing 0.64% of total fatty acids from ARA plus one of three different DHA compositions: either 0.32%, 0.64%, or 0.96% of total fatty acids. The parent trial followed children out to 18 months of age. Following the parent trial, 81 families re-enrolled and gave consent to be followed to 6 years of age. Families of 72 children (age: 5.51 ± 0.04 years) took part in the ERP portion of the follow up study. Three children refused to participate. ERP experiments were conducted in 69 children (23 male, 46 female). Sixty children (age: 5.51 ± 0.03 years) provided data of sufficient quality for further analysis (Table 1).

Procedures

The ERP experiment was conducted in a child-friendly setting. The ERP task was designed by Wiebe, Sheffield, and Espy (2012) using Neuroscan Stim2 software (Compumedics, Charlotte, NC). The task was described to children as a “fishing game” in which cartoon images of fish and sharks were shown. Children were told that they were going to play a game where the object was to “catch fish” but “don't catch a shark.” The experiment is

illustrated in Figure 1. Children were shown the stimuli and then instructed to press a button on the response pad when they saw a cartoon fish picture (Go). When a shark picture was presented (No-Go), instructions were reinforced to not press the button. In a beginning training phase, children practiced for several trials in order to become familiar with the experiment. After the training session, sixty-nine trials were presented in the test session, including 51 fish trials (74% Go) and 18 shark trials (26% No-Go). In each experimental trial, the fish or shark cartoon pictures were displayed on the screen for up to 1500 milliseconds or until the child pressed the response pad button. After the response (button press), the stimulus picture disappeared. If the child responded correctly for the Go (fish) trial, a feedback image displayed immediately, showing a fish caught in a net. In the event that they incorrectly pressed the button for the shark, a negative feedback picture appeared of a shark ripping through the net. No feedback was provided if no response was made during the stimulus presentation (incorrect fish, correct shark) (Wiebe et al., 2012).

Electrophysiologic recording

Electrophysiological brain activity was recorded via electroencephalogram (EEG) by using a NeuroScan system (Synamps 2 and Neuroscan software version 4.3, Neuroscan Inc., USA). Prior to the experiment, silver/silver chloride electrodes were placed on the child's scalp at 29 active recording sites using Elefix paste according to the International 10–20 standardization. Active electrodes were Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T3, C3, Cz, C4, T4, CP5, CP1, CP2, CP6, T5, P3, Pz, P4, T6, O1, Oz, and O2. Scalp impedances were kept at or below 10 k ohm. Electrodes were referenced to the left mastoid with a forehead ground. Vertical EOG was recorded from infra- and supraorbital electrodes placed above and below the center of the left eye. Horizontal EOG was recorded from electrodes placed near the outer canthi of each eye. EEG activity was continuously recorded with sampling rate of 1000 Hz and online filtered from 0.05 to 70 Hz with a roll-off of 6 dB per octave. Behavioral data (reaction time when the subjects pressed the button) were recorded simultaneously with the EEG.

Data Analysis

Behavioral Measures

We obtained measures of mean hit rate reaction time (correct Go responses) and response accuracy. Accuracy was defined as the percentage of correct responses to both Go and No-Go trials. We also determined d' , a metric that represents subjects' sensitivity to signal in noise; this was calculated in the traditional manner, by subtracting the z-score of the false alarm rate from the hit rate (Tottenham, Hare, & Casey, 2011). The behavioral measures for each study group are listed in Table 1.

EEG and ERP analysis

EEG data were filtered offline using a high pass filter of 0.1 Hz and a low pass filter 40 Hz. All electrodes were re-referenced to an average reference. This method renders the recording reference independent and free of the ambiguity due to use of different references (Micah et al., 2009). EEG data were then decomposed using the Independent Component Analysis method, employing the Infomax ICA algorithm from EEGLAB toolbox (Delorme &

Makeig, 2004). After ICA decomposition, the independent components representing the event-unrelated artifacts such as eye movements, blinking, heartbeat, and electromyography (EMG) were removed from the continuous EEG dataset. Noisy electrodes were replaced using spherical interpolation from their neighbors for one electrode of a single subject. Each trial was examined for residual artifacts. Trials with extreme values were excluded from the average if they exceeded a threshold of 250 μ V. Then the artifact-free, continuous EEG was divided into epochs ranging from –100 ms pre-stimulus to 1500 ms post-stimulus with zero being stimulus onset. This was based on the event trigger at stimulus onset (fish or shark picture presentation). Only trials with correct responses were included in the averaged response for each subject, for each condition (button press to Go; no button press to No-Go). The numbers of artifact-free trials were 42.75/7.16 (mean/SD, range 21–51) in the Go condition and 15.13/3.23 (range 5–18) in No-Go condition. Epochs of Go/No-Go trials were averaged for each subject individually by experimental group and stimulus condition and the grand average ERPs were generated based on the average of subjects' mean data.

Subjects' data of insufficient quality (excessive head movement, non-completion of experiment) were excluded from further analysis. The total number of children who participated in this study with data of sufficient quality (87% usable) is listed in Table 1. Data from 9 children were excluded or not acquired: 3 could not complete the experiment; 2 were excluded due to use of an incorrect electrode montage; 1 recording was aborted due to equipment failure; and 3 children's recordings were excluded because of excessive noise due to head and/or body movement.

We also assessed the LCPUFA effect on the temporal dynamic difference of topography to determine when different neuronal populations were active between the two experimental conditions (Murray, Brunet, & Michel, 2008). This spatio-temporal analysis method allows us to identify moments of global neuronal synchronization based on the scalp locations within the whole trial period. In this analysis, we also utilized microstate segmentation and statistical topographic analysis (Brunet, Murray, & Michel, 2011).

ERP components

Based on previous reports (Burden et al., 2009; Johnstone & Clarke, 2009; Jonkman, 2006) of ERP peak latencies in children and observed regions of maximal activity in the current data, three ERP components, P2, N2 and P3, were identified from the grand-averaged ERPs data: P2; 150–300 ms, N2; 300–500 ms, and P3; 500–700 ms. Based on observed waveforms and areas of activation on the EEG contour maps (Figure 3), we selected different clusters of electrodes for each component and used the same cluster scheme in different groups to compare the effects of between conditions and groups. For component P2, we used three electrodes in the fronto-central region (FC1, FC2 and Cz). For component N2, eight frontal electrodes (Fp1, Fpz, Fp2, F7, F3, Fz, F4 and F8) were selected. For component P3, five centro-parietal electrodes (P3, Pz, P4, CP1 and CP2) were used. Peak amplitudes and latencies were averaged within the assigned electrode clusters for each component. The grand-averaged ERP waveforms of Go and No-Go conditions at Fpz, Fz, Cz and Pz are shown in Figure 2.

Statistical analysis

Individual LCPUFA-supplemented groups were first tested using one-way analysis of variance (ANOVA) to determine whether there were differences as a function of LCPUFA dose; if no dose effect was found among the LCPUFA-supplemented groups, the three supplemented groups were collapsed into a single group and then compared with the control group to determine overall LCPUFA supplementation effects. Due to the unequal group sample size after collapsing the supplemented groups (LCPUFA $n=47$, control $n=13$) the assumption of homogeneity of variance was tested using Levene's test for each outcome measure.

A two-way ANOVA was used to test for behavioral differences between group and sex (male, female). We included sex in the analysis due to the preponderance of female subjects (39 female, 21 male).

A three-way mixed-design ANOVA was employed to test for differences in ERP components. Group and sex were included as two between-subject factors; the trial condition (Go, No-Go) was a within-subject factor. The ANOVA model was built and computed using SPSS software version 20 (IBM Corp., Chicago, IL). The Greenhouse-Geisser correction was adopted when the sphericity assumption was violated. An alpha significance level of $p.05$ was adopted across all statistical comparisons.

Topographic pattern analysis

Based on the ERPs, we utilized the microstates segmentation method to assess the neuronal information processing from epoch beginning to the end for both control and LCPUFA-supplemented groups. Group-averaged ERPs across all conditions/groups at each time point were subjected to a spatio-temporal clustering method, i.e., the topographical atomize-agglomerate hierarchical clustering (TAAHC) (Pascual-Marqui, Michel, & Lehmann, 1995), to identify the dominant topography maps. In order to define the optimal topographies number, the Krzanovski-Lai criterion (Murray et al., 2008) was used.

To further examine the temporal dynamics of ERP components across the Go and No-Go conditions within each group, we ran additional statistical tests on average voltage topographies. According to the method of Britz and Michel (2010), we constrained the criterion for minimum map duration at >10 ms, as durations less than it are not considered physiological plausible. This test employed a topographic ANOVA (TANOVA) (Murray et al., 2008), which measures the global dissimilarity between two electric fields at a given time point using an iterative, non-parametric randomization method.

After the segmentation of microstates on the grand-averaged ERP data, the microstate maps were fitted back into the individual ERPs of each subject and condition in order to reveal which microstate best represented that condition, relative to the set of all identified microstates. The spatial correlation of microstate topographies (Murray et al., 2008) was calculated and each time point of data was labeled with the microstate map with which it best correlated. In the fitting test, the obtained frequency of microstates present was subjected to a mixed-design ANOVA with the group as between-subject factor and microstates as within-subject factor when comparing between the groups for the same

condition, or a repeated measure ANOVA with condition (Go/No-Go) and microstates as repeated measurements when comparing the two conditions within the group.

In this study, the CARTOOL software (Brunet et al., 2011) was used for topographic analysis, in which the microstate segmentation and the TANOVA test were implemented.

Results

Behavioral Response

The LCPUFA-supplemented groups were comparable in behavior in that there was no significant difference in reaction time or accuracy between the different dose groups (one-way ANOVA, three supplemented groups, reaction time: $F(2,44)=0.11$, $p=.90$, $\eta_p^2=.01$; accuracy: $F(2,44)=0.98$, $p=.39$, $\eta_p^2=.04$). Therefore, the three LCPUFA-supplemented groups were collapsed and compared to the control group. The homogeneity of variance assumption was not violated for either reaction time or accuracy measures (Levene's test, $p>.05$).

While the collapsed LCPUFA-supplemented group had faster reaction time than the control group, the result fell just short of conventional measures of significance, $F(1,56)=3.46$, $p=.068$, $\eta_p^2=.06$. There was a significant main effect of Sex, $F(1,56)=8.99$, $p=.004$, $\eta_p^2=.14$ with faster reaction time in males (mean: 698.7 ms, SE: 31.5 ms) than females (mean: 823.6 ms, SE: 27.2 ms). However, there was no Group X Sex interaction, $F(1,56)=0.73$, $p=.398$, $\eta_p^2=.01$.

For response accuracy, there were no significant main effects for Group, $F(1,56)=0.06$, $p=.815$, $\eta_p^2=.001$, or Sex, $F(1,56)=1.77$, $p=.189$, $\eta_p^2=.03$, nor was there a significant Group X Sex interaction, $F(1,56)=1.19$, $p=.281$, $\eta_p^2=.02$.

Finally, for d' , there was no group difference between the three LCPUFA-supplemented groups, $F(2,44)=1.1$, $p=.342$, and so the three LCPUFA-supplemented groups were collapsed (homogeneity of variance assumption was not violated, Levene's test, $p>.05$) and compared with the control group. There were no significant main effects for Group, $F(1,56)=0.01$, $p=.94$, $\eta_p^2<.001$, or Sex, $F(1,56)=2.82$, $p=.098$, $\eta_p^2=.05$, nor was there a significant Group X Sex interaction, $F(1,56)=0.64$, $p=.427$, $\eta_p^2=.01$.

ERP components

ANOVA comparisons testing for differences between the three LCPUFA-supplemented groups yielded no significant group effect for ERP component amplitude and latency (Table 2), and so the LCPUFA-supplemented groups were collapsed and compared to the control group. Again, the assumption of the homogeneous variance for both the amplitude and latency of the three ERP components was not violated (Levene's test, $p>.05$).

Mean voltage maps of the P2, N2 and P3 components for both Go and No-Go conditions in the control and LCPUFA-supplemented group are depicted in Figure 3. The mean amplitude and latency for each group and condition is shown in Table 3. Significance test results of the three ERP components using repeated measure ANOVA between the control and collapsed

LCPUFA-supplemented groups are shown in Table 4. For clarity in the presentation of the ERP results, only the significant results will be discussed in the following text.

P2 Component

Amplitude—P2 amplitude showed a significant Group effect, $F(1,56)=6.31$, $p=.015$, $\eta_p^2=.10$, with greater P2 amplitude in the supplemented group (Control: 2.14 μV , Supplemented: 5.89 μV). There was also a significant main effect of Condition, $F(1,56)=8.97$, $p=.004$, $\eta_p^2=.14$, as the P2 amplitude was higher in the No-Go condition than in the Go condition.

N2 Component

Amplitude—For N2 amplitude there was a significant main effect for Condition, $F(1,56)=31.08$, $p<.001$, $\eta_p^2=.36$, as the No-Go N2 amplitude was greater than that observed for the Go stimuli (No-Go: 15.07 μV , Go: 11.39 μV). The main effect for Condition was qualified by a significant Group X Condition interaction, $F(1,56)=5.91$, $p=.018$, $\eta_p^2=.10$; the effect of Condition was observed only in the supplemented group, $t(46)=8.80$, $p<.001$, Cohen's $d=1.06$, and not in the control group, $t(12)=1.57$, $p=.143$, Cohen's $d=.35$.

To further explore the Group X Condition interaction for N2 amplitude, the Condition difference potential within each group was calculated and illustrated in Figure 4. The grand average ERP waveforms for each condition and each group were generated using the 8 electrodes in the N2 component cluster scheme as previously detailed. The LCPUFA-supplemented group shows a large condition difference for the N2 component between 300–500 ms (shaded in blue), in which No-Go amplitude is larger than Go) while the control group does not (shaded in red). Topographical maps of the N2 ERP difference potential show enhanced negativity in the fronto-central electrode cluster for the LCPUFA-supplemented group, while the control group shows minimal topographical differences.

Latency—A significant main effect of Condition emerged, $F(1,56)=4.1$, $p=.048$, $\eta_p^2=.07$, such that the No-Go condition had a longer latency than Go condition (Go: 388.7 ms, No-Go: 412.3 ms).

P3 Component

Amplitude—A significant main effect of Condition emerged for P3 amplitude, $F(1,56)=37.30$, $p<.001$, $\eta_p^2=.4$, with amplitudes significantly higher on No-Go trials than on Go trials.

Topographical analysis

Microstate segmentation of the grand average ERPs of control and collapsed LCPUFA-supplemented group of Go/No-Go condition yielded an optimal set of twelve microstate maps that explained 90.9% of the variance of the whole epoch. Fig. 5 shows the microstate segmentation (Fig. 5.a) and time of occurrence from stimulus onset to 700 ms along with the corresponding topography maps (Fig. 5.b–d).

Comparison within groups for the Go and No-Go conditions yielded qualitatively different results for the control and LCPUFA-supplemented groups. The control group showed very

similar microstate segmentations with near equivalent temporal duration for both Go and No-Go conditions; indeed, no topographical difference between the two conditions was detected by TANOVA within 0–700 ms. In contrast, the LCPUFA-supplemented group showed an intermediate microstate with a different duration between Go and No-Go conditions (Fig. 5a). During the early stimulus processing stage (around 200–300 ms), the brain activity changed from a microstate where intensive activity was observed at the occipital recording sites (Fig. 5b) to a state with broader activation in the fronto-parietal region (Fig. 5c), then brain topography returned back to the previous state. This intermediate microstate (Fig. 5c) represented a period of greater neuronal synchrony during early information processing following the visual stimulus, but prior to the motor response (button press) for the Go condition. Although the same microstate occurred in both Go and No-Go conditions for the collapsed LCPUFA-supplemented group, a longer duration was seen in the No-Go condition, where the correct response was to inhibit the prepotent response (Fig. 5a). Topographic test TANOVA consistently indicated a significant difference in the duration of the intermediate microstate, marked in red rectangle (166–297 ms in Fig. 5a). The microstates-fitting test yielded an interaction between condition and microstate in this range, $F(1,46)=11.19$, $p=.002$, $\eta_p^2=.2$, suggesting significantly greater intermediate microstate occurrence in the No-Go than Go condition, where the prepotent response was inhibited; again, this was only observed for the LCPUFA-supplemented group.

When comparing the microstate segmentation results between groups, within condition (Fig. 5a), a unique microstate (Fig. 5c) occurred only in the collapsed LCPUFA-supplemented group data (around 200 ms). For the control group, topographical activation was mainly in the occipital lobe. In contrast, the intermediate microstate in the collapsed LCPUFA-supplemented group (Fig. 5c) showed broader activation in parietal and central recording sites. The intermediate microstate appeared around 150 ms to 300 ms, reflecting initial stimulus processing in the collapsed LCPUFA-supplemented group. When comparing the two groups' No-Go condition, the TANOVA indicated the significant topographic difference between two groups from 160 to 238 ms (marked in the blue rectangle in Fig. 5a). In microstates-fitting test, a significant interaction between the two microstates and groups was obtained, $F(1,58)=10.507$, $p=.002$, $\eta_p^2=.153$, suggesting that microstate (b) better represented the control group data and microstate (c) better represented collapsed LCPUFA-supplemented group ERPs in the significantly different time period. When comparing the Go condition result of microstate segmentation between groups, the TANOVA test did not show a significant topographical difference in [0–700] ms time period. Despite the novel microstate (c) observed in the LCPUFA-supplemented group Go condition, the TANOVA confirmed that both groups had no significant difference in processing Go condition trials.

Discussion

Data from this cohort are unique in that they represent one of the last opportunities to examine long-term effects of LCPUFA supplementation within a randomized clinical trial of infant formula in which the control formula included no added LCPUFA (Birch et al., 2010). In this sample, LCPUFA supplementation was observed to positively affect the development of visual acuity (Birch et al., 2010), lower heart rate, and increase sustained attention (Colombo et al., 2011). As this cohort entered childhood, supplemented children were

observed to score higher on executive function tasks and on standardized tests of verbal IQ (Colombo et al., 2013). In the continued follow-up of this cohort, we now report differences in brain electrophysiology to contrasting conditions (Go vs. No-Go) between control and LCPUFA-supplemented groups when children were required to inhibit a prepotent response. Further, we found activation of a larger synchronized neuronal network in the LCPUFA-supplemented group using microstate analysis. The supplemented group trended towards faster reaction times, in line with other neurocognitive performance studies that measured the effect of LCPUFA supplementation (Fontani, Lodi, Migliorini, & Corradeschi, 2009; McNamara et al., 2013).

The analysis time windows for ERP components N2 (from 300 to 500 ms after stimulus onset) and P3 (from 500 to 700 ms) were selected as these components are known to have longer latencies in children compared to adults. To provide a priori constraints on the time-windows of consideration, these values were selected to correspond to previous publications with children of similar age-ranges (Espinat, Anderson, & Zelazo, 2012, 2013). Age is an important consideration for the latency of such ERP components. In one developmental study (Jonkman, 2006), the No-Go N2 activity was maximal from 240 to 260 ms in adults aged 19 to 23 years, from 280 to 320 ms in 9 to 10 year old children, and from 320 to 360 ms in the 6 to 7 year old children. The longer latencies associated with younger ages was also observed in the No-Go P3 activity, which ranged from 360 to 400 ms in adults, from 380 to 420 ms in the 9 to 10 year old children, and from 440 to 480 in the 6 to 7 year old children. Compared to the referenced report, children in our current study were even younger (mean/SD age: 5.5 years, ± 0.04), which may account for the longer latencies of the inhibition-related ERP components observed in this cohort.

Results of this study showed typical outcomes for differences between Go and No-Go conditions: P2, N2, and P3 amplitudes were all significantly larger to No-Go than Go stimuli, and N2 latency was longer on No-Go than on Go trials (Broyd et al., 2005). The magnitude of the condition difference was greater for the LCPUFA supplemented group, best illustrated by the voltage maps in Figure 3. In visual cognitive tasks, P2 is thought to be related to initial visual processing and attention allocation (Luck & Hillyard, 1994). Thus, greater P2 amplitude may be an indication of enhanced cognitive processing (Freunberger, Klimesch, Doppelmayr, & Höller, 2007; Zeng, Wang, & Zhang, 2012). Children in the control group had lower P2 amplitude than children in the LCPUFA-supplemented groups to both conditions. This finding may be highlighting group differences in the ability to perceptually discriminate between the visual stimuli, resulting in slightly longer reaction times, thus setting the stage for the lack of clearly differentiated N2 and lack of a novel microstate between conditions in the control group.

Our finding that No-Go N2 amplitude was greater than Go N2 amplitude in frontal recording sites is consistent with other study using a Go/No-Go task on children at similar age between 7 to 8 years (Lewis et al., 2006). With respect to the N2 component, the LCPUFA-supplemented group generated a distinct response to No-Go versus Go trials in this study; while the control group did not. Adopting the view that No-Go N2 reflects a conflict monitoring process (Nieuwenhuis, Yeung, Wildenberg, & Ridderinkhof, 2003), then the increase of No-Go N2 amplitude in the LCPUFA-supplemented group could be interpreted

as the engagement of areas involved in more mature forms of inhibitory control (Kenemans et al., 2005). The lack of a stimulus condition difference in the control group suggests equivalent processing of the Go and No-Go stimuli in these children. The longer reaction time in the control children to the Go stimuli indicates altered processing of the Go-stimulus which is designed to generate a prepotent response, although the group difference fell just short of statistical significance ($p=.068$). Finally, the equal microstate to both Go and No-Go stimuli in the control group is also consistent with the N2 ERP response showing no difference between stimulus conditions.

In our study, the P3 component was more evident in central-parietal recording sites (Jonkman et al., 2003; Liu, Xiao, Shi, & Zhao, 2011), with maximal amplitude in Pz and P4 electrodes. The condition effect that No-Go P3 amplitude was greater than Go condition was in line with another previous study (Lewis et al., 2006). No-Go P3 latency has also been interpreted as a measure of processing speed (McEvoy, Pellouchoud, Smith, & Gevins, 2001), but this component did not differentiate LCPUFA-supplemented versus unsupplemented children.

The current paper also detailed the results of ERP microstate analyses. Such microstates were considered to reflect coherent synchronized activity of a quasi-stable functional neuronal network. From the beginning to the end of an ERP epoch, ERP topography can be segmented into several microstate maps, with qualitatively distinct maps potentially representing a discrete information processing step moving from perception of the stimulus to action (Micah et al., 2009). The microstate analyses reported here provided a quantitative assessment of changes in electrical field topographies across time, experimental conditions and groups. Although the LCPUFA-supplemented group generated a novel microstate to the Go condition, no significant between group difference emerged for stimuli that required a response (button press). The topographical map associated with the novel microstate in the LCPUFA-supplemented group suggests stronger fronto-parietal activation after processing of visual stimuli ~100 ms but prior to the behavioral response, i.e., the button press >700 ms. For the No-Go condition, the LCPUFA-supplemented group generated the same microstate, but it was significantly longer in duration than the Go condition. The longer duration may indicate further evaluation and processing of the stimuli needed to inhibit the prepotent response; it may be important to note that such evaluative-based processing was observed even though the supplemented group tended to have faster responses. Further, this novel microstate differed significantly from the same period of information processing in the control group for the No-Go condition, suggesting an inequality between the two groups when processing the condition requiring inhibition of the response. This phenomenon suggests that the LCPUFA-supplemented group generated different functional microstates, that is, different brain activity, to discriminate between the experimental conditions, whereas the control group did not. As noted above, evidence of a distinct intermediate microstate in the LCPUFA-supplemented group at a point in time following visual presentation may also contribute to the marginally faster reaction times in this group.

Difference in cognitive function found in measures of brain electrophysiology contrasted somewhat with behavioral outcomes in this study. It might be that the behavioral assessment were not sensitive and specific enough to detect subtle nervous system developmental

differences (Uauy & Peirano, 1999). Electrophysiologic measurements were able to offer insights into brain implicit responses to external events, even when no external behavior is observed (Thierry, 2005). Differences in ERP components with no behavioral difference in reaction time had been reported in other studies using a Go/No-Go task (Dong, Lu, Zhou, & Zhao, 2010; Liu et al., 2011) and in a cued Go/No-Go task (Spronk et al., 2008). Other Go/No-Go studies in children also did not yield differences in behavioral accuracy and reaction time between testing groups but did find difference in electrophysiological measures (Burden et al., 2009; Burden et al., 2011; Steinmann et al., 2011).

In this study, a set of independent investigation goals were set up for experimental data analysis to evaluate the performance difference due to LCPUFA supplementation. More specifically, the response speed, accuracy and sensitivity (d') for behavioral difference were considered. Meanwhile, executive functions of early stimulation processing, conflict monitoring and response inhibition reflected by the electrophysiological ERPs measures (P2, N2 and P3 components) were investigated. Each investigation goal was associated with one specific hypothesis that constrained a single comparison for consideration and therefore no correction for multiple comparisons was needed.

The primary aim of this study was to determine the effect of LCPUFA supplementation that occurred in the first year of life on behavioral response inhibition and subsequent electrophysiological difference at age 5.5 years. This pattern of findings emerging from these ERP analyses was consistent with previous data from the same cohort in which LCPUFA-supplemented group outperformed controls in executive function tasks involving rule learning and inhibition tasks at ages from 3 to 5 years (Colombo et al., 2013).

We recognize several limitations of the current trial. First, due to the longitudinal nature of the trial, the sample size is smaller due to subjects who were either not interested in re-enrolling in the long-term follow up, moved away, or were lost to follow up. Second, recording ERPs in children is inherently challenging. More ERP trials and longer recording sessions would have been preferred, but at age 5.5 years, children would not tolerate a longer test time. Third, the sample is disproportionately female.

In summary, results of the current study showing significant differences in brain electrophysiology at 5.5 years, along with previously reported results of several measures of cognitive development into early childhood, suggest that LCPUFA supplementation during the first 12 months of life has a programming effect on the developing brain that can be demonstrated using both behavioral and electrophysiological measures. Early life supplementation in this sample resulted in higher visual acuity (Birch et al., 2010), lower heart rate, improved early measures of attention (Colombo et al., 2011), positive effects on rule-learning and inhibition tasks from 3–5 years, and positive effects on the Peabody Picture Vocabulary Test at 5 years and the Weschler Preschool Primary Intelligence Scale at 6 years of age (Colombo et al., 2013). Understanding the relationship between pre- and postnatal LCPUFA supplementation and the impact on brain development will require further prospective, longitudinal studies of both brain anatomical structure and cognitive function.

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Research Highlights

- The present study sought to determine whether long-chain polyunsaturated fatty acid (LCPUFA) supplementation during the first year of life would result in group differences in behavior and event-related potentials (ERPs) while performing a Go/No-Go task requiring response inhibition at 5.5 years of age.
- While the LCPUFA-supplemented group was marginally faster in their response to the Go stimuli than the control group, there was no significant behavioral difference between the groups for response latency or accuracy.
- There was a significant ERP N2 amplitude difference between conditions (Go vs. No-Go) within the LCPUFA-supplemented group. Those children in the control group did not show the same ERP component difference. The LCPUFA-supplemented group developed a novel ERP topographical microstate to No-Go trials involving broader activation with a putative frontal locus.
- These results suggested that LCPUFA supplementation during the first 12 months of life had a developmental programming effect that resulted in differences in childhood brain electrophysiology while performing task involving response inhibition.

Schematic illustration of the fish/shark Go/No-Go experimental design. Random images of fish or sharks appear. Children press the button to catch a fish and receive positive feedback as an image of a fish in the net. If the child presses the button when the shark appears (incorrect response), they receive negative feedback; an image of a shark ripping through the net. If they respond correctly to the shark image, no feedback appears.

Figure. 2.

Grand average ERP waveforms for Go/No-Go task from four midline electrodes. Scales of X- and Y-axis are shown at the Pz electrode. Data is displayed from 0 to 1000 ms after the stimulus. ERP components are marked at their representative electrodes (P2 at Fpz and Fz, N2 at Fpz and Fz, P3 at Cz and Pz).

Figure. 3.

Mean voltage maps of P2, N2 and P3 activities of Go/No-Go conditions in Control and LCPUFA-supplemented group. P2 data is from maxima of Cz electrode. N2 data is from minimum of Fz electrode. P3 data is from maxima of Pz electrode. The electrodes used in statistical analysis of each ERP component were circled. (a) Control group. (b) LCPUFA-supplemented group.

Figure. 4.

N2 ERP and its condition differences in Control and LCPUFA-supplemented group. (a) Grand averaged ERP waveforms. Color shaded areas indicate the condition differences between 300-500 ms for Control (red) and LCPUFA (blue). (b) Topography differences between Go and No-Go conditions (No-Go minus Go) at the time when difference is maximal. Top: Control ($t=301$ ms). Bottom: LCPUFA ($t=488$ ms).

Figure 5.

Illustration of microstate analysis of Control and LCPUFA-supplemented group Go/No-Go condition data. (a) Temporal assignment of different microstate maps (0–700ms), plotted under the global field power curves. Different colors indicate different microstates. The red rectangle marks the range with significantly different topographies between the Go and No-Go conditions in the LCPUFA-supplemented group that was detected by TANOVA test. Note that the microstate for the No-Go condition in the LCPUFA-supplemented group is longer in duration than that for the Go response. The blue rectangle marks the significantly different range between Control group and LCPUFA-supplemented group for the No-Go condition. (b) Denotes the topographic map of the microstate color-coded green. (c) Denotes the topographic map of microstate in color-coded yellow, representing the novel microstate only seen in the LCPUFA-supplemented group. (d) Denotes the topographic map of the later microstate color-coded blue.

Table 1

Participant numbers and behavioral data in each study group

Variable	0.00% DHA 0.00% ARA	0.32% DHA 0.64% ARA	0.64% DHA 0.64% ARA	0.96% DHA 0.64% ARA
Participants	16	16	15	22
Usable datasets	13	15	14	18
Males	6	5	2	8
Females	7	10	12	10
Reaction time				
M(SD)	806.1 (146.4)	747.1 (120.5)	724.4 (121.7)	742.4 (164.8)
Range	604.8–1,082.2	526.2–917.3	569.3–1,022.7	429.5–1,116.4
Accuracy				
M(SD)	88.6% (8.6%)	89.4% (8.3%)	91.7% (5.7%)	87.0% (12.2%)
Range	72.5%–97.1%	73.9%–100%	79.7%–100%	52.2%–100%
d'				
M(SD)	.005 (1.51)	.07 (1.58)	.45 (0.91)	–.41 (2.09)
Range	–3.55–1.46	–4.08–1.86	–1.29–1.86	–6.52–1.86

Note. The unit of reaction time is ms.

Table 2

F-values of ANOVA to test for group differences in ERP components between the three LCPUFA-supplemented groups

Condition	P2		N2		P3	
	Amplitude	Latency	Amplitude	Latency	Amplitude	Latency
Go	1.14	0.82	0.82	0.68	1.57	0.02
No-Go	0.61	0.44	0.61	0.77	0.90	0.61

Note. The between-group and within-group degree of freedom of F-values are 2 and 44 respectively. No statistically significant difference in ERP component amplitude or latency between the 3 LCPUFA supplemented groups was detected.

Table 3

ERP component amplitude and peak latency for Go and No-Go conditions. [M (SD)]

Variable	P2			N2			P3		
	Amplitude	Latency		Amplitude	Latency		Amplitude	Latency	
Control									
Go	1.1 (5.0)	195.1 (30.6)		13.1 (5.4)	397.1 (36.6)		10.1 (5.0)	576.1 (35.9)	
No-Go	3.2 (5.6)	187.8 (26.7)		15.2 (6.3)	402.8 (42.1)		13.5 (4.5)	596.1 (45.7)	
Total	2.1 (5.3)	191.4 (28.4)		14.2 (5.8)	399.9 (38.8)		11.8 (5.0)	586.1 (41.5)	
LCPUFA-supplemented									
Go	4.5 (4.6)	187.5 (30.6)		9.87 (3.8)	386.4 (42.3)		9.0 (4.1)	571.6 (34.4)	
No-Go	7.2 (5.5)	192.9 (27.7)		14.9 (5.6)	414.9 (36.4)		15.0 (5.8)	574.6 (38.4)	
Total	5.9 (5.2)	190.2 (29.1)		12.4 (5.4)	400.7 (41.8)		12.0 (5.8)	573.1 (36.3)	

Note. The units of ERP component amplitude and latency are μ V and ms respectively Only significant results will be discussed in the text.

Table 4
F-values of three-way repeated measure ANOVA comparing Group, Condition and Sex for ERP components

Variable	Group	Condition	Sex	Group X Condition	Condition X Sex	Group X Sex	Group X Condition X Sex
P2							
Amplitude	6.31 *	8.97 *	0.17	0.12	0.71	0.05	0.01
Latency	0.05	0.12	0.07	1.33	1.24	0.01	0.31
N2							
Amplitude	1.31	31.08 ***	0.59	5.91 *	0.61	0.63	0.05
Latency	0.19	4.10 *	2.08	1.70	0.60	0.52	0.14
P3							
Amplitude	0.004	37.30 ***	1.88	2.43	1.47	0.40	1.02
Latency	1.51	2.65	0.01	1.84	0.41	0.11	1.17

Note.
* p< .05;
** p< .01;
*** p< .001