

**Familiarity and novelty responses infants of very low  
birth weight:**

**A psychophysiological study of early  
supplementation of human milk with  
docosahexaenoic acid and arachidonic acid.**

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## Abstract

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The present thesis is based on data collected as part of a large multi-centre and multi-disciplinary longitudinal study initiated by the Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo.

Objective: The aim of the present study was to investigate the effects of human milk supplemented with docosahexaenoic acid and arachidonic acid to preterm infants. Cognitive processes at six months corrected age served as the primary end point.

Methods: The study was a randomized, double-blind, placebo-controlled study of infants of very low birth weight ( $\leq 1500$  g). The intervention started one week after birth and lasted until discharge from the hospital (on average 9 weeks). A group of infants born at term represented a normal control group. A modified oddball paradigm eliciting event-related potential thought to reflect visual recognition memory and attentional processes (the Negative Component; NC), in addition to the parent-administered Ages and Stages Questionnaire (ASQ) served as outcome measures.

Key results: At six months of age, the VLBW intervention group exhibited NC amplitudes comparable to the full-term infants in response to the familiar stimuli. The VLBW control group differed significantly on this measure from the VLBW intervention group, with the former group showing higher amplitudes. No differences were found between the VLBW groups in the novelty condition. In addition, the VLBW groups differed significantly in the problem solving subsection of the ASQ.

Conclusion: Supplementation with docosahexaenoic acid and arachidonic acid for VLBW infants in the early neonatal period may improve attentional and/or memory processing, as well as problem-solving skills. The NC may prove useful in investigations of information processing skills in at-risk developmental populations.

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## Introduction

Tremendous progress has been made in the field of neonatal medicine and technology over the past decades. In addition to a decline in perinatal insults, mortality rates of infants born preterm and of sub-optimal birth weight have been greatly reduced. Improved survival rates of low birth weight (LBW;  $\leq 2500$  g), very low birth weight (VLBW;  $\leq 1500$  g), and extremely low birth weight (ELBW,  $\leq 1,000$  g) infants have resulted in increased emphasis on their neurodevelopmental outcomes (Aylward, 2005). The number of infants of low birth weight is increasing, and the causes may be related to a growth in twin-and triplet born, which in turn may correlate with assisted fertilization (Pedersen, 2003). Scientific investigations of the health of at-risk infants are to an increasing extent becoming a multidisciplinary project. Medical doctors, nurses, nutritional scientists and developmental psychologists are among the broad specter of researchers that have and are collaborating in this endeavor. A parallel development in the field of cognitive neuroscience has provided theoretically based paradigms that are especially useful in the studies of cognitive processes in developmental populations. There is still a great need for knowledge of the causes of premature births, mechanisms of development and factors that promote positive outcomes. Major disabilities such as mental retardation, sensory disorders and cerebral palsy are often detected during infancy and their incidence has remained constant. However, low severity dysfunctions such as learning disabilities and Attention Deficit Hyperactivity disorder (ADHD) become more apparent when children reach school age (van de Weijer-Bergsma, Wijnroks & Jongmans, 2008). Symptoms suggestive of ADHD are reported 2.6 to four times more frequently in VLBW/ELBW infants than in controls, with some estimates indicating almost a six fold increase (for a review on neurodevelopmental outcome in preterm infants, see Aylward, 2005).

### *Brain development and nutrition*

The infant brain changes at a very rapid pace as axons and dendrites grow and become myelinated, synaptic connections are made and broken through the process of synaptic pruning, neuronal pruning, and changes in neurotransmitter sensitivity (Webb, Monk & Nelson, 2001). These changes occur under the control of both genetic factors (nature) and experience (nurture), and depend on adequate nutrition and metabolism (Picton and Taylor, 2007). Nutrition is the sum of all processes involved from the acquisition an ingestion of food and water through their digestion and assembly into metabolically functional substances (i.e.

nutrients) for energy, growth/development, tissue repair and replacement, or elaboration of products (e.g. human milk). Thus, a complex sequence of events precedes any measurable nutrient-related outcome (Rosales & Zeisel, 2008).

The optimum nutritional plan for the developing brain – what will allow it to make the most of what it has inherited and what it perceives – is not known (Picton and Taylor, 2007). Present recommendations for preterm infant nutrition are designed to approximate the growth and development of a normal fetus of the same postconceptional age. Docosahexaenoic acid (DHA) and arachidonic acid (AA) are important for growth and neurodevelopment of the fetus and preterm infants (Carlson & Neuringer, 1999, in Henriksen, Haugholt, Lindgren et al., 2008, for a review see Heird & Lapillonne, 2005). These essential fatty acids are transferred to the fetus by specific placental proteins during pregnancy and are incorporated into cell membranes of all tissues of the body, in particular those of the retina and central nervous system. Preterm infants are deprived of the optimal length of this supply. Preterm infants receiving human milk had higher IQ scores than did formula-fed infants at 8 years of age in a study by Lucas, Morley et al. in 1992. The authors attributed this effect to the higher content of DHA in human milk. Most preterm formulas are now supplemented with DHA and AA to approximately the same levels as found in human milk. Several randomized, clinical trials showed beneficial effects on growth, visual function and cognitive development, but all of those studies were performed with formula-fed infants (O'Connor, Hall, Adamkin et al, 2001; Innis, Adamkin, Hall et al., 2002; Fewtrell, Abbott, Kennedy et al, 2004, Clandinin, Van Aerde, Merkel et al., 2005). Behavioral studies have suggested that nutrition may have significant effects on the development of language and cognition (e.g., Innis, Gilley, & Werker, 2001; Helland et al., 2003)

### *Measures of infant cognition*

When behavioral development is viewed through the lens of neuroscience, it becomes possible to shed light on the mechanisms that underlie behavioral development, which permits a move beyond the descriptive level to the process or mediating level. For example, instead of attributing the concept of infantile amnesia to the repression of early traumatic memories, we might instead attribute it to the immaturity of the neocortex, which we know is responsible for the long-term storage of memories (Nelson et al., 2006).

The evaluation of event-related potentials (ERPs) is the most effective current way to look at infant brain function (Picton & Taylor, 2007). At present, ERP studies can show differences between groups of subjects that can demonstrate developmental disorders or elucidate mechanisms of development. However, because of their variability, individual ERPs are less helpful in determining whether an infant is developing abnormally. Direct measures of stimuli-induced potentials in the brain, as measured at the scalp surface, are especially useful in the study of infants, since the recording does not require a behavioral response other than the ability to keep their attention on the presented stimuli. The combination of standardized behavioral measures and ERP can provide new knowledge of the developing child and the neural correlates associated with this development.

### *Infant memory and attention*

Theories of infant memory differ as to whether they emphasise continuities or discontinuities in memory development across the lifespan (de Haan, 2007). Behavioral studies of recognition memory and its development show many similarities in the characteristics of infant and adult recognition memory. These results are suggestive of remarkable continuity of this memory process. Some authors conclude that a considerable stability exists in memory development, at least at the level of basic processes and mechanisms. Furthermore, whatever these “basics” necessary for storage and retrieval of information are, they are clearly present and operating in the first year of life (Howe & Courage, in deHaan, ed., 2007). On the other hand, theories of the neural bases of development of recognition memory propose that discontinuity best describes the nature of human memory development (Nelson, 1995).

#### *Two types of infant memory.*

Nelson (1995) proposed that there may be two types of memory processes in the infant. One is an early form that mainly depends on the medial temporal lobe (the hippocampus in particular) and results in preferences for novelty that are reflexive in nature. This pre-explicit form of memory accounts for performance on any task that involves a novelty preference, but for which task demands or delays are minimal. Furthermore, this early form is extended and modified by a second and explicit type of memory which emerges some time between 6 and 12 months. Depending on cortical structures, this latter form is likely responsible for the emergence of function in more complex tasks such as cross-modal recognition memory tasks. Some authors define recognition memory as a type of explicit memory (Broadbent et al., 2002; in deRegnier, 2005).

### *Visual recognition memory.*

Visual recognition memory has been defined as an early emerging and fundamental form of memory (Rose, Feldman & Jankowski, 2004). Its defining feature, namely, responsiveness to novelty, reflects a core biological adaptation. Preference for novel stimuli is thought to arise when infants, having completed assimilation of the information in the familiar stimulus, turn their attention to encoding information in the new one. It is presumed that infants form a mental representation of the stimulus during familiarization. This presumption is based on the observation that attention wanes with the repetition of an event and recovers when the event changes. Research on infant visual recognition memory grew out of Robert Fantz' work, where he found that infants over 2 months of age gave decreasing attention to frequently presented photo and increasing attention to a new photo which was paired with the familiar target. This novelty preference method was further developed by Fagan in the visual-paired-comparison task (VPC). Here, infants are first familiarized with two identical stimuli presented simultaneously (or a single visual stimulus) and then tested for recognition by pairing the previously viewed stimulus along with a new one. Recognition memory is inferred from the differential responsiveness to the two stimuli (Rose et al., 2004). The response to novelty is central to several theories of cognitive development (Piaget) and intelligence (Sternberg).

In 1963, Sokolov proposed a comparator model in which a mental representation is formed over time. When infants first encounter a new stimulus, they attempt to match it with a stored representation. Attention is inhibited if a match is found. If no match is found, attention remains engaged until sufficient information is assimilated from the stimulus to render it no longer novel. Thus, preference for a new stimulus can be taken as evidence for a stored representation of the old one (Feldman & Jankowski, in Rose et al., 2004). Early in processing, or when the infants are young, using brief familiarization times, or complex targets, a transitory preference for the familiar stimulus is sometimes seen. Regardless of circumstances, novelty responses generally emerge with lengthier familiarization times (Rose et al., 2004). Studies that have examined performance as a function of age have found that there is a systematic increase in recognition memory between the age of three and twelve months (e.g. Richard, 1997, Rose, 1983). There also seems to be a decrease in familiarization time needed to achieve comparable novelty scores as age increases (Fagan, 1974; in Rose et al., 2004). Visual recognition memory at the ages of 29 and 56 weeks, as measured by The

Fagan Test of Infant Intelligence has also been shown to predict intellectual performance in VLBW infants at 8 years of age (Smith, L., Fagan, J. & Ulvund, S. E., 2002).

Factors known to increase risk for cognitive impairment in later childhood, such as premature birth and nutritional deficiency, have also been found to depress infant visual recognition (e.g., Rose et al., 2001; Rose, 1994). In studies using preterms, infants have generally been tested at their corrected age, that is, age from the expected date of birth, so that performance differences are not confounded with biological maturity. Compared to full-term infants, many of these studies indicate that preterm infants do show recognition memory, but that slower information processing speed might be the factor that, if not provided increased familiarization time, results in lower novelty scores for this group (Rose, 2004).

Variations among infants' visual recognition memory are often explained as due to differences in processing speed. This construct is generally thought of as a basic aspect of the human cognitive architecture (Kail & Salthouse, 1994; in Rose et al., 2004). In a longitudinal study by Rose, Feldman and Jankowski (2002), preterm infants required about 20% more trials and 30% more time than full-terms to reach criterion in a VPC task. Speed of information processing is a factor that can explain differences in recognition memory.

### *Attention*

Visual attention and recognition memory in infants are closely related. Measurement of infant visual recognition memory has been closely tied to visual attention, as the contents of infants' memories are inferred on the basis of their allocation of visual attention, operationalised as looking time in behavioral studies (de Haan, 2007). Infants demonstrate greater memory for presented events while in an attentive state than for events they were exposed to in an inattentive state (Richards, 1997). Events that have been partially encoded into memory or events that are novel elicit larger attention responses than those events that have been fully encoded (Fantz, 1961; in Reynolds & Richards, 2005). Several neuropsychological models of attention development agree that the development of attention is accompanied by a gradual shift from subcortical processing to increasing cortical control over attention (van de Weijer-Bergsma, 2008). Colombo (2001) proposes four attentional functions that are relevant to infancy – alertness, spatial orienting, attention to object features and endogenous attention. Endogenous, or internally directed attention involves the ability to voluntarily direct and hold (or inhibit shifts of) attention as a function of the tasks in which the individual is engaged.

Furthermore, the author suggests that the assessment of infants' attentional responses to visual stimuli at any one point during the first year will necessarily be the result of an interaction of various systems, with each system at different levels of maturity.

#### *Event-related potentials in developmental populations*

A growing body of studies of infant recognition memory development has used the electroencephalogram (EEG) to measure event-related potentials (ERPs) that are related to recognition memory. ERPs are changes in voltage that are time locked to a sensory stimulus or a motor response. Measured by electrodes at the scalp-surface, ERPs reflect the synchronous firing of neuronal populations in the cortex. These neurons need to be sufficiently aligned so that the electrical fields associated with their activation generate fields at a distance. These fields must therefore overlap in both time and space (Picton & Taylor, 2007). ERPs have excellent temporal resolution, and thus can provide milliseconds by milliseconds information about ongoing cognitive processes. Due to the physical and physiological properties of the skull, scalp, and the brain, however, the signal-to-noise ratio during this type of recording can be quite low. In order to increase the signal relative to the noise, stimuli are presented such that the responses to a particular condition or category can be averaged across multiple trials. In order to create a stable average, psychophysicists who study adults often collect hundreds of trials from an individual participant. As would be expected, the requirements for developmental populations are reduced and the total number of trials for many infant-based experiments is between 40 and 100 (Snyder et al., 2002). The recording and analysis of ERPs is complex. However, this should not be surprising since the human brain works in complicated ways (Picton & Taylor, 2007).

Studies of infant cognition using ERPs can be traced to an exploratory study of visual attention in infants from 7 weeks to 12 months of age by Schulman-Galambos and Galambos in 1978 (in Ackles & Cook, 2007). A relatively large amplitude negative component between 500 and 650 ms named the Negative component (NC) was discovered in the infant waveform as response to visually presented stimuli. This ERP appeared in all of the stimulus conditions; its presence or absence, furthermore, was correlated with whether or not the baby seemed interested in the stimuli. An increasing number of studies of infants across the first year of life have replicated and extended these results in a variety of attention and memory tasks.

### *Process specificity.*

A variety of interpretations regarding the functional significance of ERP components have been proposed in the literature. This is perhaps a natural consequence of the fact that studies have shown mixed results for the effects of stimulus probability, familiarity, and novelty on ERP components in six-month-old infants. The fundamental theoretical assumption guiding much of this research is the principle of process specificity. According to this principle, each component in an ERP waveform is a manifestation of the activation of a relatively specific cognitive process (Ackles & Cook, 2007).

### *The Negative Component*

Studies of infant visual recognition memory using ERPs have found that infants show differential brain wave activity to novel compared to familiar visual stimuli as young as 1 month of age (Karrer & Monti, 1995). The NC is a negative deflection most prominent over midline fronto-central electrodes that peaks about 600 ms in 6-month-olds. It is a reliable and robust component in infants at this age (Ackles & Cook, 2007). The ERP component is elicited for virtually all types of visual stimuli (de Haan, 2007). A study by Reynolds & Richards (2005) suggests that the cortical source of the NC is located in areas of the prefrontal cortex and anterior cingulate cortex. In a longitudinal study by Webb et al. (2005), the NC was shown to decrease in latency and increase in amplitude over the first year of life. One theoretically significant challenge currently confronting investigators of infant ERPs is the question of what specific cognitive processes underlie the NC in six-month-olds. The prevailing views of this component revolve around attention and memory processes although the exact relationship of these processes and the NC remains open.

### *Underlying processes of the NC.*

There seems to be a general consensus that the NC component reflects an attention-related process (Ackles & Cook, 2007). The component appears to reflect both aspects of sustained attention and orienting of attention to salient stimuli. The salience may be due to their novelty, or to some other factor, such as the salience of their mother's face to young infants (de Haan & Nelson, 1997). Some authors have argued that the recognition occurs after the NC, while others argue that stimulus recognition must occur on at least some level by the time of the NC (de Haan & Nelson, 1997). The latter opinion relies on findings that show that its amplitude can differ for stimuli presented with equal probability, and which differ only in

prior familiarity. Modulations of NC amplitude may reflect more controlled allocation of attentional resources based on memory or other factors (de Haan, 2007).

A basic inclusion criterion for trial data is that the infant has actually seen the presented stimuli. Although not necessarily in a sustained attentive state, attention at some level is obviously involved. Opinions on the functional significance of the NC differ as to whether it reflects a bottom-up, automatic orienting response (e.g. Richards, 2003) or a more controlled top-down attentional process (e.g. Ackles & Cook, 1998). Investigators also disagree on whether it reflects primarily attentional processing (Richards, 2003) or also reflects primarily aspects of memory (e.g., Ackles & Cook, 1998; de Haan & Nelson, 1997). Nelson & de Haan (1996) suggested that NC amplitude reflects the degree of allocation of attention or selective attention to external stimuli.

Nelson and Collins (1991) proposed that the electrophysiological components elicited by frequent vs. infrequent stimuli represent different information processes in the human brain. More specifically, they suggest that ERPs invoked by an infrequently presented familiar stimulus reflect the updating of working memory, whereas a frequently presented familiar stimulus reflects stimulus encoding. In their study, 6-month-olds were first familiarized with two different faces. One of these was presented 60 % of the trials, the other 20 % of the trials, and for the remaining 20 % trials, novel and trial-unique pictures of faces were shown. On the basis of the results, the authors also suggest that ERPs to the novel stimuli represent a non-specific and possibly automatic process of novelty-detection.

Using the NC in response to a visual perceptual priming paradigm as a means of group comparison, Stolarova et al. (2003) compared a group of low-risk premature at the corrected age of 4 months to infants in two control groups. The latter two groups consisted of healthy full-term infants representing respectively the corrected and chronological ages of the premature group respectively (4- and 6-month olds). The results showed that the brain responses of the preterm infants at the chronological age of 6 months, specifically the topography and the latency of the NC, were more similar to those of their corrected age peers than to those of the chronological age controls. Their aim was to investigate the relative influence of experience versus maturation on the processes of neural and behavioral development. Given that the present study tested the VLBW infants at their corrected age, it

lends support to the interpretation that group differences are caused by environmental contributions rather than maturational factors.

### *The present study*

The aim of the present study was to investigate the effects of early supplementation of DHA and AA to VLBW infants on cognitive processes. A group of full-term infants served as a normal control group. The hypothesis was that a beneficial effect of the intervention would be manifested in group differences in ERP correlates of attention and recognition memory, and performance on The Ages and Stages Questionnaire. The NC in response to frequently presented stimuli was hypothesised to discriminate group differences in visual recognition memory, and novel stimuli could potentially show differences in attentional processes.

## Methods

### *Participants*

The participants were recruited from four Norwegian hospitals (Rikshospitalet-Radiumhospitalet Medical Centre, Akershus University Hospital, Buskerud Hospital and Vestfold Hospital.) All VLBW infants born at these hospitals between December 2003 and November 2005 were eligible for inclusion. Infants with major congenital abnormalities and cerebral hemorrhage (grade 3 or 4 as determined by ultrasonography) were not included in the study. Written informed consent was obtained from the parents, and the study was approved by the regional ethics committee. The infants were assigned randomly to either the intervention group or the control group by using computer-generated randomization schedules. All personnel recruiting infants, parents, hospital staff and laboratory staff were blinded to the group allocation. Over a period of two years (August 2004 to August 2006), 98 infants of very low birth weight (VLBW), at a mean corrected gestational age of 6 months and 4 days, participated at the Electroencephalographic Laboratory at the Institute of Psychology, University of Oslo. The Ages and Stages Questionnaire at the 6 months level was completed by the infant's parents.

### *DHA and AA Supplementation*

The infants received human milk (from either the mother or a donor) from the first or second day after birth. As enteral feeding was increased, the milk was fortified with proteins, minerals, vitamins, iron, and folic acid according to the local routines. In addition, the infants

received a daily dose of 0.5 ml of study oil per 100 ml of human milk. The intervention group received a study oil with AA and DHA as triacylglycerol (Martek Biosciences, Colombia, MD). The study oils were dispersed in a mixture of soy oil and medium-chain triglyceride oil at the hospital pharmacy, packed, and numbered according to the randomization list. The control group received the same mixture of soy oil and medium-chain triglyceride oil as the study group, but without DHA or AA. (For more in-depth information concerning the nutritional data, see Henriksen, Haugholt, Lindgren et al., 2008)

### *ERP stimuli*

In a modified oddball paradigm, based on Goldman, Shapiro & Nelson (2004), infants were presented a pseudo-randomized series of 31 images in which a frequent image occurred on 70 % of the trials, and novel, trial-unique images occurred on the remaining 30 % of the trials, with a restriction that an oddball did not occur on two consecutive trials. The stimuli were selected with the primary intention of capturing the interest of a 6 month old infant, and thereby serve as a measure for comparing attention and memory processes across the groups of interest in the present study. Thus, complex and colorful images of various objects and animals, with and without faces, were chosen. The frequent image was of a colorful ball. As a fixation point, a small, blue circle was presented after each image. The first 10 images (8 standard and 2 novel) were presented for 5 seconds each in order to familiarize the infant to the stimuli. The duration of the remaining stimuli was 2 seconds per image. The theoretical justification for using this paradigm is based on the assumption that those events occurring more frequently would become familiar to the infant. Moreover, it was assumed that a discrepant level of attention by the infant to the familiar and novel images would produce different cognitive and neural processes. These processes were expected to be reflected in different patterns of ERP activity. A traditional oddball paradigm would include two stimuli which are repeated (usually one 80% of the time and one 20%). Because the paradigm uses repeating stimuli, it does not allow for assessment of the individual's response to a novel event (Goldman et al., 2004). Thus, a choice of trial-unique images will produce cognitive and neural processes more purely related to novelty. All infants were shown the same series of images. Stimuli were presented on a color computer monitor.

### *Procedure*

Prior to the EEG recording session the parents completed the Ages and Stages Questionnaire appropriate for the age of 6 months. They were informed about the preceding events and were

encouraged to ask any question they might have concerning the study. During the EEG recordings, participants were seated on their parent's lap, 1 meter in front of a 30 x 40 cm monitor, in an electrically shielded and sound-insulated experimental room. A small video camera was placed above the monitor, and a mirror was placed behind the child so the presented stimuli were through the mirror recorded by the camera. This made possible looking judgments at a later time. The parents were instructed to refrain from speaking or in any way direct the child's attention toward the monitor. The experiment lasted for circa 10 minutes. The total time to complete administration of electrodes and impedance measures was about 30 minutes, resulting in sessions of approximately 40 minutes duration.

### *EEG recording*

Using an EasyCap (EasyCap, Herrsching, Germany) the EEG was continuously recorded from 30 silver–silver chloride electrodes from the sites F3, F4, C3, C4, P3 and P4 according to the 10–20 International System of Electrode Placement. The vertical electrooculogram (VEOG) was recorded from supraorbital (X1) and infraorbital (X2) electrodes on the right eye and the horizontal electrooculogram (HEOG) from electrodes located lateral to the left (X3) and right eye (X4). Impedances were kept below 5 k $\Omega$  and all electrodes were referenced to the average of the left (M1) and right (M2) mastoid bones. EEG traces were recorded using Neuroscan software, the signals were amplified through a Neuroscan Nuamps amplifier (Compumedics Neuroscan, El Paso, TX) and digitized on-line at a rate of 500 Hz.

### *EEG analysis*

Continuous EEG traces were scanned for artifacts, and segments where the EEG signals exceeded 150  $\mu$ V were excluded from additional analysis. Furthermore, trials where the infants were not looking at the screen, as judged from the video recording were also excluded from further analysis. EEG results were bandpass filtered from 0.5 to 30 Hz, with a 12-dB roll-off. Epochs from -100 to 1500 milliseconds were formed and baseline-corrected by using the prestimulus interval of -100 ms. Averages were calculated separately for standard and novel stimuli. A minimum of 10 artifact-free trials in each condition was set as an inclusion criterion for further analysis. The mean number of accepted trials was 21 novel and 48 standard (SD 4,2 and 10,7) for the full-term group, 20/43 (SD 5,0 and 11,4) for the preterm intervention group and 21/44 (SD 5,1 and 11,9) for the preterm control group.

### *Statistical analysis*

The data of 66 VLBW infants, comprising the VLBW intervention group (n=33), the VLBW controls (n=33), and the full term control infants (n=23) were included in the statistical analysis. The recordings from the remaining infants were excluded due to visual impairment (n=2), fussiness during recording session (n=7), failure to meet the minimum criterion of 10 accepted trials per condition (n = 11), or technical failure of the recording equipment (n=12). Based on visual inspection of the component of interest, the mean aggregated amplitude values in the interval 400 to 650 milliseconds were used for statistical analysis. Amplitude peaks and latencies were compared across the groups using repeated measures ANOVA for peak amplitude in the pre-identified time-window (i.e. the mid-latency negative component, NC) at the four fronto-central leads used CONDITION (novel vs. familiar) and LOCATION (F3, F4, C3, C4) as within- subjects variables. GROUP (full-term, VLBW intervention, VLBW control) represented the between subjects variable. LSD post hoc analysis was performed in order to disentangle significant group effects. Repeated measures ANOVA for peak amplitudes for the familiar stimuli were computed separately in a second line of analysis. AREA (frontal vs. central) and HEMISPHERE (left vs. right) were used as within subjects variables, and GROUP as the between subject variable.

### *The Ages and Stages Questionnaire*

In addition to electrophysiological data, a measure at the behavioral level was included in the present study. The Ages and Stages Questionnaire (ASQ) is a parent-administrated standardized questionnaire which consists of five sections with each having six questions. The five sections assess the following domains of development: Communication, gross motor, fine motor, problem solving and personal-social. The parents or other caregivers are asked to observe and record whether the child performs the various age-appropriate behaviors. The ASQ now exists in a Norwegian version, and has been found to be an effective diagnostic tool of developmental delay and/or disturbances (Janson & Squires, 2004; Richter & Janson, 2007). Parents or caregivers received the questionnaire by mail, and completed it at home. MANOVA test using the five sections (communication, gross motor, fine motor, problem solving and personal-social) as dependent variables, and group (full-term: n = 28, VLBW intervention: n = 48, VLBW control: n = 55) as fixed factor was performed on the ASQ data. LSD post hoc analysis was performed to investigate how the groups differed from one another. Total scores were statistically analysed using a one-way ANOVA.

## Results

### *Event-related potentials*

The grand average ERPs for the familiar and novel stimuli at electrode site C3 for the three groups are shown in Figure 2 and 3. The NC was defined as occurring during the pre-selected 400- to 650-ms interval, and a repeated measure analysis of variance (ANOVA) was performed for the fronto-central leads F3, F4, C3 and C4.

There was a significant main effect of condition ( $F(1, 86) = 101.55, p < .001$ ). Also, there was a significant main effect of group ( $F(2, 86) = 7.49, p < .001$ ). Post-hoc analysis showed that this effect existed between the full-term group and the VLBW control group (mean difference = 8.05,  $p < .001$ ). In addition, there was a significant mean difference between the two VLBW groups (mean difference = 4.93,  $p < .05$ ). There was no significant difference between the full-term group and the VLBW intervention group (mean difference = 3.12,  $p = .15$ ).

### *Familiar stimuli.*

A significant main effect of group was found ( $F(2, 86) = 7.11, p < .005$ ) for the familiar stimulus condition. Post hoc test of group revealed a significant difference between the two VLBW groups (mean difference = 6.27,  $p < .01$ ), as well as a significant difference between the full-term group and the VLBW control group (mean difference = 7.1,  $p < .01$ ). There was no significant difference between the full-term group and the VLBW intervention group (mean difference = .83,  $p = .71$ ). There were no significant differences in latencies between the groups for the familiar stimuli.

### *Novel stimuli.*

An identical analysis of variance was computed for the novel stimuli. Results showed a significant main effect of group ( $F(2, 86) = 5.87, p < .01$ ). Post hoc test revealed a significant difference between the full-term group and the VLBW intervention group (mean difference = 5.42,  $p < .05$ ). Also, a significant difference was found between the full-term group and the VLBW control group in both hemispheres (mean difference = 9.0,  $p < .01$ ). No significant difference of peak amplitudes to the novel stimuli was found between the two VLBW groups (mean difference = 3.58,  $p = .14$ ). There were no significant differences in latencies for the novel stimuli.

Table 1.

Observed means in microvolts for NC amplitudes at site C3 for familiar and novel stimuli (Standard deviations are in parentheses).

Lead	Group		Familiar stimuli	Novel stimuli
		n	Mean (S.D.)	Mean (S.D.)
F3:	Full-term controls	23	-13.6 (6.7)	-19.8 (7.9)
	VLBW intervention	33	-15.6 (8.0)	-26.2 (9.6)
	VLBW controls	33	-21.9 (10.3)	-30.5 (11.1)
F4:	Full-term controls	23	-14.1 (6.0)	-19.8 (7.9)
	VLBW intervention	33	-15.7 (8.5)	-26.3 (10.8)
	VLBW controls	33	-21.9 (9.4)	-30.1 (11.1)
C3:	Full-term controls	23	-14.7 (7.2)	-22.7 (7.3)
	VLBW intervention	33	-14.5 (7.9)	-26.2 (9.7)
	VLBW controls	33	-21.6 (10.9)	-30.2 (14.0)
C4:	Full-term controls	23	-15.5 (6.9)	-21.7 (7.0)
	VLBW intervention	33	-15.5 (7.9)	-27.7 (11.5)
	VLBW controls	33	-21.0 (8.1)	-30.9 (11.7)

*The Ages and Stages Questionnaire*

Figure 1 shows the distribution of group scores on the five subsections of the ASQ.

The results showed a significant effect of group for the problem solving section ( $F(2, 128) = 3.56, p < .05$ ). Post-hoc LSD analysis revealed that the observed differences were between the two VLBW groups (mean difference = 3.79,  $p < .05$ ), and between the full-term infants and the VLBW controls (mean difference = 3.88,  $p < .05$ ). No difference at a significant level was

found between the full-term controls and the VLBW intervention group (mean difference = .06,  $p = .96$ ).

In addition, group differences were found for the personal/social subsection ( $F(2, 128) = 5.45, p < .001$ ). Post-hoc analysis showed a significant difference between the full-terms and the VLBW intervention group (mean difference = 8.11,  $p < .001$ ), as well as between the full-terms and the VLBW controls (mean difference = 9.1,  $p < .001$ ). There was no significant difference between the two VLBW groups (mean difference = .99,  $p = .69$ ). There were no group differences in the total scores of the ASQ.

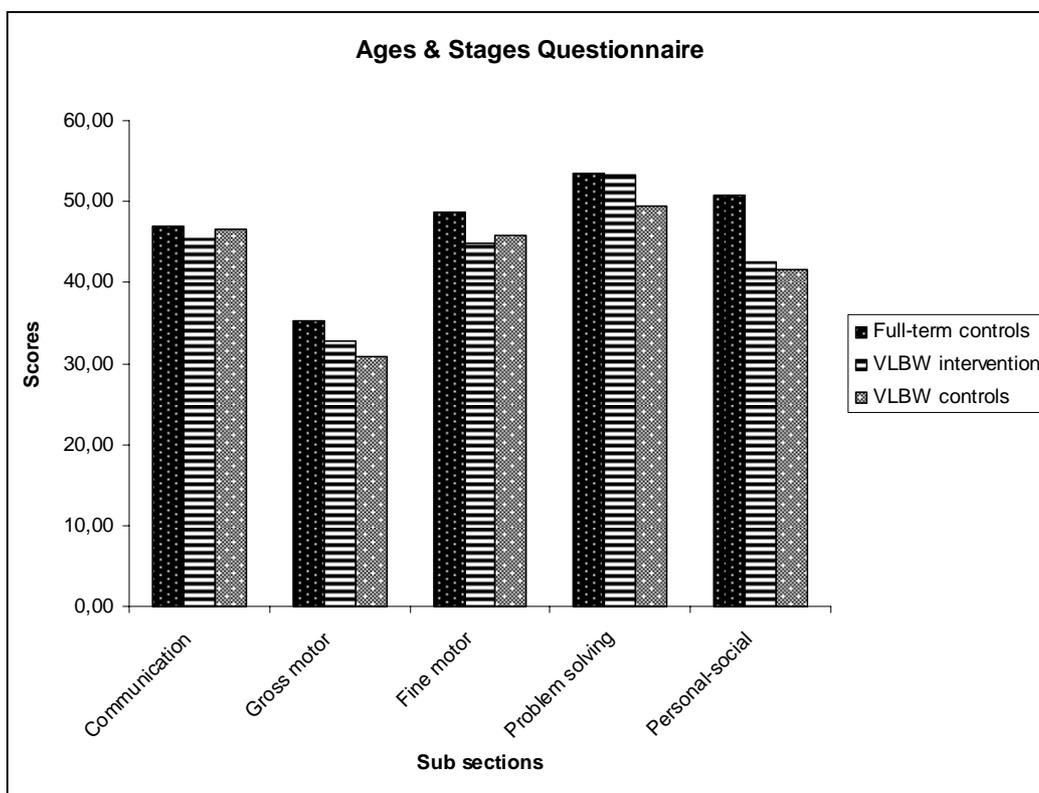


Figure 1. Group scores on the 5 subsections of the ASQ.

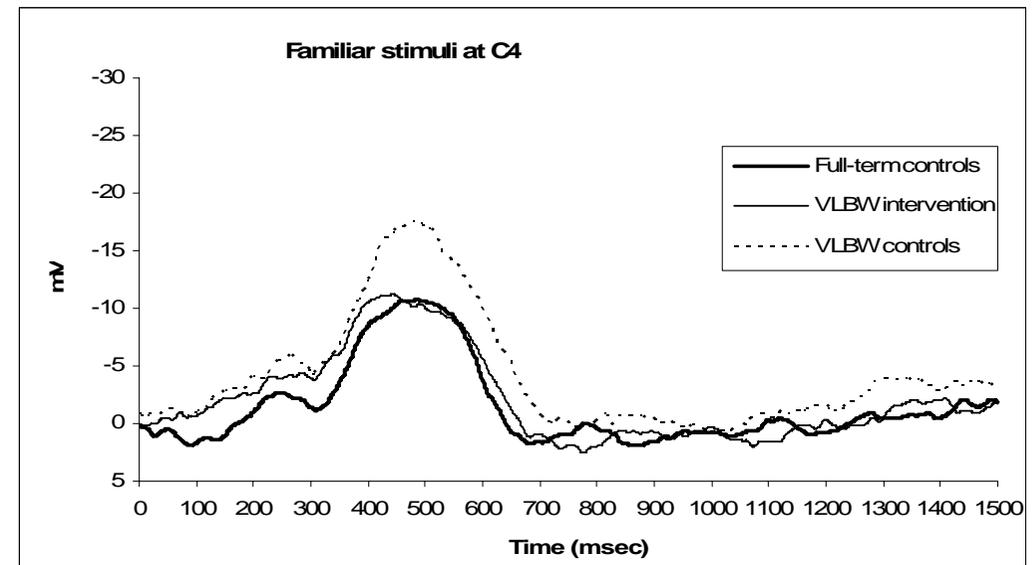
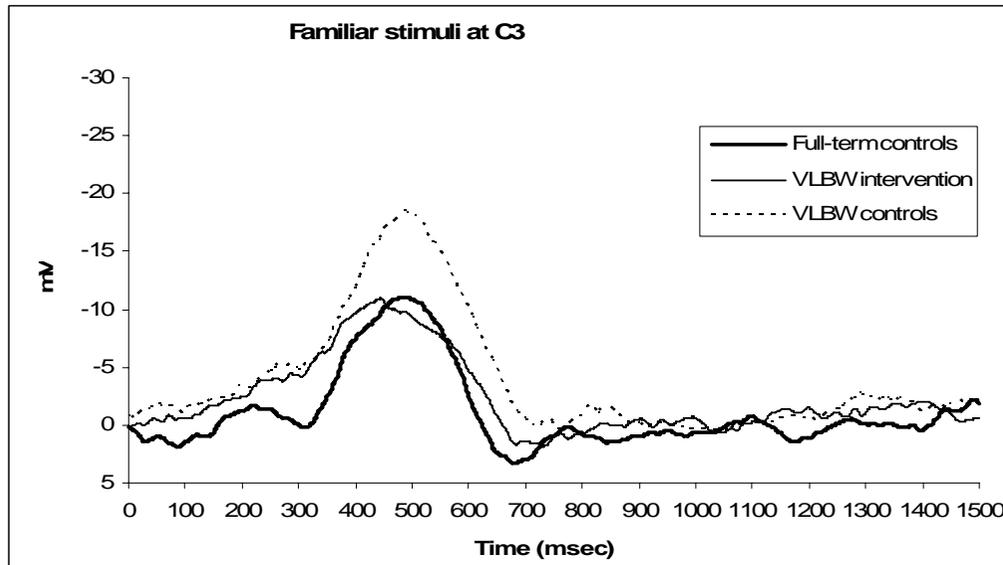
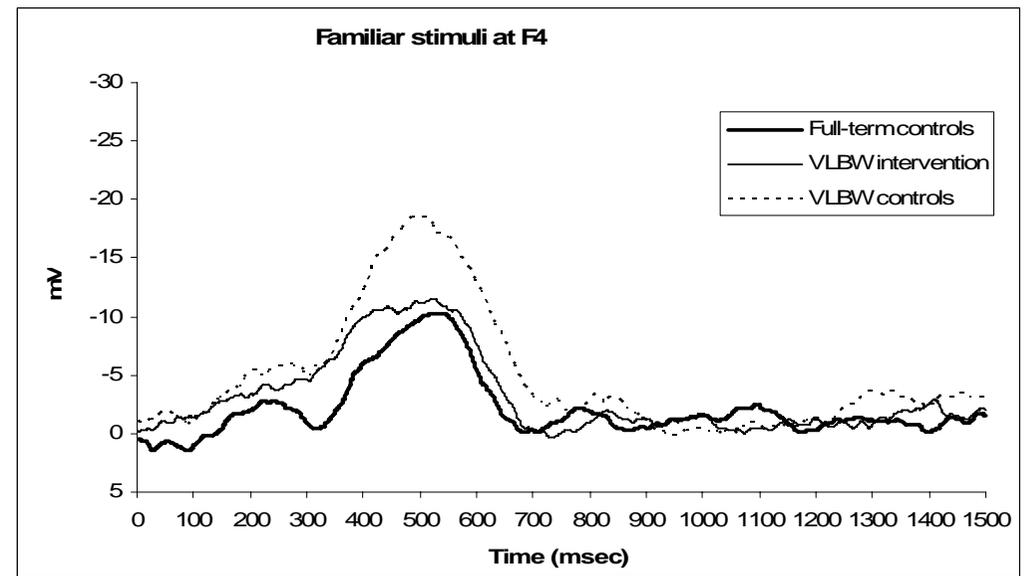
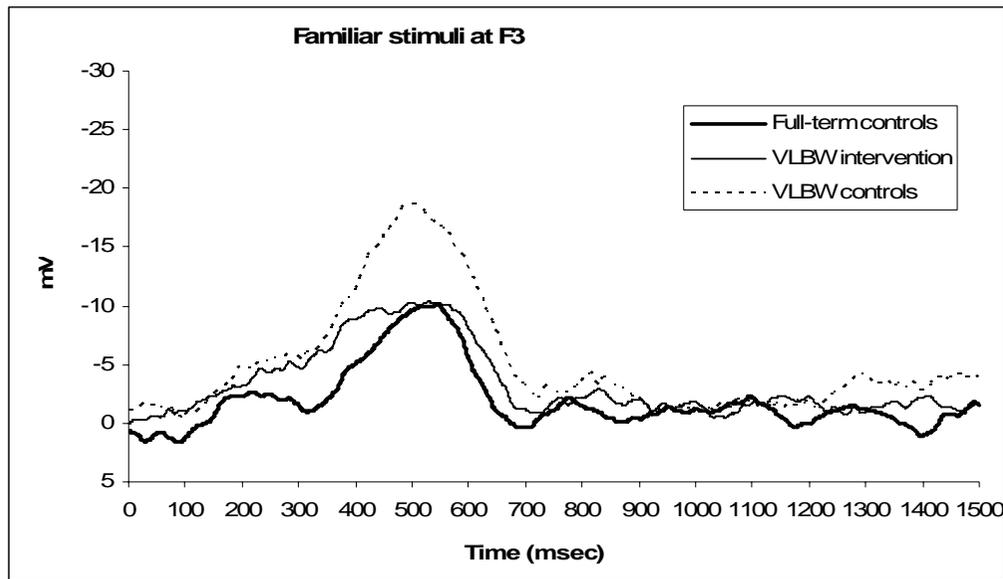


Figure 2. Grand average ERPs for the 3 groups in response to the familiar stimulus condition at the fronto-central leads, measured in microvolts (mV) and time (msec). The NC is the most prominent negative deflection that peaks at approximately 500 msec. The amplitude scale has negative values upward.

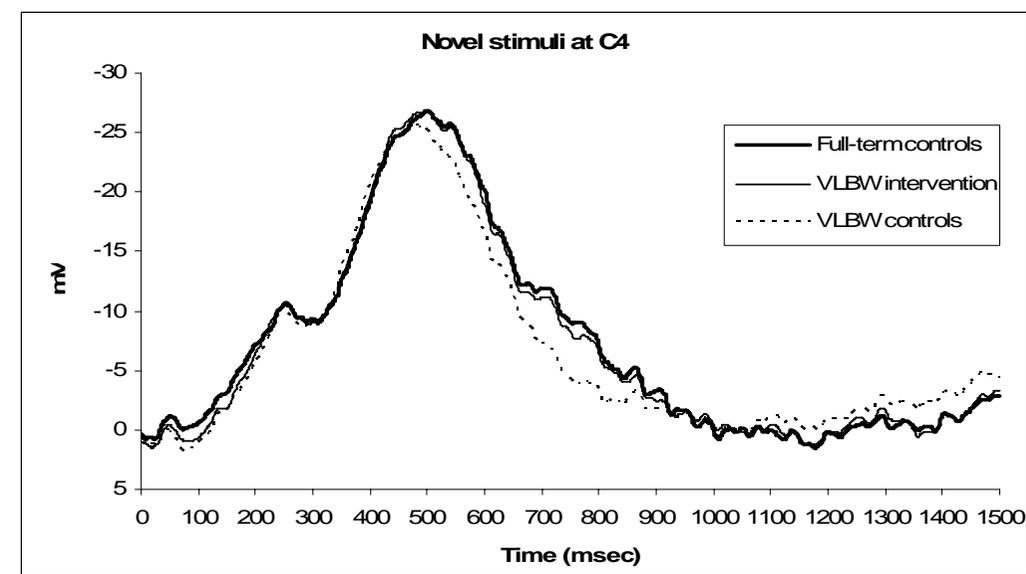
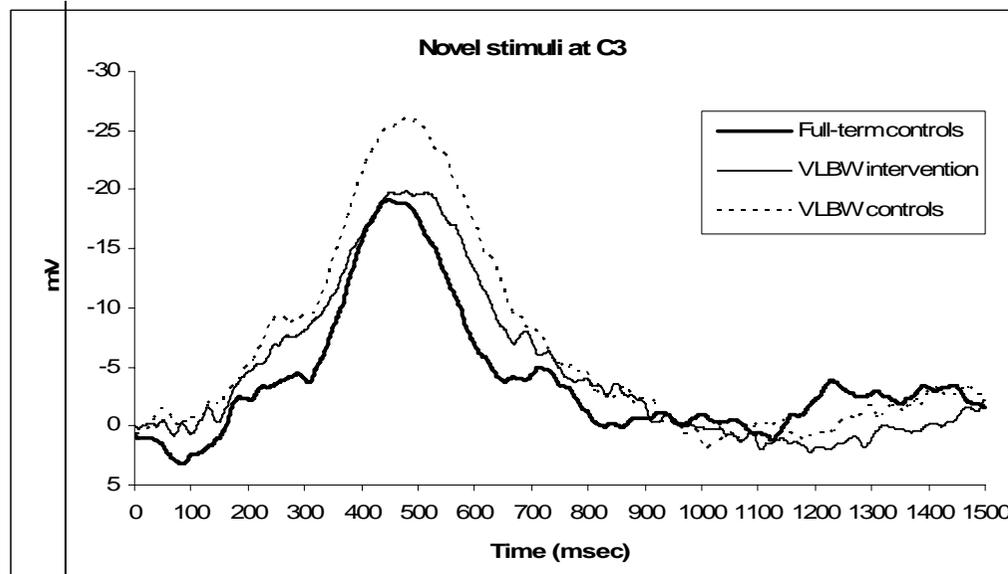
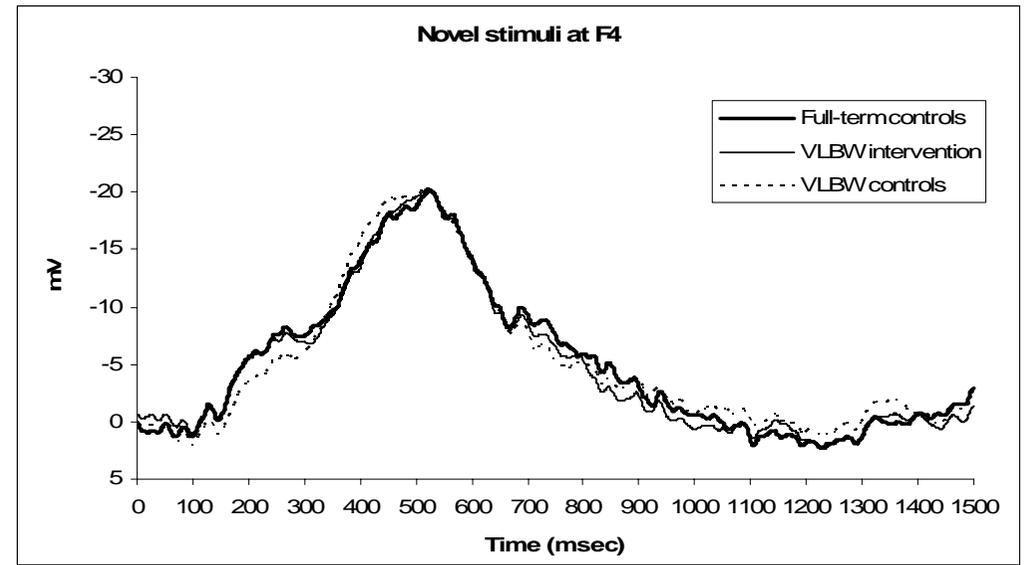
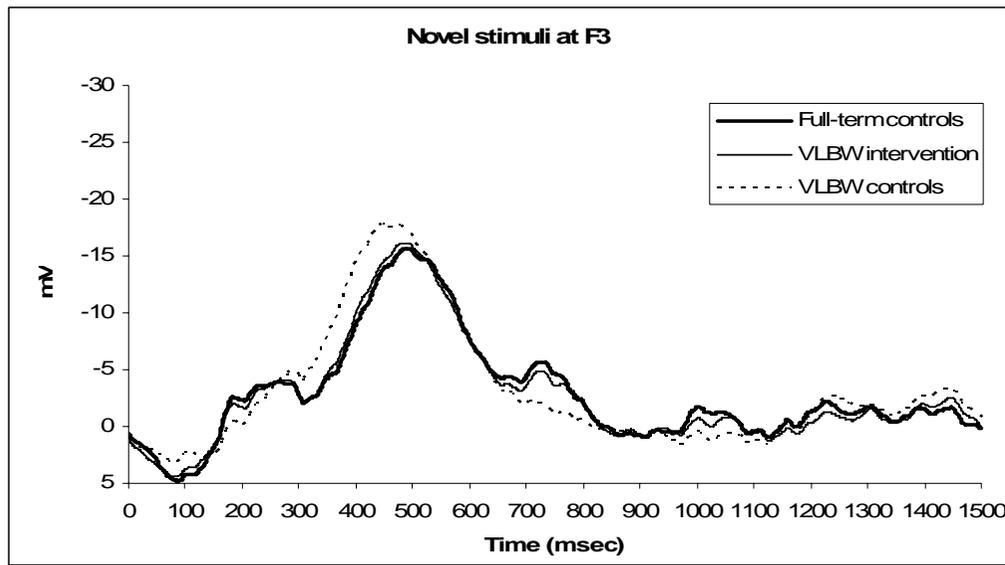


Figure 3. Group responses to novel stimuli condition at the fronto-central leads, measured in microvolts (mV) and time (msec). The NC is the most prominent negative deflection in the waveform. The amplitude scale has negative values upward.

## Discussion

Early assessment of cognitive development may assist in understanding the immediate impact of nutritional deficiencies as well as allow for ongoing evaluation of the effectiveness of nutritional remediation (deRegnier, Long, Georgieff & Nelson, 2007). The main goal of the present study was to compare the cognitive processing of a two groups of VLBW infants, where one received early supplementation of DHA and AA and the second served as a control group. The full-term infants served as a normal comparison group. The groups were compared on electrophysiological indices of attention and/or visual recognition memory and the parent-administered Ages and Stages Questionnaire (ASQ). As far as we know, no other study has investigated the effect of early supplementation of DHA and AA in human milk with these outcome measures.

As predicted, the modified oddball paradigm of the present study was successful in eliciting the NC at the frontal and central electrode sites. Given that most studies of the NC show that the amplitude is greatest at these locations, parietal electrodes were not included in the current analysis. Stimulus familiarity modulated the NC in all three groups, indicating that all infants demonstrate the same type of processing in response to the stimuli. Nonetheless, the amplitudes of the groups did differ.

The results of the present study show several differences between the full-term infants, the VLBW infants who received the supplement of DHA and AA, and the VLBW control infants. With regards to the ERP findings, a significant effect of group was revealed in the amplitude peaks in response to the familiar stimulus at the frontal and central leads. This was in line with the hypothesis that possible group differences as a result of the nutritional intervention and birth weight would be manifested in an electrophysiological correlate to attentional and recognition memory processes. The NC of the familiar condition did in fact discriminate between the two VLBW groups, with amplitudes being significantly greater in the VLBW control group. Furthermore, a significant group difference was found in response to the novel stimuli at the frontal and central leads. However, this effect was not found between the VLBW infants. Instead, the NC in response to the novel stimuli discriminated between the full-term infants and the VLBW infants as a whole, with full-term amplitudes being significantly smaller. In the novelty condition, no effect was found as a result of the supplementation; rather an effect of birth weight is indicated in the data. Differences in

latencies were not found between any groups, or between the two stimuli conditions. The results of the parent-administered ASQ showed a significant difference in the problem-solving subsection. This subsection discriminated the VLBW intervention group from the VLBW controls. The full-term infants did not differ from the VLBW intervention group at a significant level. In addition, the two VLBW groups differed from the full-term infants on the personal-social subsection of the ASQ.

### *ERPs in special populations*

In healthy infants, ERPs have been used to gain further insight into early cognitive development and the effect of experience on brain function. Increasingly, the ERP technique is being used to study differences in memory and attention between clinical and normally developing groups. Studies of infants at risk for cognitive impairments with electrophysiological methods have recently elucidated atypical memory development in infants of diabetic mothers (deRegnier, Long, Georgieff & Nelson, 2007), difficulties with perception and discrimination of speech sounds in infants at risk for dyslexia (Lyytinen et al., 2004), and multiple areas of cognitive differences in extremely premature infants. With respect to ERP studies of premature infants, most have used measures of auditory recognition memory. Also, examinations of infants with global developmental delay, such as Down syndrome, infants believed to be generally at risk for cognitive impairment (deRegnier, Georgieff, & Nelson, 1997), or at particular risk for memory disorders (deRegnier, Long, Georgieff & Nelson (2007) have all used ERP correlates of infant visual recognition memory (Karrer et al., 1998).

Black, deRegnier, Long et al. (2004) studied 34-38 week gestation intrauterine growth-restricted (IUGR) newborns with head-sparing, using an auditory recognition memory paradigm (speech and non-speech). Compared to age-matched controls, the IUGR showed a much larger area of the Negative Slow Wave component at lateral leads (T4, CM3, and CM4) in response to the familiar speech condition (their own mother's voice). The authors concluded that IUGR newborns with head-sparing showed evidence of accelerated maturation of cognitive processing suggesting an atypical process of maturation that may not support typical cognitive development. The results of the present study indicate that none of the VLBW groups showed atypical development in the waveform in response to visually presented stimuli. The VLBW infants, at the group level, showed the same general pattern as the full-term infants. D

In a study by deRegnier, Long, Georgieff & Nelson (2007), newborn, 6-month-old, and 8-month-old infants of diabetic mothers (IDMs), who are at an increased risk of perinatal iron deficiency, were compared on several ERP components. In animal models, severe perinatal iron deficiency targets the explicit memory system of the brain, and cross-sectional ERP studies have shown that infants of diabetic mothers have impairments in recognition memory from birth through 8 months of age. However, deRegnier et al. (2007) did not find effects of maternal diabetes on the development of the NC recorded over the midline and anterior temporal electrode sites. It is, however, important to note that little is known about the specific pattern of this component in at-risk developmental populations.

The paradigm used to elicit the NC in the present study was based on Goldman, Shapiro & Nelson (2004). Although the familiar image was different (ball vs. stuffed animal) as well as the total amount of trials presented (100 vs. 66), our paradigms match in frequency of presentation (70% and 30%) in addition to the presentation of trial-unique stimuli in the novelty condition. Infant ERP studies have typically used one of two paradigms. The first is a standard oddball paradigm, in which two or more stimuli are presented repeatedly, with different frequencies of presentation, while ERPs are recorded. The second is a modified habituation paradigm in which the infant is first familiarized to a stimulus, and then presented with the familiar and a novel stimulus repeatedly while ERPs are recorded. The paradigm of the present study is defined as a modified oddball paradigm for several reasons. First, a short familiarization phase was included at the beginning of the presentation of the stimulus material. This phase included 8 trials with the familiar stimulus type, and 2 novel stimuli. These first ten images were presented for 5 seconds each (remaining stimuli had a duration of 2 seconds per image). The intention was not ensure habituation in this phase of the task, rather to give the infants an introduction of the stimuli that would spark their interest for the remaining part of the presentation. On the other hand, the presentation rate of the present study was slower than most other NC studies, where the average rate seems to be 500 milliseconds (for an excellent overview, see de Haan, ed., 2007). Second, the novel stimuli were trial-unique, i.e. never repeated.

Infant ERPs offer a challenge to interpretation for many reasons. A lack of consistency in the choice of paradigms makes comparison across studies difficult. Different paradigms may elicit the same ERP components, but variations in probabilities of the stimuli presentations and choice of stimuli create uncertainty as to what cognitive processes are required. In infant

ERP studies, ecologically relevant stimuli are preferred so that the stimuli are successful in keeping the child's attention focused on the task.

### *Interpretations of amplitude and latency*

The main measurements of an identified component are its latency and its amplitude. The most important determinants of latency are the conduction velocity in the pathway from the sensory receptor to the generator of the ERP, as well as the length of this pathway (Picton & Taylor, 2007). Conduction velocity depends mainly on the degree of myelination of the axons in the pathway. To a lesser extent than myelination, latency may also be affected by synaptic efficiency. The concept of processing speed has been related to latencies of ERP components. Overall postnatal improvements in maturation of synapses and myelination should lead to decreases in information processing speed over the first year of life, a hypothesis that is consistent with the results of Webb et al. (2005). The interpretation of response amplitude in terms of underlying neural mechanisms is less clear (Picton & Taylor, 2007). A determinant of importance to amplitude size is the number of synapses activated in the region generating the response. At the cognitive level, amplitude size is generally interpreted as related to the amount of attentional resources spent on the particular event. However, factors such as the degree of synchronization, the alignment of the responding neurons, the volume of cortex activated, and the amount and impedance of the tissue between the generators and the recording electrodes, also play important roles. In comparing groups of infants of the same age, it has been suggested to consider latency of an ERP as reflecting myelination (white matter) and the amplitude as reflecting a combination of synaptogenesis and synchronization (gray and white matter.) (Picton & Taylor, 2007).

The results of a longitudinal study by Webb et al. (2005) suggest that there is significant developmental change in the ERP response to visual stimuli during the first year of life. For instance, they found that the NC amplitude showed a significant linear increase over time (i.e. more negative). From 6 to 8 months the NC amplitude tended to become less negative. This is confirmed in the present study, in that the full-terms showed smaller amplitudes in response to both stimuli conditions as compared to the VLBW infants.

### *The NC: Reflective of different processes*

The NC has been proposed to represent a variety of processes, although all under the umbrella of attention and memory processes. Differences in the distribution of activity across the scalp (topography) are thought to reflect different underlying neural generators and hence different

cognitive processes. In fact, since the topography of ERP components changes with age, one can infer that important changes are still taking place in the neural substrate generating the components of interest throughout development (DeBoer, Scott & Nelson, in de Haan ed. 2007). Many studies involving the NC do not report such differences, but some do. For instance, Snyder et al. (2002) report that differences in topography indicate that the familiar and novel stimuli are being processed differently. They suggest that the habituation of an infant's visual attention may be due, in part, to the decrement in neuronal activation of structures in the temporal lobe which encode specific perceptual properties of a stimulus, a form of short-term perceptual memory (Snyder et al., 2002). Reynold & Richards (2005) The data of the present study do not indicate clear topographical differences, as was expected due to choice of using a limited number of active electrodes<sup>1</sup>

Infants demonstrate greater memory for presented events while in an attentive state than for events they were exposed to in an inattentive state (Richards, 1997). Events that have been partially encoded into memory or events that are novel elicit larger attention responses than those events that have been fully encoded (Fantz, 1961; in Reynolds & Richards, 2005). Several neuropsychological models of attention development agree that the development of attention is accompanied by a gradual shift from subcortical processing to increasing cortical control over attention (van de Weijer-Bergsma, 2008). Oddball effects, as in the differential ERPs in response to familiar and novel events, appear to indicate that stimulus discrimination and recognition memory processes are likely to occur early in the information processing stream and are perhaps completed by the time of the peak of NC rather than later in the time range (Ackles & Cook, 2007).

### *Familiar stimuli*

The peak amplitudes of the two VLBW groups were found to differ in response to the familiar stimuli, but not to novel stimuli.

The argument by Snyder et al. (2002) that familiar and novel stimuli are processed differently, possibly by different neural circuit, does shed light on the possible effect of the DHA and AA supplementation. It is conceivable that the processing of familiar stimuli taps cognitive processes that are of a higher order compared to novel stimuli. According to the comparator

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<sup>1</sup> The main goal of the study was group comparison and the NC would most likely be visible at the leads chosen. Also, choosing a limited number of leads would benefit in keeping the preparation and recording session as short as possible, which in turn improves the chances of collecting data of sufficient quality.

model by Sokolov (in Rose, 2004) attention is inhibited if a match of a stimulus representation is found. If no match is found, attention remains engaged until sufficient information is assimilated from the stimulus to render it no longer novel. Decrements of the NC may reflect the efficiency of assimilation and storage of information among infants. Successful stimulus encoding and retrieval could lie at the heart of the group difference found in this study. This would imply that the intervention group, as a result of the supplementation, was as successful in forming a representation of the familiar stimulus as the full-terms. This could be seen either as improved endogenous attentional capacities in the intervention group, or a combination of superior attentional capacities with the resulting enhancement in visual recognition memory. The finding that the VLBW controls had significantly greater NC amplitudes in the familiar condition, suggests that the stimuli was not as familiar to them as it was to the other groups. This could be the manifestation of a developmental lag in effective processing. Alternatively, the stimulus presented frequently did become familiar to these infants, but the processing was made in a more effortful way. Perhaps is this response reflective of a more bottom-up, orienting attentional response. Altered inhibitory responses could also explain this result.

Inhibition could be seen as equivalent to endogenous attention, or a top-down controlled attentional process. A study of six-month-old infants with Down syndrome (DS) by Karrer, Karrer, Bloom, & Davis (1998) does provide another interpretation of the amplitude differences found in the present study. They used an oddball paradigm with two different female faces that were presented with probabilities of 80% and 20%. Their results showed that the DS infants evinced the same pattern of responses to the two stimulus conditions as the controls. Interestingly, they found similar differences in amplitudes peaks in response to the familiar stimuli as has been described in the present study. The DS infant group had significantly higher amplitudes in response to both stimulus conditions. The authors interpret these results as support of altered inhibitory processes in the brain of infants with Down syndrome. One might speculate that this is the same mechanism as affected in the VLBW infants, and perhaps the early supplementation of DHA and AA is beneficial for inhibitory processes.

### *Novel stimuli*

It has been suggested that ERPs to novel stimuli represent a non-specific and possibly automatic process of novelty-detection (e.g. Nelson & Collins, 1991). The novel stimuli presented in this study were trial-unique, which ensures that the responses seen are correlates

of novelty-related processes and not confounded with familiarity (which could be the case if only one stimulus was presented in the novelty condition, as in the standard oddball paradigm). Richards (2003) found that NC amplitude increased with age during attention. However, these results were not replicated in a later study (Reynolds & Richards, 2005). Here, a simple effect was found with 20-week-olds demonstrating greater NC during inattention than during attention. The authors interpreted this as a possibility of an obligatory orienting response for younger infants to the presentation of stimulus during inattention, and that this orienting response decreases with age.

No differences were revealed in the NC in the novelty condition between the two VLBW groups. Thus, an effect of the DHA and AA supplementation was not established in this condition. However, the full-terms showed significantly lower amplitudes in response to the novel stimuli. Although attentional state was not controlled for in the present study, the results could be interpreted as a maturity-dependent decrease of the orienting response. Perhaps is this the result of more developed processing of novelty in that the full-terms “figured out” that new images are presented every now-and-then, and that the decrement in amplitudes represents a matching of this expectancy to the novel stimuli. Expectancy would, in turn, imply some form of explicit memory. Possibly are the responses of all groups a manifestation of Nelson’s (1995) proposed pre-explicit memory type, with the full-terms showing greater influence of top-down, endogenous attention.

### *Processing speed*

In behavioral studies, variations in recognition memory are often explained as due to differences in processing speed. Processing speed, defined as a basic aspect of the human cognitive architecture, has been linked to results showing a less developed visual recognition memory in preterm infants.

The concept of processing speed has been related to latencies of ERP components (e.g. Picton & Taylor, 2007). There were no group differences with respect to latency of the NC in response to familiar or novel events in the present study. This indicates that processing speed was not affected by the DHA and AA supplementation. On the other hand, one can speculate that processing speed was a contributing factor to the differential amplitudes seen in the groups of the present study. Perhaps in familiar stimulus condition, the amplitude decrements seen in the VLBW intervention group was a result of more effective and speedy encoding and retrieval. Given the well-known need of preterms for longer familiarization time (Rose et al

2004), it could be that the VLBW controls did exhibit this greater need in the relatively greater amplitudes in this condition.

### *Individual differences*

Individual differences in maturation are to be expected in infants due to ongoing development of the brain. Longitudinal changes in infant ERP components correlate with synaptogenesis and myelination of the brain. Furthermore, the range of normality has not been well defined for scalp distribution, amplitudes, and latencies of specific ERP components (deRegnier, 2005). Infant ERPs tend to show more variable responses than ERPs of older children and adults. Infants have a larger and more variable background EEG, and the actual ERP of the infant is likely more variable from trial to trial than in older children. The causes of this variability are not completely understood today. Variability has been linked to a combination of individual differences in brain maturation, state of arousal, or attentional differences, and to the fact that it is not possible to directly instruct infants on the task to be performed in the study (deRegnier, 2005; Picton & Taylor, 2007). The data of the present study indicated that within-group variability of NC amplitude peaks was greater in the VLBW control group. The full-terms and VLBW intervention group had similar variability in their responses with respect to the peak of the NC. Speculatively, the variability in the VLBW control group reflects the same attentional differences that are revealed in the data. It could be that there exists a link between the supplementation of DHA and AA and a more stable level of arousal, which in turn increases the possibility of successfully encoding the presented stimuli. Understanding individual differences in typically developing children is vitally important in interpreting differences that are seen in studies of low- and high-risk groups of infants (deRegnier, 2005).

de Haan (2007) notes that there are important factors to consider when using ERPs to compare typically developing and at-risk or atypical groups. Various aspects of procedure and data processing can affect the amplitude or latency of components other than true differences in cognitive processing between groups. For instance, one study showed that the amplitude of the NC differed for infants who saw a different total number of trials in the recording procedure, even when there were no differences in number of trials used to create averages (Snyder et al., 2002). This factor was not controlled for in the present study. However, the finding that the VLBW differed at a significant level in the problem solving subsection of the ASQ lends support to the presented ERP results.

### *Improvements at the behavioral level*

At the neural level, differences were found between the VLBW intervention group and VLBW controls. Infant ERP components are not standardized measures; therefore a standardized questionnaire was included in this study. The results from the parent-administered ASQ indicate group differences on the problem-solving subsection (see Figure 1). Thus, the ERP results are supported at the behavioral level. The VLBW control infants had significantly lower scores than the intervention group. Moreover, the intervention group did not differ at a significant level from the full-term group on this measure. The parents/caregivers were blind to the group allocation of their child. Hence, the observation they made of the infants problem solving skills could not be biased by their beliefs with regards to the effect of the intervention. Problem solving is generally thought of as abilities related to frontal cortical areas of the brain. It is conceivable that infant visual recognition memory and attention are a pre-requisite for the different problem solving behaviors measured in the ASQ. Alternatively, the ASQ has tapped other processes that have also been enhanced by the supplementation. The six questions of the problem solving subsection are related to the manipulation of objects. The intervention could have improved motor skills, but no group differences were found in the fine or gross motor skills subsection. In light of the ERP results previously discussed, the ASQ findings support the conclusion that cognitive processes have been enhanced as a result of the DHA and AA supplement. This also underlines the importance, and strength of the present study, of including a standardized measure to supplement the ERP findings of group differences.

Another strength of the present study is the inclusion of a full-term control group. ERPs in infants are variable, and thus interpretations of group differences in clinical populations can be difficult. The ERPs of the full-term group lends support to the conclusions that the VLBW intervention group has in fact benefitted cognitively from the supplementation of DHA and AA, given that the NC in the familiar condition was not significantly different between these two groups.

The present study tested the VLBW infants at their corrected age. In accordance with the conclusions of Stolarova et al. (2003), this lends support to the interpretation that group differences are caused by environmental contributions rather than maturational factors (see introduction). The main difference between the two VLBW groups was, presumably, the

supplementation of the essential fatty acids DHA and AA. The original distribution of sex and birth weight (and other confounding variables) in the two VLBW groups did not differ (for more information, see Henriksen, et al., 2008). Unfortunately, these factors were not controlled for in the remaining group members that were included in the ERP study. There is a possibility that the differences found in the brain-activations of infants in this study are a result of gender and/or other variables. On the other hand, there is little documentation of specific differences related to gender and within-category birth weight on the cognitive abilities measured here.

### *Conclusion*

The main purpose of the present study was to investigate the effect of early supplementation of DHA and AA in human milk on the cognitive development of VLBW infants. The hypothesis was that an information processing benefit of this supplementation would manifest itself as an improvement of attentional processes and/or visual recognition memory, as measured with ERPs using a modified oddball paradigm. The NC was shown to distinguish between the VLBW infants who received supplementation and the VLBW controls in response to the familiar stimuli. In addition, the problem-solving subsection of the ASQ provided further evidence of the beneficial effect of the DHA and AA supplementation. A double-blind prospective design strengthens the notion that the differences in attentional and memory processes revealed here are caused by the experimental manipulation.

The optimum nutritional plan for the developing brain is not known. The findings of the present study suggest that that early supplementing of DHA and AA has a positive effect on cognitive abilities such as attention, visual recognition and problem solving skills. The long-term effect of this nutritional intervention has yet to be investigated. The results of this study have shown that infant ERPs provide important knowledge of the effect of nutritional intervention on the developing brains of VLBW infants.

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## Appendix

ERP stimuli presented to 6-month-old infants in the present study.

Familiar stimulus:



Novel stimuli:

