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Fish oil-supplementation from 9 to 12 months of age affects infant attention in a free-play test and is related to change in blood pressure

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ABSTRACT

Introduction: This intervention examined whether fish-oil-supplementation in late infancy modifies free-play test scores and if this is related to blood pressure (BP) and mean RR interval.
Patients and methods: 83 Danish 9-month-old infants were randomized to ± fish oil (FO) (3.4 ± 1.1 mL/d) for 3 months and 61 of these completed the free-play-test before and after the intervention.
Results: Most of the free-play scores changed during the intervention, but the intervention affected only the number of looks away from the toy, which was increased in +FO and decreased in −FO ($p=0.037$). The increased numbers of looks away were associated with an increase in erythrocyte eicosapentaenoic acid ($r=0.401$, $p=0.017$, $n=35$) and were also associated with a decrease in systolic-BP ($r=-0.511$, $p<0.001$, $n=52$).
Conclusions: The results indicate that *n*-3 fatty acid intake also in late infancy can influence brain development and that the cognitive and cardiovascular effects may be related.

1. Introduction

Long-chain *n-3* polyunsaturated fatty acids (LCPUFA) are accumulated in the brain during the brain growth spurt occurring in the perinatal period—mainly in the form of docosahexaenoic acid (DHA). During the 1990s DHA accretion of the brain was shown to be lower in formula-fed infants, who at that time did not receive any LCPUFA [1,2]. Observational studies rather consistently show that breastfeeding is associated with cognitive advantages [3], and it was hypothesized that this could be due to differences in the DHA-accumulation. During the last decades, several trials have been performed in order to test this hypothesis; some of the studies do find that the performance on different neurodevelopmental tests differ between the randomized groups, but the results are not yet conclusive [4]. The brain growth spurt continues during late infancy and early childhood, and the DHA-accretion—in terms of the absolute amount of DHA incorporated in the brain—is as high in late infancy as it is in the last trimester of pregnancy [5]. We hypothesize that DHA supplementation could have an effect on brain development also during late infancy. To our knowledge; no one has tested whether fish oil supplementation during late infancy can affect infant performance on cognitive tests.

Recently researchers have re-considered the use of global, standardized measures of development as a means for detecting the potential cognitive effects of *n-3* LCPUFA. Many clinical studies used Bayley Scales of Infant Development as this is validated for clinical use; however, these assessments provide only global indicators of cognitive functioning and are not designed to measure specific cognitive processes, which may reveal subtle differences in children [6,7]. Some psychologists have used non-standardized laboratory measures that are designed to tap specific cognitive processes at given ages—among them the free-play paradigm. This paradigm examines attention in the context of toy play, which shows marked change across development. The development of attention is complex and nonlinear and thus not a uniform construction [8]. Research has shown a positive correlation between attention measured in free-play tests and later cognitive abilities such as vocabulary [9]. We have chosen to modify the coding of the paradigm to include the assessment of looks at parent that may characterize some of the looks away, specifically in late infancy [10]. Looks at parent may be interpreted as social referencing which is a phenomenon where infants reference their mothers (*e.g.* her facial expressions) as a guide in novel situations (in the current project, the novel toy play context) [11].

Two studies in infants have shown that formula with DHA and arachidonic acid reduced heart rate (HR) [12,13] and a meta-

Abbreviations: ANS, autonomic nervous system; BP, blood pressure (systolic SBP & diastolic DBP); CNS, central nervous system; DHA, docosahexaenoic acid; ECG, electro-cardiogram; EPA, eicosapentaenoic acid; FA%, fatty acids given as area percentage; FO, fish oil; HR, heart rate; LCPUFA, long-chain polyunsaturated fatty acid; RBC, red blood cell

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analysis have shown that fish oil supplementation reduce HR in adults [14]. Scandinavians have an old tradition of giving rather large doses of cod liver oil (up to 5 mL/d) to their children and this praxis is still recommended in Norway. In a study where we tested the effect of this praxis on health parameters in infants, we found that fish oil supplementation affected mean RR interval, which was 6% longer among the fish oil supplemented boys [15]. Data from the same study also showed that fish oil lowered systolic blood pressure (SBP) in the infants [16]. Both mean RR interval and SBP are influenced by the autonomic nervous system (ANS) and these influences may be correlated with effects on cognitive development. We therefore hypothesized that the cardiovascular and cognitive effects of *n-3* LCPUFA could be associated.

This hypothesis was tested in the before mentioned trial where 83 healthy Danish infants were randomized to ± fish oil (FO) from 9 to 12 months of age. We examined whether the FO-supplement in late infancy had an effect on cognitive development in late infancy, assessed by the free-play test, and furthermore, if this effect was related to the previous reported changes in mean RR interval and SBP.

2. Subjects and methods

2.1. Design

The study design was a 2 × 2-factorial intervention, in which healthy Danish infants were randomized to receive either 5 mL fish oil (+FO) daily or no supplementation (−FO) from 9 to 12 months of age. To examine the effects of recommending fish oil no control oil was used. Infants in both groups were also randomized to drink either whole cow's milk or standard infant formula. The milk intervention had no effects on the outcomes reported in the present paper. The study was approved by the Ethical Committee of the Municipalities of Frederiksberg and Copenhagen (J.No. KF 02-014/03) and registered in a clinical trial database (ClinicalTrials.gov NCT00-379171). Both parents of all participating infants gave written consent to participate after the study had been explained to them orally as well as in writing.

2.2. Recruitment and subjects

Participants were recruited by random extraction from the National Danish Civil Registry throughout May–October 2003. The inclusion criteria were singleton infants born ≥37 weeks of gestation, with birth weight >2500 g and ≥5th percentile for gestational age according to Danish reference material, a 5-min Apgar score ≥7, no major complications during pregnancy or at birth, and no chronic diseases. Only infants who had a daily consumption of infant formula or cow's milk and whose parents agreed to the principle of randomization were included.

2.3. Fish oil supplementation

The infants in the fish oil group (+FO) were given fish oil in 105 mL-bottles of the brand Eskimo-3® (Cardinova supplied by Anjo A/S). The parents were given five bottles and were asked to give the infant a daily dose of 5 mL fish oil starting the day after the examination at 9 months and until the examination at 12 months. The parents were told to keep open bottles refrigerated, to return any unused oil and to report any waste. The daily mean intake of fish oil was estimated to be 3.3 mL (range 0.77–4.97 mL/d), corresponding to a daily dose of 1162 mg *n-3* LCPUFA (range 271–1749 mg/d) distributed with about 465 mg DHA and 697 mg EPA daily.

2.4. Study protocol

Interviews and examinations were performed at the Department of Nutrition, Exercise and Sports, Faculty of Sciences, University of Copenhagen. The parents were interviewed about diet, growth, and health of the infant and given instructions to register the infant's diet 7 days before each examination visit. The infants were examined before (baseline) and after the intervention when they were 9 months ± 3 weeks and 12 months ± 3 weeks, respectively. Examinations included assessment of both clinical (blood pressure, mean RR interval and venous blood samples) and anthropometric measures and a cognitive free-play test. Ninety-four infants were included in the study, and 83 of these completed both the 9 and 12 months examination. The overall drop-out rate was 12% ($n=11$) and this did not differ between the groups ($p=0.149$).

2.5. Red blood cell (RBC) fatty acid analysis

Fatty acid composition was analyzed on RBC from 1-mL heparinized blood samples taken before and after the intervention. RBC lipids were extracted by the Folch procedure [17] in the presence of butylated hydroxytoluen (0.005%), trans-methylated with BF_3 and analyzed by gas liquid chromatography as described in [16]. The content of specific fatty acids is given as an area percentage (FA%) relative to the overall identified chromatogram area. Blood sampling and determination of RBC fatty acid composition was successful in 69% ($n=56$ and 57) of the infants at each visit and in 55% ($n=45$) of the infants at both visits.

2.6. Measurement of BP and mean RR interval

Arterial blood pressure was determined with an automated oscillometric device (model 506 N, Critcare Systems) during cuff inflation as previously described [16]. Blood pressure was recorded in triplicate in 26% of the infants, 48% were measured in duplicate, and 26% were based on single measurements. Means or single values are reported. The mean RR interval was measured as the mean length of all normal RR intervals during a 0.5 h-ECG recording as previously described [15]. The ECG was recorded continuously for 0.5 h by a two-channel tape recorder (Tracker Reynolds, Reynolds Medical, Hertford, UK), while the infant was doing the free-play task. The recordings were analyzed with commercially accessible software (from Diagnostics Monitoring Santa Ana, CA). Mean RR interval was successfully collected from 68% ($n=56$) at both examinations and BP in 78% ($n=64$).

2.7. Cognitive development testing

A single object free play task [9,18] was used to assess cognitive development at 9 and 12 months of age. This task has been used to measure endogenous (higher level, voluntary) attention and the ability of infants and toddlers to hold and maintain their attention [9,18]. The same electronic toy was used for assessment at both 9 and 12 months, and infants received the toy to freely explore for 5 min. The toy had multiple functions, buttons, and a telephone receiver to explore, and the infants could manipulate the toy to produce sounds. The infant was seated on their parent's lap throughout the free-play test and the toy was placed on a table in front of the infant and the infant was encouraged to play, explore and manipulate the toy. The free-play task was timed using a handheld stopwatch and recorded with a video camera placed at the opposite side of the table so the infant could be seen from the front. The parents were asked not to speak or seek contact with the infant during the test and if they did, this time was subtracted from the coded test time.

The free-play task videos were coded by an experimenter who was blinded to group allocation. For coding purposes a time