Prostaglandins, Leukotrienes and Essential Fatty Acids ∎ (∎∎∎∎) ∎∎∎–∎∎∎

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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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Q6 *Keywords:*
Infant diet
n-3 Polyunsaturated fatty acids
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Cognitive development
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A B S T R A C T

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 heart inter beat 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-analysis have shown that fish oil supplementation reduce HR in

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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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 $\pm$ 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 $\geq$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 $\pm$ 3 weeks and 12 months $\pm$ 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 Q3 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 generator was used to mark on the video film the elapsed time (accurate to 0.001 s). The jog-shuttle dial on the VCR was used to determine the exact timing of the start and end of each look at the toy and each look away from the toy. An individual look was defined as a look at the toy in >1 s. Furthermore, looks at the toy that were separated by a glance away from the toy <1 s were combined to one individual look at the toy. From the videos we determined: the percentage of time spent on task, mean time per look on task, and the total number of looks away from the toy. The total number of looks away from the toy was sub-categorized as looks at the parent and unspecified looks away. All free-play videos were successfully coded, but some children were not tested or recorded due to time constraints and equipment issues. Q4 Successful data was available from 89% ($n=73$) of the infants at 9 months and 85% ($n=$) at 12 months and in 74% ($n=61$) at both examinations.

2.8. Statistics

All data were analyzed with the Statistical Package for the Social Sciences (SPSS, version 20.0, IBM) and Stata 12.0 (Stata Institute, Texas). Results are presented as means ± SD for normally distributed variables or as medians and inter-quartile range (IQR, 25 and 75 percentiles) for not normally distributed variables. The level of significance was set to $p<0.05$. Normality was examined by visual inspection of histograms. Paired *t*-tests were used to examine a possible age effect. This was done by comparing free-play test scores at 9 months with 12 months scores adjusted for intervention and sex. FO-intervention effects were examined by ANCOVA adjusted for baseline, sex and duration of breastfeeding, the latter being somewhat skewed between the randomized groups. If necessary, transformation of data was done to ensure normality. Modification of the intervention effects by sex was examined by including an interaction term between intervention group and sex. Effects of sex were examined by ANCOVA adjusted for baseline, intervention group and duration of breastfeeding. Homogeneity of variance was assessed by Levene's test. The effect of the FO-intervention on free-play test scores was further examined by scatter plots and Pearson's Product Moment Correlation to test a possible dose-dependent association with RBC-EPA. Correlation analyses were furthermore done for changes in free-play test scores *versus* changes in mean RR interval and SBP. With approximately 35 infants with successful assessments in the single object free-play task in each group, this study was powered to show an effect of 0.62 × SD with a 5%-level of significance and a power of 80%.

3. Results

Table 1 shows the socio-demographic, birth, gender, nutrition, and anthropometric characteristics of infants with at least one successful free-play test in the two groups. The infants were predominantly breast-fed as only one infant had never been breast-fed and 10 were breast-fed for less than 4 months (equally distributed in the groups). The only major difference between the two groups was in the number of children who were still partially breast-fed during the intervention (28% in the +FO *versus.* 56% in the −FO-group). Growth of the children in the two groups did not differ (data not shown).

3.1. RBC fatty acid levels

The RBC fatty acid composition of the +FO and −FO groups was similar at baseline, and fish oil supplementation significantly increased the RBC content of *n-3* LCPUFA and decreased the *n-6* LCPUFA content [16]. The estimated daily fish oil intake was linearly associated with the *n-3* LCPUFA content of the RBC at 12 months, particularly with RBC-EPA ($r=0.86$, $n=53$, $p<0.001$) [16]. The content of RBC-EPA increased by 2.64 ± 1.50 FA%-point ($n=24$, $p<0.001$) in the +FO group and by 0.24 ± 0.26 FA%-point ($n=22$, $p<0.001$) in the −FO group and was at 12 months 3.20 ± 1.54 FA% and 0.71 ± 0.22 FA% in the +FO and −FO groups, respectively.

3.2. Free-play scores

The free-play scores did not differ significantly between the two groups at baseline and furthermore, did not differ between boys and girls at 9 months, except that girls tended to spent relative less time in total looking on the toy during the test than boys ($p=0.075$, *data not shown*). All infants spent significantly less time on task and exhibited shorter average look lengths at 12 months compared to 9 months ($p<0.001$ for both scores) (Table 2). The total number of looks off on the other hand increased with age ($p<0.001$) and further analysis of the data revealed that this was due to a greater number of looks to the parent with age ($p<0.001$). There were no age differences in number of looks away. At 12 months of age, girls

Table 1
Baseline characteristics of infants with free-play data.

	+FO	−FO
	Mean ± SD (n^a)	Mean ± SD (n^a)
Gender, F:M, *n*	21:17	21:23
Maternal age, y	31.4 ± 3.6	31.8 ± 4.2
Q5 Maternal education, y	15.9 ± 3.5	16.4 ± 3.7 (42)
Duration of gestation, weeks	39.7 ± 1.3	39.6 ± 1.3
Birth weight, kg	3.67 ± 0.45	3.67 ± 0.45
Duration of breastfeeding, months	6.8 ± 3.8 (34)	8.3 ± 3.7 (42)
Weight at 9 months, kg	9.3 ± 0.9	9.1 ± 0.8
Length at 9 months, cm	72.2 ± 2.1	72.4 ± 2.3 (43)
Body Mass Index at 9 months, kg/m2	17.7 ± 1.3	17.3 ± 1.3 (43)
Head circumference at 9 months, cm	46.1 ± 1.3	46.0 ± 1.5
Systolic blood pressure at 9 months, mmHg	110 ± 12 (32)	107 ± 12 (41)
Diastolic blood pressure at 9 months, mmHg	65 ± 9 (32)	63 ± 12 (41)
Mean arterial blood pressure at 9 months, mmHg	80.2 ± 9.6 (32)	79.5 ± 10.2 (41)
Mean RR-interval at 9 months, ms	443 ± 31 (35)	450 ± 27 (39)
RBC-EPA at 9 months, FA%	0.62 ± 0.41 (29)	0.50 ± 0.21 (28)
RBC-DHA at 9 months, FA%	5.57 ± 1.85 (29)	5.29 ± 1.97 (28)
Est. mean intake of fish oil, mL/d	3.25 ± 1.06 (33)	

[a] If different from 38 in +FO and 44 in −FO.

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Table 2
Free-play scores at 9 and 12 months in the fish oil-supplemented (+FO) and the control group (−FO).

	+FO Mean ± SE (n=27)			**−FO** Mean ± SE (n=34)			p_{age}	$p_{intervention}$	p_{sex}
	9	12	Δ	9	12	Δ			
Time on task (%)	78 ± 4	67 ± 3	−11 ± 4	77 ± 3	72 ± 2	−6 ± 3	< **0.001**	0.419[a]	**0.050**[b]
Mean time per look on the toy (s/look)	20.9 ± 3.2	11.0 ± 0.9	−9.9 ± 3.0	19.9 ± 2.9	12.5 ± 0.8	−7.5 ± 3.0	< **0.001**	0.374	**0.041**[c]
Total no. of looks off	11.8 ± 1.1	19.1 ± 1.2	7.3 ± 1.4	14.1 ± 1.2	18.7 ± 1.0	4.6 ± 1.5	< **0.001**	0.812	0.106
No. of looks off due to looks on parent	9.0 ± 1.1	15.0 ± 1.2	6.0 ± 1.4	9.4 ± 0.9	15.7 ± 1.0	6.3 ± 1.4	< **0.001**	0.479[c]	0.075[d]
No. of looks off unspecified	3.3 ± 0.7	4.3 ± 0.6	1.0 ± 0.7	5.2 ± 0.6	3.3 ± 0.4	−1.9 ± 0.7	0.432	**0.037**	0.563

p_{age} paired t-test (scores at 12 months adjusted for intervention and sex compared with scores at 9 months).
$p_{intervention}$ ANCOVA of differences at 12 months adjusted for baseline, sex and duration of breastfeeding.
p_{sex} ANCOVA of differences at 12 months adjusted for baseline, intervention group and duration of breastfeeding.

[a] Analysis performed on transformed data raised to the third power.
[b] Boys used more time on task compared to girls (analysis performed on transformed data raised to the third power).
[c] Boys had a longer mean time per look on toy compared to girls.
[d] Girls tended to have more looks off due to looks on parent compared to boys.

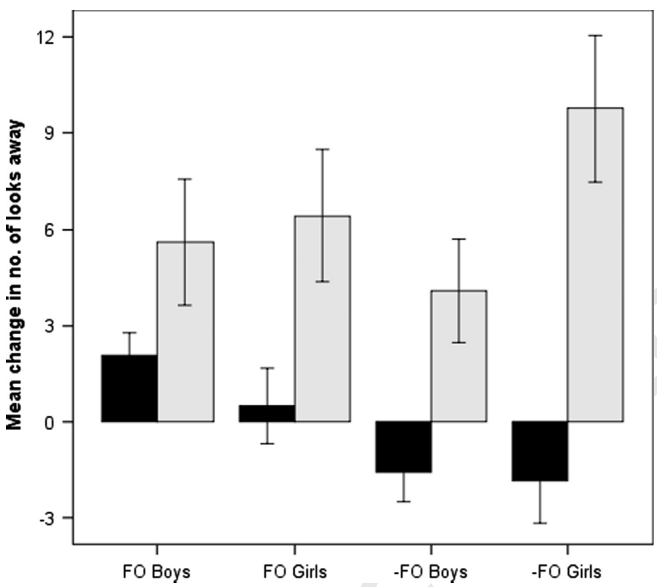

Fig. 1. Mean change from 9 to 12 months (±SE) in number of unspecified looks away from toy (black) and looks at parent (gray) among boys and girls, who received fish oil (+FO) or no fish oil (−FO). There was significant difference in change in unspecified looks away between the two intervention groups (p=0.007). A post hoc t-test comparison of delta-values in gender sub-groups, resulted in a significant difference only among boys (p=0.009).

Table 3
Correlations between RBC-DHA and cognitive functions at baseline and between the RBC-EPA and cognitive response to the fish oil intervention.

	RBC-DHA *versus* scores at 9 months (n=55)		**Δ RBC-EPA *versus* Δ scores (n=35)**	
	Pearsons *r*	***p***	**Pearsons *r***	***p***
Time on task (%)	−0.365	**0.006**	−0.226	0.191
Mean time per look at toy (sec/look)	−0.154	0.260	0.073	0.678
Total no. of looks off	0.037	0.786	0.107	0.541
No. of looks off due to looks on parent	0.034	0.804	−0.102	0.558
No. of looks off unspecified	0.011	0.935	0.401	**0.017**

p-Values and r-values from Pearson's test of bivariate correlations.

tended to have more looks at parent than boys (p=0.075, adjusted for baseline intervention and duration of breastfeeding, *data not shown*). Boys used significantly more time on task and had a longer mean time per look on toy compared to the girls (p=0.048 and p=0.041, respectively, adjusted for baseline intervention and duration of breastfeeding, *data not shown*).

The fish oil-supplementation was only found to affect one free-play score, namely the number of looks away from the toy without referencing the parent. The infants in the +FO-group exhibited an increase in looks away between 9 and 12 months, whereas those in the −FO-group showed a decrease from 9 to 12 months. The increase in looks away seemed to be the most pronounced among the boys; although no difference in response (*i.e.* no significant interaction term) was observed between boys and girls (Fig. 1).

3.3. Associations

The increase in number of looks away was found to be significantly correlated with the increase in RBC-EPA (Table 3), and it still was when adjusted for sex and breastfeeding (p=0.041). In the cross-sectional analysis at baseline, we found significant associations between RBC-DHA and relative time spent looking on the toy.

We found a significant association between change in SBP and change in the percentage of time spent on task, mean duration of looks on toy, and number of looks away during the intervention (Table 4 and Fig. 2). No significant correlations were seen between the change in free-play scores and change in mean RR interval determined by 0.5 h-ECG, although an increase in mean RR interval tended to be associated with a decrease in looks away (p=0.093).

4. Discussion

To our knowledge this study is the first randomized trial to investigate the effects of fish oil on cognitive scores in infants during the period of complementary feeding. We wished to explore possible effects of providing fish oil to infants in line with the old Nordic tradition and thus provided one daily spoonful of fish oil, which with respect to n-3 LCPUFA was equivalent to what is currently recommended from 1 months of age in Norway in the form of cod-liver-oil. Although high (actually equivalent to that used in many studies in adults), the fish oil dose given to the infants is therefore presumed to be safe.

Our results show that infants intervened with fish oil showed an increase in the number of looks away from the toy from 9 to 12 months. In addition, we observed a significant dose-response association between the change in RBC-EPA during the intervention and the change in number of looks away. The effect on looks away was strengthened by an inverse association between change

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Table 4
Correlations between changes in cognitive scores during the fish oil intervention and changes in systolic blood pressure (SBP) and RR interval, respectively.

	versus Δ SBP (n=52)		versus Δ RR (n=39)	
	Pearsons r	***p***	**Pearsons r**	***p***
Δ Time on task (%)	0.495	< **0.001**	−0.059	0.719
Δ Mean time per look at toy (sec/look)	0.276	**0.047**	−0.105	0.524
Δ Total no. of looks off	−0.150	0.289	0.082	0.618
Δ No. of looks off due to looks on parent	0.127	0.371	−0.040	0.810
Δ No. of looks off unspecified	−0.511[a]	< **0.001**	0.272	0.093

p-Values and r-values from Pearson's test of bivariate correlations.
[a] Also correlated with change in DBP ($r = -0.334$, $p = 0.015$).

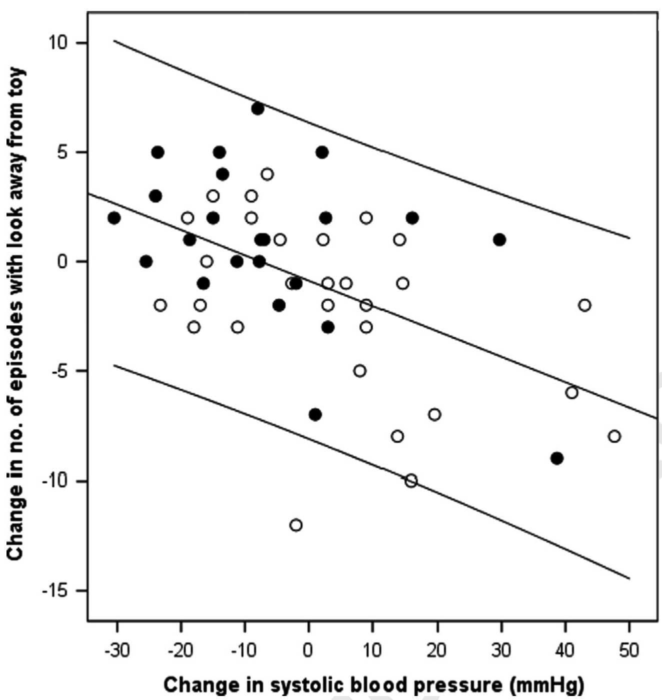

Fig. 2. The bivariate association between change from 9 to 12 months in systolic blood pressure and number of looks away from the free play task without focus on the parent in the fish oil-supplemented (•) and the control (○) group ($r = -0.511$, $n = 52$, $p < 0.001$).

in number of episodes due to looks away and change in SBP during the intervention. Additionally, a decrease in SBP was further significantly correlated with shift in looks away from the toy. We also observed an increase in looks at parent between 9 and 12 months. An association between free-play scores and DHA has previously been reported in two observational studies, which found that a high maternal-DHA was positive correlated with attention at 1 year compared to infants of mothers with a lower level of DHA [18,19], but the effect changed with age, resulting in an inverse association at 1.5 years [18].

The present study is, to our knowledge, the first study that examined looks at parent as a variable in relation to a free-play test. Looks at parent was the dominant type of activity when the infants looked away from the toy at both 9 and 12 months, but the frequency of this type of activity was not affected by the intervention. As hypothesized, we found that the infants spend significantly more time looking at their parent with age. Looks at parent may be interpreted as social referencing and the increase with age reflects a natural maturation in late infancy [10] and is in accordance with results from Walden and Kim [20], who found that infants looked toward their mothers with greater frequency and shorter latency with increasing age.

The development of attention is complex and has been explored and discussed by many researchers (*e.g.* [8,9,21,22]). It is difficult to compare the present results with results from other studies as the studies vary in different types of paradigms and measures, study design, testing ages, number of participants and follow-up periods. It is hard to say if the effects on looks away interpreted as inattention are beneficial or not. Look duration, which at younger ages reflects speed of processing, typically declines with age across the first year of life (meaning more looks away), whereas look duration increases again in the second year of life, where longer looking (and thus less looks away) may reflect attention span [18]. The exact age of the turning point is expected to differ between children depending on their developmental stage. Generally when looking away from the toy the infants showed few signs of refusal to play with the toy (either pushing the toy away or trying to move away from the toy) and generally sat quietly on the parents lap. The observed association between looks away and mean RR interval and SBP could also indicate that the infants are in a stage of increased calmness, while looking away from the toy. It is however not from the present study possible to know whether the effect of FO on infant attention and BP are two parallel effects or two reflections of a common underlying effect. We did not observe any significant correlations between the change in free-play scores and change in mean RR interval. But it is interesting that the only variable showing an association was a change in number of looks away which tended to be correlated with change in mean RR interval. The lack of significant associations between mean RR interval and free-play scores could be due to the lower number of HR assessments and thus a decrease in power and an increase in the possibility of type II errors.

Past work from the present study, showed that the 3 months of fish oil-supplementation reduced SBP and increased mean RR interval in the infants [15;16]. In addition, Forsyth et al. [23] found a blood pressure lowering effect in later childhood after supplementation with LCPUFAs in early infancy and maternal fish oil supplementation has also been shown to reduce fetal HR [24]. Additionally, Pivik et al. [12] found increased HR and decreased HRV in infants who had no supplement with DHA from formula compared to supplemented or breast-fed infants. This indicates that DHA could be an important factor in programming the autonomic regulation of HR via increased parasympathetic activity. A hypothesis could thus be that the effect of FO on mean RR interval (and BP) could be secondary to an effect of DHA in the autonomic nervous system. Other studies have found correlations between mean RR interval, HRV and cognitive function such as attention, social skills and IQ [25–28]. Previous studies have also argued about a possible early relation between autonomic nervous system functions and cognition and a potential bidirectional link between these [26,29,30]. *E.g.* a relationship between DHA, cognition/attention and HR via the autonomic nervous system has been suggested by Gustafson et al. [29]. This is concordant with a Swedish observational study with 248 healthy school children which found a correlation between psychological health and an early predictive marker for CVD (endothelial function) [31]. Several studies have found effects of *n-3* LCPUFA on HR (single measure, average of multiple resting measures, or 24-h measure) [32] and BP in adults [33], which ultimately could be due to similar effects.

The level of RBC-DHA measured at 9 months of age reflects the intake of DHA through breast-milk, infant formula and fish in the diet. The relatively large variation in the level of RBC-DHA at 9 months in the present study could result from diversity in the diet during weaning such as length of breastfeeding and timing of the introduction of fish in the complementary feeding. Formulas on the Danish market at the time of the study did not contain any LCPUFA. In a double-blinded controlled randomized study with healthy infants born at term, Schwartz et al. [34] found that the plasma LCPUFA concentration decreased continuously during the transition from breastfeeding or infant formula to the replacement with complementary feeding. Schwartz et al. [34] further found that an intervention with *n-3* fatty acid-rich oil (in form of α-linolenic acid) increased the content of *n-3* LCPUFA in plasma, also reflected in plasma DHA. A large European cross-sectional study shows that Danes have a high intake of fish compared to other European countries [35]. This may have affected the outcomes of the intervention since infants with a low RBC-DHA level may not have been represented adequately.

A limitation in the current study is that the infants had not eaten for several hours when they were tested with the single object free-play task and also had to undergo various physical examinations before and after the free-play test such as determination of blood pressure, anthropometric assessments and blood sampling. Several of the infants appeared tired during the tests which could have had an influence on their motivation and thus on their performance in the test. One could speculate that more significant results had been obtained under different circumstances. Another limitation is the reduced reliability of the behavioral scores since they were only quantified by one experimenter. However quantification was done in a very thorough manner and a number of the infants were scored several times to minimize the intrapersonal variation. The observed number of unspecified looks away from the toy during testing episodes were low and in that context the effect size is considered to be substantial although as before mentioned not easy to interpret. Despite these limitations it is the first randomized study of its kind and it brings new results about a possible relationship between cognitive and cardiovascular functions.

In conclusion, we found that fish oil-supplementation to infants in the second half of infancy has an effect on their attention scores in a free-play test. Furthermore, the observed cognitive effect of *n-3* LCPUFA occurred in parallel with the cardiovascular effects, which makes us hypothesize that the effect of *n-3* LCPUFA on both functions could be related via a common effect in the autonomic nervous system. This underlines the importance of further studies to explore if the effects of fish oil are transient or could have beneficial long-term effects. Further studies are also needed to clarify the reliability and importance of the found effects.

Contributions

The study was designed by LL, who consulted with KK and JHC for the free-play testing and scoring and the mean RR interval assessment, respectively. HLH have performed the scoring of the free-play test results, the statistical analysis and the drafting of the manuscript under supervision by LL and LBSH. All authors took part in the final interpretation of the results and approved of the final manuscript.

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