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Neither n–3 Long-Chain PUFA Supplementation of Mothers through Lactation nor of Offspring in a Complementary Food Affects Child Overall or Social-Emotional Development: A 2 × 2 Factorial Randomized Controlled Trial in Rural Ethiopia

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Abstract

Background: The n–3 (ω-3) long-chain polyunsaturated fatty acid (LC-PUFA) docosahexaenoic acid (DHA) is essential for optimal brain development. There is a lack of evidence on the effect of postnatal n–3 LC-PUFA supplementation on child development in low-income countries.

Objective: We evaluated the efficacy of fish-oil supplementation through lactation or complementary food supplementation on the development of children aged 6–24 mo in rural Ethiopia.

Methods: We conducted a double-blind randomized controlled trial of n–3 LC-PUFA supplementation for 12 mo using fish-oil capsules [maternal intervention: 215 mg DHA + 285 mg eicosapentaenoic acid (EPA)] or a fish-oil–enriched complementary food supplement (child intervention: 169 mg DHA + 331 mg EPA). In total, 360 pairs of mothers and infants aged 6–12 mo were randomly assigned to 4 arms: maternal intervention and child control, child intervention and maternal control, maternal and child intervention, and maternal and child control. Primary outcomes were overall developmental performance with the use of a culturally adapted Denver II test that assesses personal-social, language, fine-motor, and gross-motor domains and social-emotional developmental performance using the Ages and Stages Questionnaire: Social Emotional at baseline and at 6 and 12 mo. We used mixed-effects models to estimate intervention effects on developmental performance over time (intervention × time interaction).

Results: The evolution in overall and social-emotional developmental performance over time did not differ across study arms (intervention × time: $F = 1.09$, $P = 0.35$, and $F = 0.61$, $P = 0.61$, respectively). Effects did not change after adjustment for child age, birth order, and nutritional status; maternal age and education; wealth; family size; and breastfeeding frequency. Children's developmental performance significantly decreased during study follow-up (β: −0.03 SDs/mo; 95% CI: −0.04, −0.01 SD/mo; $P < 0.01$).

Conclusions: n–3 LC-PUFA supplementation does not affect overall or social-emotional development of children aged 6–24 mo in a low-income setting. Follow-up of the cohort is recommended to determine whether there are long-term effects of the intervention. This trial was registered at clinicaltrials.gov as NCT01817634. *J Nutr* 2019;149:505–512.

Keywords: fish oil, docosahexaenoic acid, child development, breastfeeding, complementary feeding, developing country

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Introduction

DHA is an n–3 long-chain PUFA (LC-PUFA) essential for the structural and functional development of the brain (1, 2). DHA deficiency during the brain growth spurt has been shown to exert deleterious effects on learning, mood, and motor development in animals (3, 4).

Human milk is the predominant source of n–3 LC-PUFAs for infants and young children in low- and middle-income countries (LMICs) (5–7). However, the adequacy of the DHA supply remains a concern because breast-milk DHA concentrations, which largely depend on the maternal diet, show considerable variations across and within populations (8–10). Mothers living far from coastal areas have limited access to dietary DHA sources to ensure the recommended intake of 200 mg DHA/d during lactation, which could be achieved by consuming 1–2 portions of sea fish/wk (6, 11–13). Furthermore, with the introduction of complementary foods, children in LMICs gradually shift from dependence on breast milk to a complementary diet that is often very low in DHA (6, 14). Although DHA can be synthesized de novo from its precursor, α-linolenic acid (18:3n−3), the conversion is typically very low and is further hampered by a high dietary n–6 to n–3 PUFA ratio and common genetic polymorphisms (15, 16). Studies have confirmed that infant DHA status can be more efficiently improved by supplementation with preformed DHA compared with α-linolenic acid (17).

Observational studies have shown that breastfed compared with formula-fed children have better neurodevelopment, which has been attributed to the lack of preformed n–3 LC-PUFAs in previous infant formulas (18, 19). However, it remains uncertain whether n–3 LC-PUFA supplementation of children born at term can have neurodevelopmental benefits because systematic reviews of randomized controlled trials did not produce any conclusive evidence (20–24). Furthermore, it is currently still unclear whether these findings from predominantly high-income countries can be extrapolated to children living in LMICs. LMICs have a higher prevalence of other developmental risk factors, such as intrauterine growth restriction, stunting, inadequate stimulation, infections, and poor water and sanitation conditions (25). Some studies have reported differential responses to n–3 LC-PUFA supplementation in which subgroups with a higher developmental risk—such as very-low-birth-weight infants and children with reduced caregiver interactions and psychomotor stimulation—benefited more from supplementation (26–28). This might suggest that any neuroprotective effects of n–3 LC-PUFA supplementation could be more pronounced among children living in LMICs. To date, only one clinical trial in The Gambia evaluated n–3 LC-PUFA supplementation of breastfed infants in LMICs and found no benefit on cognitive development (29). However, the infants had a rather adequate dietary n–3 LC-PUFA supply, and breast-milk DHA concentrations of mothers were surprisingly high and matched those of populations with high fish consumption.

Ethiopia is a landlocked country and has been estimated to have the lowest dietary DHA intake in the world (i.e., 7.0 mg/d per capita) (11). The median DHA intake from complementary foods in children aged 6–36 mo is estimated at 1.1 mg/d, which is negligible compared with the worldwide median of 14.6 mg/d (14, 30). Thus, breastfed children's DHA status is expected to be low due to suboptimal DHA intakes from complementary foods and breast milk. In this study, we hypothesized that an increased intake of n–3 LC-PUFAs would improve developmental performance of breastfed children aged 6–24 mo in a rural setting in Ethiopia. The intervention was delivered through 2 channels: *1*) supplementation of children with a complementary food supplement enriched with fish oil and/or *2*) supplementation of their lactating mothers with fish-oil capsules.

Methods

Subjects and measurements

This study was a randomized, double-blind, placebo-controlled trial that assessed the independent and combined effects of fish-oil (n–3 LC-PUFA) supplementation of lactating mothers using fish-oil capsules (maternal intervention) and their breastfed children using a fish-oil–enriched complementary food supplement (child intervention) (31). The trial was conducted from November 2013 to February 2015 in the districts of Deneba, Assendabo, and Serbo of Jimma Zone, Ethiopia. Previous dietary surveys conducted in the study area showed that a negligible number of infants and young children consumed fish, whereas 95% of children continued breastfeeding throughout the second year of life (32). Children and their lactating mothers were randomly assigned to receive either the fish oil (intervention) or a placebo control without fish oil. Thus, there were 4 study arms: both mother and child received the fish-oil intervention (MCI), only the mother received the fish-oil intervention and her child received a placebo control (MI), only the child received the fish-oil intervention and the mother received a placebo control (CI), and both mother and child received the placebo control (C). Study participants, development assessors, and researchers remained blinded to the intervention allocation until the end of data analysis (31).

All of the mothers with infants aged 6–12 mo living in the study districts were invited to attend a screening session. Inclusion criteria included infants who were singleton, currently breastfed, not wasted (weight-for-length z score ≥ -2 SDs), and no bilateral pitting edema, and whose mother had no plan to leave the study area for >1 mo. Exclusion criteria included chronic illness, taking other nutritional supplements, and infants with a congenital abnormality or severe anemia (hemoglobin <7.0 g/dL). Eligible mothers were asked for their written consent after an information session detailing the study, voluntary participation, and study withdrawal.

On the day of study enrollment, a researcher opened the next sealed randomization envelope in the presence of an eligible mother-child pair (in order of arrival) and provided them with the first month's supply of supplements. The maternal supplements were airtight soft-gel oil capsules, which, at a daily dose of 2 capsules, provided either 500 mg n–3 LC-PUFAs (intervention: fish oil providing 215 mg DHA + 285 mg EPA) or no n–3 LC-PUFAs (control: corn oil). The intervention and control capsules were identical in appearance (Biover NV). The child complementary food supplements were extruded corn-soy blends

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: ASQ-SE, Ages and Stages Questionnaire: Social Emotional; C, control (both mother and child received placebo); CI, child intervention (child received fish oil and mother received placebo); Denver II-Jimma, culturally adapted and standardized Denver II Developmental Screening Test; LC-PUFA, long-chain PUFA; LMIC, low- and middle-income country; MCI, maternal and child intervention (both mother and child received fish oil); MI, maternal intervention (mother received fish oil and child received placebo); TF, total fat.

fortified with 19 micronutrients that were either enriched with a daily dose of 500 mg n–3 LC-PUFAs (intervention: fish oil providing 169 mg DHA + 331 mg EPA) or not enriched with n–3 LC-PUFAs (control: corn oil) (Michiels Fabrieken NV and Fortitech, Inc.). The intervention was provided for a total duration of 12 mo; hence, children's ages ranged from 6 to 24 mo. Community workers—female high school graduates recruited from the community and trained—conducted weekly home visits to complete compliance assessments.

The primary study outcomes were the performance score on overall development using a culturally adapted and standardized Denver II Developmental Screening Test (Denver II-Jimma), which assesses personal-social, language, fine-motor, and gross-motor developmental domains, and the performance score on social-emotional development using an adapted Ages and Stages Questionnaire: Social Emotional (ASQ-SE) (33). Secondary outcomes included performance scores on the individual Denver II developmental domains (i.e., personal-social, fine-motor, language, and gross-motor development) to evaluate effects on different aspects of child development and the risks of suspected global developmental delay (using Denver II-Jimma) and social-emotional developmental delay (using ASQ-SE) to evaluate effects on the proportion of children with poor developmental performance. Outcomes were assessed at baseline, midline (after 6 mo), and endline (after 12 mo) of the intervention. The Denver II-Jimma has 125 test items that evaluate a child's skills in 4 domains of development (personal-social, fine-motor, language, and gross-motor). The number of test items to be administered per domain depends on the age and performance of the child as described in the official Denver II manual (34). Some of the test items were assessed by maternal report ("report item"), whereas others required an observation of the child carrying out the task ("test item"). Test items were scored as "pass," "fail," "refusal," or "no opportunity." "Refusal" indicates that a child refused to attempt a "test item" 3 times, and "no opportunity" indicates that a child did not have the chance to perform a "report item" at home. Children's skills in overall development and for each developmental domain were then evaluated by applying a scoring approach on a continuous scale. Children's performance scores were calculated as the ratio of the total number of passed test items to the total number of test items expected to be passed by ≥75% of same-age children in the Ethiopian benchmark (33). Children were also assessed for risk of suspected global developmental delay by categorizing each child as "normal," "suspect," or "untestable" according to the criteria in the official Denver II manual (**Supplemental Table 1**). The ASQ-SE evaluates a child's skills in social-emotional development and therefore complements the Denver II-Jimma. The ASQ-SE tool contains age-specific questionnaires including for the age ranges of 3–8, 9–14, 15–20, and 21–26 mo (35). Each questionnaire contains a set of questions that refer to critical adaptive and maladaptive behaviors for the target age interval and focuses on skills related to attachment, autonomy, and self-development. The ASQ-SE questionnaire was completed by asking the mother how frequently her child performed a given behavior (i.e., "most of the time," "sometimes," or "rarely/never"), which were each assigned a score. Social-emotional performance scores were then calculated as the ratio of the sum of all scores over the maximum attainable score on the age-specific questionnaire, with a higher value indicating poor developmental performance. Each child was also screened for suspected social-emotional developmental delay using the recommended age-specific cutoffs (Supplemental Table 1) (35). Two clinical nurses conducted the developmental tests throughout the study after receiving 1 mo of intensive training, with practical sessions and standardization exercises, and a refresher training before the start of midline measurements. Two experienced developmental psychologists provided the training and alternately supervised data collection in the field. Both nurses worked in all of the 3 study districts and were randomly assigned to perform an assessment in order of children's arrival at the sites. Assessments were conducted in quiet and convenient rooms and took ~30–45 min/child. Assessments were postponed to the next day when a child was sick or refused to start the test.

Institutional review board approval was obtained from Jimma University in Ethiopia, the ethics committee of the Ghent University Hospital in Belgium (registration number: B670201214299), and the National Health Research Ethics Committee of Ethiopia. The study supplements were approved by the Food, Medicine, and Health Care Administration and Control Authority of Ethiopia. The trial was registered at clinicaltrials.gov as NCT01817634.

Statistical analysis

A sample size of 360 subjects (90 subject/study arm) was required to detect an effect size of 0.038 SDs in monthly change of the overall and social-emotional developmental performance score over 12 mo of intervention follow-up, assuming an autocorrelation of 0.50, 80% statistical power, and a type I error of 5% and taking into account an anticipated 20% attrition rate (36). This effect size on the monthly change in child development is equivalent to an effect size of 0.12 SDs after 12 mo of intervention using an estimated SD of 0.03 for the mean monthly change in child development and an SD of 0.12 for the mean developmental score (37). These estimates are derived from a study conducted in a similar population in the study area using the same Denver II tool (38). Data were entered in duplicate using EpiData version 1.4.4.4 (EpiData Association), and consistency checks and statistical analysis were conducted using Stata version 13.1 (StataCorp). Developmental performance scores were standardized to *z* scores on the basis of the distribution of the data. The Denver II-Jimma–derived risks of suspected developmental delay were categorized as either "normal" or "suspect," with the latter including the "untestable" category.

The effect of the interventions was assessed with mixed-effects linear regression models for the continuous outcomes and mixed-effects linear probability models for the binary outcomes, with child identifier as random intercept. The use of linear probability models for binary outcomes is well established and allows for a straightforward interpretation of the average intervention effect expressed as risk difference using percentage points (39). Fixed effects in the models included study arm (C, MI, CI, or MCI), intervention time (in months), and child sex. Child sex was added due to an imbalance among study arms at baseline. We evaluated the interventions' effects on the evolution of child developmental performance taken over the 3 measurement rounds. For this purpose, we tested interaction terms between study arms and intervention time that estimate the difference between each intervention arm and the control arm on monthly changes in an outcome over time.

As a secondary analysis, we analyzed the intervention effect adjusted for relevant time-invariant covariates such as child age, birth order, and length-for-age *z* score at enrollment; maternal age and education; household wealth and family size; and frequency of breastfeeding at baseline. We further explored if there were any effect modifications of the intervention by our chosen covariates by testing triple interaction terms between study arm, intervention time, and a covariate. Analyses were performed by the intention-to-treat principle (i.e., including all children initially enrolled into the study). For this purpose, we conducted multiple imputations of missing data using chained equations under the missing-at-random assumption. Fifty imputations of missing data were conducted to estimate the regression coefficients. All of the tests were 2-sided, and the level of significance (α) was set at 0.05.

Results

The numbers of mother-child pairs who were screened for eligibility, randomly assigned, and lost to follow-up are presented in **Figure 1**. A total of 360 mother-child pairs were randomly assigned to the study arms and 329 (91%) attended either the midterm or the endline developmental test. Eighty-seven percent of the participants received all 12 distributions of the supplements. Baseline characteristics of study participants were comparable across the study arms, except for an imbalance in child sex (**Table 1**).

There was no significant difference between the study arms on the evolution of children's performance score over

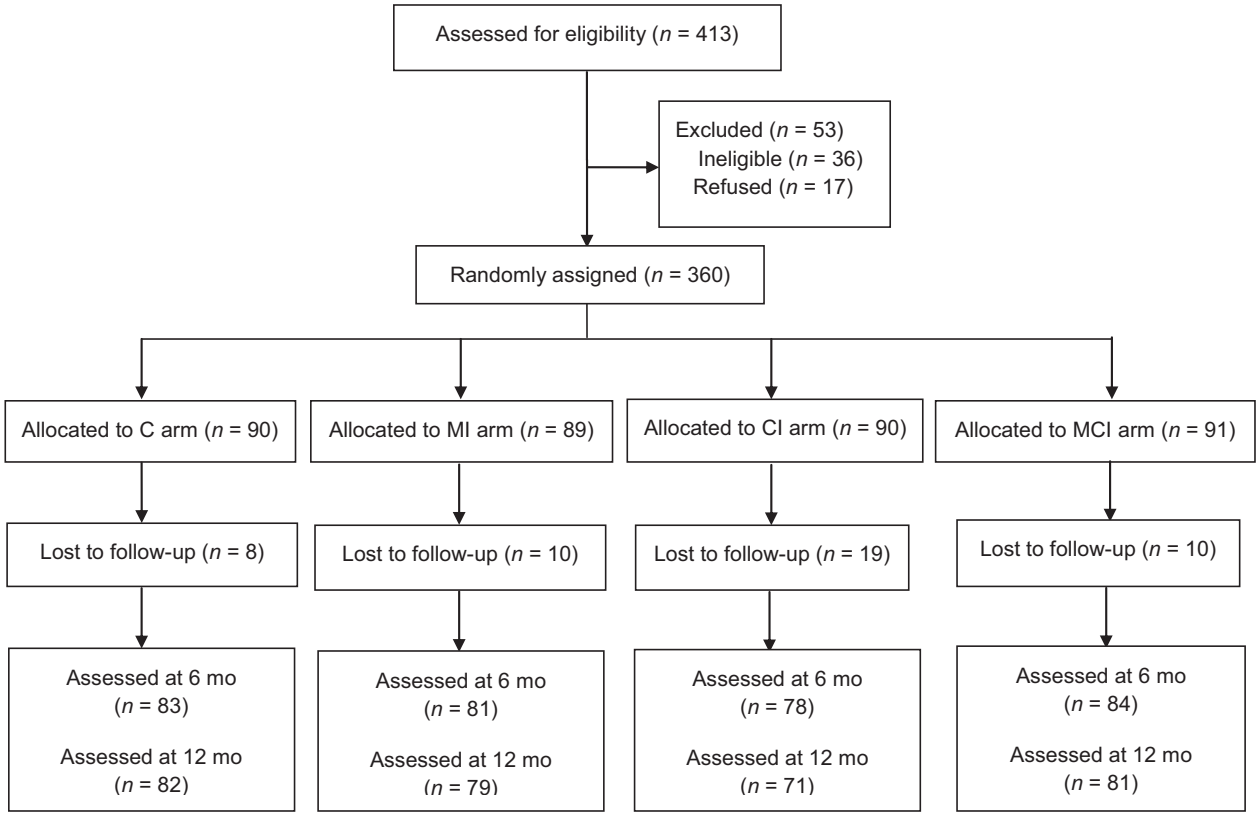

FIGURE 1 Trial flowchart. C, control (both mother and child received placebo); CI, child intervention (child received fish oil and mother received placebo); MCI, maternal and child intervention (both mother and child received fish oil); MI, maternal intervention (mother received fish oil and child received placebo).

time for both overall development using the Denver II-Jimma (intervention × time: $F = 1.09$, $P = 0.35$) and social-emotional development using the ASQ-SE (intervention × time: $F = 0.61$, $P = 0.61$) (Table 2). Similarly, we found no significant effect of any of the interventions on developmental performance scores on the personal-social, fine-motor, language, and gross-motor domains as well as on the risks of suspected global developmental delay (using Denver II-Jimma) and social-emotional developmental delay (using ASQ-SE). The interventions' effects on developmental outcomes remained unaffected when we adjusted the analysis for relevant covariates such as child age, birth order, and length-for-age z score at enrollment; maternal age and education; household wealth and family size; and frequency of breastfeeding at baseline. There was no significant interaction between the study arms and our chosen covariates on the developmental outcomes.

The age-adjusted overall developmental performance of children significantly decreased with time during the study follow-up (β: −0.03 SD/mo; 95% CI: −0.04, −0.01 SD/mo; $P < 0.01$). The prevalences of suspected global developmental delay and social-emotional developmental delay were 19.2% and 67.5% at baseline, 19.7% and 60.6% at midline, and 20.6% and 41.7% at the endline of the study, respectively.

Discussion

We previously showed that supplementation with n–3 LC-PUFA–rich fish oil through lactation and/or a child complementary food supplement significantly increased child blood DHA concentrations by 15–20% and decreased the ratio of arachidonic acid (20:4n−6) to DHA+EPA by 22–35% (31). However, the results of the current study showed no apparent benefit of the supplementation on child developmental performance assessed using the Denver II-Jimma and ASQ-SE tests.

The current study is 1 of 2 trials conducted in an LMIC setting that reports on the impact of postnatal n–3 LC-PUFA supplementation on child development. Another trial in The Gambia found no benefit of direct fish-oil supplementation of breastfed infants from ages 3–9 mo on cognitive performance using the Willatts' infant planning test (29). Several randomized studies in developed countries have examined the effects of n–3 LC-PUFA or DHA supplementation of lactating mothers or infant formulas on different aspects of child neurocognitive and behavioral development. Although some of these studies reported beneficial effects of supplementation on some aspects of child development, others studies that used similar or other developmental tools found no differences between intervention and control groups (20–24). Campoy et al. (21) and Meldrum et al. (40) proposed that the mixed results from previous trials could be due to heterogeneity among studies with regard to the timing, duration, and dose of supplementation; the methods used for outcome assessment; the age of children at outcome measurement; inadequate sample size; or potential differences in the populations studied, such as genetic polymorphisms possibly affecting DHA requirements and concentrations in breast milk.

The interpretation of the results of the current study should therefore consider the age at which supplementation was provided (i.e., 6–24 mo). The intervention did not cover the entire period of expected higher brain sensitivity to dietary

TABLE 1 Baseline characteristics of study children and their mothers by study arm[1]

Characteristics	C ($n = 90$)	MI ($n = 89$)	CI ($n = 90$)	MCI ($n = 91$)
Child sex (female), %	46.7	55.1	40.0	59.3
Child age, mo	8.89 ± 2.16	9.18 ± 2.09	8.93 ± 2.10	8.68 ± 2.00
Maternal age, y	26.0 ± 5.04	25.8 ± 4.82	26.1 ± 5.48	26.3 ± 5.28
Maternal education, %				
No formal education	46.7	49.4	51.1	52.7
Primary education	41.1	34.8	34.4	36.3
Secondary and above	12.2	15.7	14.4	11.0
Household wealth tertiles, %				
Lowest	30.0	38.2	31.1	34.1
Middle	32.2	25.8	38.9	36.3
Highest	37.8	36.0	30.0	29.7
Child weight, kg	8.18 ± 1.15	8.15 ± 1.10	8.05 ± 1.08	7.93 ± 1.10
Maternal height, cm	157 ± 4.67	157 ± 5.55	157 ± 5.95	157 ± 5.74
Maternal BMI, kg/m^2	20.2 ± 2.41	20.3 ± 2.50	20.1 ± 2.57	21.0 ± 3.31
Breastfeeding frequency, %				
4–6 times/d	11.1	6.74	12.2	8.89
7–9 times/d	23.3	31.5	28.9	25.6
≥10 times/d	65.6	61.8	58.9	65.6

[1]Values are means ± SDs unless otherwise indicated. C, control (both mother and child received placebo); CI, child intervention (child received fish oil and mother received placebo); MCI, maternal and child intervention (both mother and child received fish oil); MI, maternal intervention (mother received fish oil and child received placebo).

DHA. Rapid brain DHA accretion coincides with the brain growth spurt that spans from the beginning of the third trimester of pregnancy to the second year of life, with the majority accumulating before the first 12 mo of life (41, 42). This may suggest that supplying DHA prenatally and to younger infants could influence brain DHA status and associated functional outcomes more. On the other hand, potential impacts in older infants and young children cannot be excluded because the human brain remains sensitive to dietary DHA throughout its protracted developmental phase (43). Increased brain DHA exposure during this time may benefit the late-maturing dopamine system of the prefrontal cortex, which is selectively influenced by DHA (44) and which reaches peak maturation toward the end of the first year and reaches functional maturity at ~12–15 mo of age and beyond (45, 46). For instance, a DHA supplementation study in older children aged 8–10 y showed a significantly higher functional activity of the prefrontal cortex in the supplemented group, and erythrocyte DHA composition was inversely correlated with reaction time in a sustained attention task (47).

Another important point requiring consideration is that the Denver II-Jimma and ASQ-SE tools might not be adequately sensitive to detect subtle effects of dietary DHA on specific brain functions (48, 49). DHA selectively concentrates in the prefrontal cortex of the brain region, where cognitive processes involving attention regulation and components of short-term and working memory take place (44, 46). Thus, tools assessing specific cognitive abilities mediated by the prefrontal cortex, such as attention and problem solving, might have been more informative about the effects of increased dietary DHA intake on brain function (49). Furthermore, the impacts of early DHA exposure could be more evident on cognitive abilities that emerge at a later age. On the other hand, global developmental tests taken before the age of 2 y have been shown to have limited predictive validity for later childhood cognitive and behavioral performance (50, 51). Jensen et al. (52, 53) reported benefits of maternal supplementation with 200 mg DHA/d during lactation on child development at 2.5 and 5 y of age but did not find any impact during infancy compared with a control group. Others found that the addition of DHA to infant formula was associated with better performance on several tasks, reflecting discrete aspects of cognitive functions between the ages of 3 and 6 y. However, no impact of this enriched infant formula was detected using standardized developmental tasks at the age of 18 mo (54).

Children from the control arm of this study still received DHA through breast milk. As such, this could have diluted the differential impact between the intervention and control arms. This contextual element is important to highlight when comparing the study results with previous findings from randomized controlled trials conducted in weaned infants in which control infants received formulas devoid of preformed n–3 LC-PUFAs. A review by Lauritzen et al. (55) suggested that DHA supplementation is more likely to result in an impact on developmental outcomes when children's basal DHA intake is <70 mg/d. From the analysis of baseline breast-milk samples we derived a mean DHA concentration of 74.0 mg/L (31). With breast milk being the sole predominant source of DHA in this setting and assuming an average breast-milk intake of 650 mL/d (56), we estimate that the study children's average DHA intake from breastfeeding would not exceed 50 mg/d. We therefore do not expect that this low DHA breast-milk concentration would have masked any potential effects of n–3 LC-PUFA supplementation in the current study.

Often, the inadequacy of the studied dose or poor compliance to the intervention is an argument to explain the lack of impact of supplementation trials on functional outcomes. In the current study, the median (IQR) compliance (i.e., the ratio of actual supplement consumption over prescribed consumption) was 79.7% (62.6–91.4%) for the child complementary food supplement and 69.9% (52.2–80.4%) for the maternal capsules, with no significant difference between the study arms. The frequency of breastfeeding remained high throughout follow-up, with an average frequency of ≥7 times/d in 84.2% of the participants (31). The n–3 LC-PUFA dose amounts for the maternal and child supplements used were determined

TABLE 2 Developmental performance score and suspected developmental delay in children, by measurement round and study arm[1]

Outcomes and group	Baseline	Midterm	Endline	P^2	P^3
Developmental performance score					
Overall development				0.351	0.312
C	1.14 ± 0.12	1.14 ± 0.09	1.10 ± 0.07		
MI	1.12 ± 0.12	1.12 ± 0.09	1.10 ± 0.07		
CI	1.12 ± 0.12	1.12 ± 0.08	1.08 ± 0.06		
MCI	1.13 ± 0.13	1.13 ± 0.10	1.11 ± 0.07		
Social-emotional				0.607	0.648
C	0.35 ± 0.23	0.32 ± 0.20	0.22 ± 0.15		
MI	0.38 ± 0.24	0.27 ± 0.22	0.23 ± 0.17		
CI	0.35 ± 0.24	0.32 ± 0.20	0.25 ± 0.15		
MCI	0.35 ± 0.22	0.30 ± 0.23	0.21 ± 0.15		
Personal-social				0.796	0.747
C	1.31 ± 0.23	1.28 ± 0.23	1.17 ± 0.11		
MI	1.28 ± 0.23	1.23 ± 0.18	1.18 ± 0.11		
CI	1.27 ± 0.22	1.25 ± 0.19	1.17 ± 0.10		
MCI	1.32 ± 0.23	1.29 ± 0.21	1.20 ± 0.10		
Fine-motor				0.720	0.771
C	1.15 ± 0.17	1.09 ± 0.09	1.07 ± 0.07		
MI	1.12 ± 0.18	1.08 ± 0.10	1.07 ± 0.07		
CI	1.11 ± 0.16	1.07 ± 0.09	1.06 ± 0.07		
MCI	1.11 ± 0.21	1.09 ± 0.09	1.08 ± 0.07		
Language				0.124	0.131
C	1.14 ± 0.20	1.10 ± 0.11	1.08 ± 0.16		
MI	1.10 ± 0.16	1.08 ± 0.10	1.08 ± 0.16		
CI	1.13 ± 0.20	1.08 ± 0.12	1.03 ± 0.13		
MCI	1.16 ± 0.20	1.09 ± 0.12	1.09 ± 0.16		
Gross-motor				0.787	0.851
C	1.04 ± 0.12	1.13 ± 0.11	1.08 ± 0.08		
MI	1.04 ± 0.12	1.12 ± 0.13	1.08 ± 0.08		
CI	1.04 ± 0.11	1.12 ± 0.10	1.07 ± 0.08		
MCI	1.04 ± 0.10	1.11 ± 0.14	1.09 ± 0.08		
Suspected developmental delay					
Global[4]				0.235	0.258
C	21.1	12.2	22.2		
MI	16.9	22.5	18.0		
CI	18.9	22.2	26.7		
MCI	19.8	22.0	15.4		
Social-emotional[5]				0.777	0.811
C	67.8	67.8	38.9		
MI	71.9	52.8	42.7		
CI	64.4	64.4	44.4		
MCI	65.9	57.1	40.7		

[1] Values are means ± SDs of developmental performance score and proportions of children with suspected developmental delay. Sample sizes: C, $n = 90$; MI, $n = 89$; CI, $n = 90$; and MCI, $n = 91$. C, control (both mother and child received placebo); CI, child intervention (child received fish oil and mother received placebo); Denver II-Jimma, culturally adapted and standardized Denver II Developmental Screening Test; MCI, maternal and child intervention (both mother and child received fish oil); MI, maternal intervention (mother received fish oil and child received placebo).
[2] P values for the intervention effect on the evolution of an outcome over time (intervention × time interaction), estimated from mixed-effects linear models for continuous outcomes and mixed-effects linear probability models for binary outcomes with child identifier as random intercept and adjustment for child sex.
[3] P values for the intervention effect, estimated from models adjusted for child sex and fixed-effects covariates including child age, birth order, and length-for-age z score at enrollment; maternal age and education; household wealth and family size; and frequency of breastfeeding.
[4] Suspected global developmental delay assessed by using Denver II-Jimma.
[5] Suspected social-emotional developmental delay assessed by using the Ages and Stages Questionnaire: Social Emotional.

on the basis of the trade-off between supplying an adequate amount of n–3 LC-PUFAs and avoiding theoretically possible adverse effects of a high-dose of n-3 LC–PUFAs, including the risk of immunosuppression among the children exposed to both maternal and child interventions. Reviews of previous randomized controlled trials of infant formula supplementation recommend a dose of ≥0.32% DHA of total fat (TF; grams per 100 g TF) to target developmental outcomes, an amount based on the global average human-milk concentration (40, 57). The dose of DHA in the child intervention complementary food supplement in our study was estimated to be 0.56% TF using the total dietary fat requirement of 30 g/d for infants

aged 7–12 mo (56). Even when considering compliance in the CI arm (median: 79.0%; IQR: 62.3–89.9%) (31), the dose was ≥0.35% TF in 75% of the children. The dose of DHA in the maternal intervention capsules was also adequate to achieve the recommended breast-milk DHA content because a previous dose-response study showed that a dosage of 167 mg DHA/d was able to enrich breast milk to 0.32% TF in lactating mothers consuming a very low n–3 LC-PUFA diet similar to a vegetarian diet (58). Furthermore, we addressed the potential for a lack of impact due to an inadequate dose by including a study arm who received the combined interventions.

We found a high risk of suspected developmental delay among children in our study sample. In addition, the age-adjusted developmental performance scores of children declined during the study follow-up. Poor nutrition and suboptimal child care and stimulation practices have been identified as important contributors of poor developmental performance in LMICs (59, 60). In a recent randomized controlled study in our study area, a home-based stimulation intervention was shown to significantly improve developmental performance in high-risk children aged <5 y (38). Therefore, there is a critical need for identifying appropriate interventions that integrate both nutrition and stimulation interventions to mitigate the developmental risks in this and other child populations.

This study has a few strengths and limitations that need to be addressed. First, we used the Denver II instrument because it has been culturally adapted and standardized to the local context by a previous study conducted in the same study area (33). Work by Rubio-Codina et al. (61) showed that this shorter tool can be used as a valid and highly feasible substitute of the more-lengthy Bayley Scales of Infant and Toddler Development for large-scale field-setting studies in LMICs. On the other hand, tests of specific cognitive tasks that can be mechanistically and theoretically related to the role of DHA on brain function may have provided more understanding of an impact of the intervention. However, the feasibility of administering such tests in young children in our study was limited by the field setting and the relatively large sample size. Second, we did not monitor dietary intake other than compliance to the intervention supplements. However, previous dietary surveys found that the consumption of fish and other sea foods was very rare in the study area (32). Finally, we did not assess the impact of n–3 LC-PUFA supplementation in infants aged <6 mo because of the universal recommendation of exclusive breastfeeding in this age group and the specific design of this study (i.e., the assessment of the concomitant impact of n–3 LC-PUFA supplementation through complementary foods and breast-milk enrichment).

In conclusion, this study did not find evidence of a positive impact of n–3 LC-PUFA supplementation on child developmental performance in breastfed children in a low-income setting. Assessing long-term effects of early dietary n–3 LC-PUFA exposure on later cognitive and behavioral skills in this population is warranted.

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