

Effect of Fish-oil Supplementation During Lactation on Maternal Milk Long-chain Polyunsaturated Fatty Acids Concentration: Results From a Sub-study of a Randomized Controlled Trial in Rural Ethiopia

Alemayehu Argaw (✉ yemariamwork2@gmail.com)

Jimma University College of Public Health and Medical Sciences <https://orcid.org/0000-0003-3982-5408>

Kimberley P Bouckaert

Universiteit Gent

Mekitie Wondafrash

Jimma University

Patrick Kolsteren

Universiteit Gent

Carl Lachat

Universiteit Gent

Bruno De Meulenaer

Universiteit Gent

Giles Hanley-Cook

Universiteit Gent

Lieven Huybregts

International Food Policy Research Institute

Research

Keywords: Human Milk, Docosahexaenoic Acid, Arachidonic Acid, Lactation, Polyunsaturated Fatty Acids, Low Income Countries

Posted Date: June 10th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-33686/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Human milk (HM) is the main source of the long-chain polyunsaturated fatty acids (LCPs) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) for infants and young children in low- and middle-income countries. While the AA concentration in HM seems rather stable, the concentration of DHA is highly variable and influenced by the maternal intake of marine foods. Mothers in low-income settings living far from coastal areas have limited access to marine foods which may put their children at risk of inadequate omega-3 (n-3) LCPs intake.

Objective: In a sub-study of an individually randomized controlled trial, we evaluated the efficacy of fish-oil supplementation of lactating mothers on HM n-3 LCPs concentrations in a rural setting in Ethiopia.

Methods: Mothers (n = 360) with children 6-12 months old were randomized to receive either an intervention fish-oil capsules [FO: 215 mg DHA + 285 mg EPA] or a control corn-oil capsules [CO: without n-3 LCPs] for 12 months. In a random subsample of 154 participants, we analyzed LCPs in HM and child capillary blood using gas chromatography at baseline, and at 6 and 12 months of the intervention.

Results: Compared to the control, FO supplementation increased HM concentrations of DHA by 39.0% ($P < 0.001$) and EPA by 36.2% ($P < 0.001$), whereas the AA/(DHA + EPA) ratio decreased by 53.5% ($P < 0.001$). We also found statistically significant associations between the changes in (DHA + EPA)/AA ratio in the maternal milk and the child capillary blood samples following the supplementation ($P < 0.001$). However, HM DHA concentrations still remained lower than international norms after the fish-oil intervention.

Conclusions: Fish-oil supplementation in lactating mothers improves n-3 LCPs status of HM. It is recommended that future studies evaluate different doses of n-3 LCP and consider the impact of potential effect modifiers such as genetic polymorphism, diet, and others.

Trial registration: ClinicalTrials.gov, NCT01817634. Registered 20 March 2013, <https://clinicaltrials.gov/ct2/show/record/NCT01817634>.

Background

Adequate supply of the long-chain polyunsaturated fatty acids (LCPs) of the omega-3 (n-3 series) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and of the omega-6 (n-6) series arachidonic acid (AA) are essential for normal growth and development (1, 2). DHA and AA accumulate rapidly in the cerebral cortex and retina during the brain growth spurt which takes place during the first 1,000 days since conception (3–5). DHA status of infants was shown to relate with visual and neurocognitive development (6). In addition, LCPs are precursors of eicosanoids and other lipid mediators modulating the expression of genes involved in inflammation processes and other immune functions and metabolic control (7–10).

Human milk (HM) remains the most affordable source of LCPs for infants and young children in low- and middle-income countries as complementary diets in these settings are usually very low in preformed LCPs (11, 12). The global average concentrations of DHA and AA in HM are estimated at 0.37% (SD 0.11) and 0.55% (SD 0.14) in w/w of total lipids (%TL) (13). The concentration of DHA in HM is highly variable and mainly explained by maternal dietary intake of preformed DHA found in fish and other marine foods, whereas the concentration of AA is relatively stable and less affected by AA in the maternal diet (13–15). Expert groups recommend that lactating women should aim for an average daily dietary intake 300 mg DHA + EPA from which 200 mg should come from DHA (16, 17). There are no indications that lactating women with an adequate dietary intake of the precursor linoleic acid (LA) need an additional dietary supply of AA (17). The recommended intake of n-3 LCPs during pregnancy and lactation can be attained by a weekly consumption of 1–2 portions of sea fish (17). For those women without access to marine foods, n-3 LCP supplementation may be required to ensure this recommended intake.

In Ethiopia, the LCPs status of HM have not been documented. The per capita DHA availability in Ethiopian diet was estimated at 7.0 mg/d, which is one of the lowest globally and extremely low to meet the recommended intake during lactation (18). Furthermore, the contribution of complementary foods to DHA intake by Ethiopian children 6–36 months old was estimated to be negligible (1.1 mg/d), compared to the worldwide average (14.6 mg/d) (11).

We conducted the OME³JIM trial, a 2 × 2 factorial randomized controlled trial to evaluate the effects of n-3 LCPs supplementation of lactating women and/or their children 6–24 months of age on child health, growth and development in rural Ethiopia. In a subsample of this trial, we collected HM and child capillary blood samples. The purpose of this sub-study was to evaluate the effect of maternal fish-oil (FO) supplementation on n-3 and n-6 LCPs concentrations in maternal milk and the association between changes in maternal milk and child blood concentrations.

Methods

The OME³JIM trial was conducted from November 2013 to February 2015 in three districts of Jimma Zone in southwest Ethiopia. Subsistence farming is the main form of livelihood in the study districts, and staple crops in the area are very low in n-3 LCPs. Mother-infant pairs with singleton infants of age 6–12 months were enrolled in the main trial if the infant was breastfeeding and not acutely malnourished (weight-for-length z score ≥ -2 SD and no bilateral pitting edema), and the mother had no plan to leave the study area for more than one month during the study period and was willing to participate in the trial. Mother-child pairs were excluded from the trial when the mother or child had a known chronic illness, was taking other nutritional supplements, when the child had a congenital abnormality or severe anemia (hemoglobin < 7.0 g/dL) at enrolment or during study follow-up.

Details of the main OME³JIM trial have previously been published (19, 20). In brief, from a total of 413 mother-infant pairs screened, 360 eligible pairs were enrolled in the main trial (Fig. 1). Study mothers were randomly assigned to either an intervention group that received fish-oil capsules (FO, $n = 180$) or a control

group that received placebo corn-oil capsules (CO, $n = 180$). A random subsample of 168 mother-infant pairs from both study arms was selected for the HM sub-study. HM samples at baseline were available from 154 mothers (n : CO = 82; FO = 72), who were finally considered for this study.

Both the fish-oil and corn-oil capsules were produced as identical airtight soft-gel capsules (Biover NV, Belgium). A daily dose of two intervention fish-oil capsules provided 500 mg/d n-3 LCs (215 mg DHA + 285 mg EPA) whereas the control corn-oil capsules contained no n-3 LCs. Each capsule additionally contained 5 mg of the antioxidant d- α -tocopherol. The intervention was provided for 12 months, with supplements distributed on a monthly schedule and compliance monitored through weekly counts of remaining capsules.

In addition to the maternal intervention, infants aged 6–12 months of the same mothers were individually randomized to either an intervention group that received a food supplement fortified with fish-oil ($n = 181$) or a control group that received the same food supplement without fish-oil ($n = 179$) during the same period. The intervention food supplement contained a daily dose of 500 mg n-3 LCs (169 mg DHA + 331 mg EPA), whereas the control food supplement contained no n-3 LCs.

Sample Collection And Lcp Analysis

HM and child capillary blood samples were collected at baseline, midline (after 6 months) and endline (after 12 months) of the intervention to determine DHA and EPA concentrations. The concentration of AA was additionally considered to evaluate any potential influence of n-3 LCP supplementation on n-6 LCP levels. Before collecting HM samples, mothers were asked to breastfeed their child for a few minutes to establish breastfeeding. Then, study nurses expressed breast milk samples (10–15 mL) manually or by using manual breast pumps into sterile plastic containers with lids. An aliquot of 9 mL homogenized milk sample was pipetted into a 10 mL cryovial containing 1 mL of an 0.01% BHT (2,6-Di-tert-butyl-4-methylphenol) acetone solution for storage, so that to limit lipolytic and oxidative degradation of milk lipids before extraction. Child blood samples were collected using dried blood spot cards. Prior to sample collection, blood spot cards (TFN, Munktell) were impregnated with BHT (2,6-di-tert-butyl-4-methylphenol) to minimize the oxidation of LCs as previously described by Ichihara et al. (21). A large drop of blood from a finger prick was collected on preprinted circles on the spot cards and then, dried overnight at room temperature, and, once dry, inserted into aluminum-coated airtight envelopes with dry desiccants before storage. HM samples and dried blood spot cards were collected in the field in the morning up to noon (between 9:00 AM and 1:00 PM), and transported in cold-chain using cooled bags before storage at $-80\text{ }^{\circ}\text{C}$ in a central laboratory at Jimma University. Samples were later air-shipped on dry ice to a laboratory in Belgium and stored at $-80\text{ }^{\circ}\text{C}$ upon arrival until analyses.

A total lipids extract was prepared from each HM sample using an aliquot of 50 μL homogenized HM according to the Bligh-Dyer method (22), and from each child whole blood sample using a disc of 8 mm diameter punched from the blood spot cards (corresponding to $\pm 21.8\text{ }\mu\text{L}$ blood) (23) according to the method detailed by Bailey-Hall et al. (24). A prior lipid extraction step was performed to guarantee the

extraction of all lipids from the filter paper. We performed saponification with NaOH and methylation into FA methyl esters with BF₃ in methanol. Then, FA methyl esters were separated by gas chromatography with cold-on column injection (0.1 µL, GC-FID 6890N, Agilent Technologies) and a FA methyl ester column (CP-Sil 88, 60 m length, 0.25 mm ID, 0.20 µm film thickness, Agilent Technologies). We used helium as carrier gas (BIP Plus-X50S, Air Products) and applied a programmed temperature gradient (hold at 50 °C for 4 min, increase temperature by 25 °C/min to 225 °C, hold at 225 °C for 25 min). Retention times were compared to the standard (GLC-68 D, Nu-Chek-Prep), and FAs were quantified relative to the 19:0 FA internal standard.

Statistical analysis

For this sub-study, a sample size of 144 participants (72 participants/group) was sufficient to detect an effect size of ≥ 0.44 SDs on HM LCP concentrations between study arms using a statistical power of 80%, a type I error of 5%, an anticipated attrition of 20%, and assuming a $\rho = 0.50$ for the correlation between the midline and endline measurements. 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 14.1 (StataCorp LLC, Texas, USA). The FA data are presented in mg/L HM or blood as the overall fat content of HM and capillary blood samples could not be determined. Data were checked for normality by visual inspection of histograms and Q-Q plots, confirmed by the Shapiro-Wilk test, and log transformed when necessary. Participant characteristics were compared between the HM sub-study sample and the main study sample, and between the FO and CO groups within the HM sub-study sample, using independent-samples t-test for the continuous and Pearson's chi-square test for the nominal variables.

Differences between study groups in HM LCP concentrations at midline and endline measurements was estimated using mixed-effects linear regression models with the study mother as random intercept to account for clustering of repeated measurements at midline and endline. A similar mixed-effects model was used to assess the relationship between the changes in maternal milk and child capillary blood (DHA + EPA)/AA ratios from baseline to midline and endline measurements. For the later analysis, data were analyzed separately for mother-child pairs where children received a FO-fortified complementary food supplement as part of the child intervention of the OME³JIM trial ($n = 81$), and pairs where children received a placebo complementary food supplement ($n = 72$). The difference in slope between the two groups by child intervention arms was compared by adding interaction terms between child intervention group and HM ratios. Models were adjusted for baseline HM LCP concentrations and additional covariates, including household wealth, child age and sex, frequency of breastfeeding, maternal age, height, parity and the occurrence of pregnancy during study follow-up. For the log transformed outcomes, the antilog of coefficients and CIs were used to express group difference as percent of the control group value. A two-sided P value of less than 0.05 was considered statistically significant.

Analyses were conducted using a modified intention-to-treat analysis which included all randomly selected mothers for the HM sub-study from whom breastmilk sample was available at baseline. For this

purpose, a multiple imputations procedure under the missing at random assumption was employed using chained equations of 50 imputations for the lost to follow-up cases at midline and endline. Predictor variables in the imputation models included baseline LCP concentration, duration of breastfeeding, time of measurement (midline/endline), maternal age and body mass index, and child age and sex.

Results

Of the total of 154 mothers who were considered for the HM sub-study at baseline, breastmilk samples were available from 129 (83.8%) and 118 (76.6%) mothers at the subsequent midline and endline follow-up measurements, respectively (Fig. 1). Baseline characteristics of mothers in the HM sub-study ($n = 154$) were similar to those of mothers in the main trial ($n = 360$) except that mothers in the sub-study had slightly younger infants (**Additional file 1**). Within the HM sub-study, the CO and FO groups were comparable in most baseline characteristics. Mothers in the CO group had better household wealth status and were slightly taller (Table 1). At baseline, mothers were on average 25.9 years old (range: 17–40 years), had been nursing for 7.66 months (range: 5.75–12.1 months), and 26.6% were primiparous. Median (IQR) compliance to daily intake of the maternal capsules (i.e., percent of the actual capsules consumed over the prescribed amount of capsules) in the HM sub-study was 71.0% (IQR: 51.3, 80.1%) with no significant difference between study groups [median (IQR): CO = 67.6% (51.1, 79.6%) vs. FO = 71.7% (54.6, 82.6%); $P_{Wilcoxon\ rank-sum\ test} = 0.45$].

Table 1
Baseline maternal characteristics and HM LCP concentrations¹

Characteristics	CO (n = 82)*	FO (n = 72)*
Maternal age, years	25.8 ± 5.43	25.9 ± 4.67
Child age, months	7.6 ± 1.1	7.7 ± 1.3
Child sex, female	37 (45.1)	42 (58.3)
Primiparous	20 (24.4)	21 (29.2)
Marital status, married	78 (95.1)	70 (97.2)
Maternal education		
No formal education	36 (43.9)	41 (56.9)
Primary education	34 (41.5)	19 (26.4)
Secondary and above	12 (14.6)	12 (16.7)
Household head education		
No formal education	26 (31.7)	24 (33.3)
Primary education	37 (45.1)	33 (45.8)
Secondary and above	19 (23.2)	15 (20.8)
Household wealth tertiles		
Lowest	20 (24.4)	33 (45.8)
Middle	35 (42.7)	22 (30.6)
Highest	27 (32.9)	17 (23.6)
Breastfeeding frequency		
4–6 times/day	10 (12.2)	6 (8.33)
7–9 times/day	22 (26.8)	16 (22.2)
≥10 times/day	50 (61.0)	50 (69.4)
Maternal anthropometry		

¹Data are presented as means ± SDs, n (%), or median (P²⁵, P⁷⁵). *The control group (CO) included 41 (50.0%) children, and the intervention group (FO) included 40 (55.6%) children who received an n-3 LCP fortified food supplement as part of the child intervention.

AA, arachidonic acid; CO, control mothers who received placebo corn-oil capsules; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, intervention mothers who received fish-oil capsules; HM, human milk; LCP, long-chain PUFA.

Characteristics	CO (<i>n</i> = 82)*	FO (<i>n</i> = 72)*
Height, cm	158 ± 5.06	156 ± 5.36
Weight, kg	50.6 ± 6.48	50.5 ± 7.58
Body Mass Index, kg/m ²	20.3 ± 2.33	20.8 ± 2.86
HM LCP		
DHA, mg/L	70.1 (50.1, 94.3)	74.9 (54.4, 116)
EPA, mg/L	24.9 (15.8, 37.3)	24.9 (16.9, 32.8)
AA, mg/L	315 (224, 442)	334 (238, 443)
AA/∑(DHA, EPA)	3.29 (2.83, 3.59)	3.22 (2.71, 3.70)
¹ Data are presented as means ± SDs, <i>n</i> (%), or median (P ²⁵ , P ⁷⁵). *The control group (CO) included 41 (50.0%) children, and the intervention group (FO) included 40 (55.6%) children who received an n-3 LCP fortified food supplement as part of the child intervention.		
AA, arachidonic acid; CO, control mothers who received placebo corn-oil capsules; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, intervention mothers who received fish-oil capsules; HM, human milk; LCP, long-chain PUFA.		

At baseline, there was no significant difference in HM LCP concentrations between the study groups ($P > 0.10$) (**Table 1**). After 6 and 12 months of supplementation, however, the DHA concentration in HM was 39.0% (95% CI: 20.6, 57.5%; $P < 0.001$) and EPA concentration was 36.2% (95% CI: 16.0, 56.4%; $P < 0.001$) higher in the FO group compared to the CO group, whereas the AA concentration decreased by 17.5% (95% CI: -31.4, -3.74%; $P = 0.013$) and the AA/(DHA + EPA) ratio by 53.5% (95% CI: -70.2, -36.7%; $P < 0.001$) (**Figure 2**).

The changes in HM and child capillary blood (DHA + EPA)/AA ratios following FO supplementation were significantly associated. The association was marginally stronger in mother-child pairs where children received no FO in the child intervention (β (SE) = 0.25 (0.03); $P < 0.001$) compared to those who received a FO-fortified food supplement in the child intervention (β (SE) = 0.16 (0.03); $P < 0.001$) (β (SE) for interaction = 0.09 (0.04); $P = 0.043$) (Fig. 3).

Compared with the global average estimates and other studies (**Additional file 2**), the average DHA concentration in HM of the study mothers was lower both at baseline [median (IQR): 74.0 (50.4, 104)] and after FO supplementation [median (IQR): 89.5 (45.9, 122)]. In contrast, the AA concentration in HM was high at baseline [median (IQR): 328 (230, 442)] and normalized towards the global average estimate after FO supplementation [median (IQR): 189 (134, 248)].

Discussion

We investigated the effect of n-3 LCPs-rich fish-oil supplementation of mothers during lactation (6–24 months postpartum) on HM LCP concentrations. Fish-oil supplementation resulted in significantly higher HM DHA and EPA concentrations and a lower AA/(DHA + EPA) ratio compared to the placebo group. Although fish-oil supplementation prevented the decline in HM n-3 LCP concentrations over time, which was observed in the placebo group, the daily dose of 500 mg n-3 LCPs (215 mg DHA + 285 mg EPA) did not result in HM DHA concentration comparable to the worldwide norms. A review of previous trials on maternal supplementation using fish-oil (i.e., DHA + EPA) or DHA alone during lactation showed similar results of improved HM n-3 LCPs status (26).

Due to the lack of a clear consensus on the optimal concentrations in HM, current recommendation on DHA intake by lactating mothers is based on observed intake levels in previous studies that were required to enrich HM to the global mean DHA value of 0.32% TL (~ 134 mg/L) (16, 17). A dose of 200 mg DHA/d increased DHA in HM from 0.21% TL to the global average when consumed during 4–6 weeks (27) and 5 days-12 weeks postpartum (28). HM DHA concentration (89.5 mg/L) in our study mothers remained below the global average after receiving a similar dose. The relatively smaller increase in our study may relate to different factors. Mainly, the stage of lactation is an important difference between the current study and most previous studies with stronger impact. Compared to this study, supplementation in the other studies usually started no later than a few weeks postpartum and lasted for a shorter period. Decline in HM DHA levels over the course of lactation has been described previously including in mothers enrolled in supplementation studies (29–34). Therefore, the relatively lower HM DHA enrichment in our study might be due to maternal depletion concurring with the intervention period; i.e., the relatively later start at 6 to 12 months postpartum as well as the longer follow-up period of up to 24 months postpartum.

Our results also raise the question of whether current intake recommendation for lactating women – which is mainly based on dose-response curves of maternal DHA intake and HM levels derived from short-term studies (28) – may need reconsideration using data from studies covering the whole period of lactation. A higher dose than the recommended intake may be required to achieve optimum HM concentrations in this and similar populations, especially during the period of late lactation. A previous study using two different doses of DHA supplementation also supports this supposition (35). In this study, although dosages of both 200 and 400 mg DHA/d significantly increased HM DHA compared to a placebo, an average HM DHA concentration closer to the global mean was achieved only in the group of mothers who received the higher dose of 400 mg/d (HM DHA in placebo vs. 200 vs. 400 mg DHA/d groups: 58.7 vs. 88.3 vs. 131 mg/L). On the other hand, the safety of a very high n-3 LCPs intake through consumption of sea fish or fish-oil supplements should be considered carefully with respect to a potential risk of exposure to environmental contaminants in the mother and her child during pregnancy and lactation (16).

The extent of HM enrichment following supplementation may also be influenced by factors that can affect PUFA metabolism and their transfer to breastmilk or elsewhere. Competition between the n-3 and n-6 fatty acid families occurs not only in the synthesis of the LCPs from their respective precursors, but also in their incorporation into tissues (36, 37). The basal AA concentration in HM of the study mothers was

very high compared to the global mean, albeit within the range of values reported in different populations (13, 14). The reason for this high value is not clear as intake of AA sources – like meat and eggs – is limited in the study area. Alternatively, milk AA mainly comes from maternal AA stores (38, 39), which in turn could have been influenced by long-term high dietary LA intake. Studies showed that a high dietary LA intake can inhibit the incorporation of preformed DHA into milk lipids (36), which might have potentially attenuated the uptake of supplementary DHA. Additionally, effects of an intervention could be modulated by single nucleotide polymorphisms that are commonly observed in the FADS gene cluster, suggesting the need for consideration of possible differences in the distribution of these gene variants while comparing results from different populations. A study in another population showed that FADS genotype modify the association between maternal DHA intake and HM concentration possibly due to limited incorporation into breastmilk in women with some gene variants (40). Currently, there is no information available on the distribution of polymorphisms in the FADS gene cluster in the Ethiopian population. However, the very high milk AA in our mothers is not indicative of an important role of genetic polymorphism as these gene variants are generally expected to have more effects on HM levels of the n-6 than the n-3 PUFAs (41).

The significant association observed in the changes in n-3/ n-6 LCPs ratio between the maternal milk and child capillary blood following the intervention shows that the increased maternal dietary n-3 LCP intake improved the status of their breastfed infants and young children. Other studies also noted positive correlations between HM concentrations and child n-3 LCP status (33, 42) as well as improved child status following supplementation of their lactating mothers (43, 44).

The low maternal DHA status in this setting corroborates the findings of previous reports on the very low availability of n-3 fatty acid in the Ethiopian diet (12, 18). It is also an indication of potential risk of a suboptimal status in their breastfed infants and young children who live in a setting where the complementary foods have a negligible contribution to n-3 LCPs intake (11). The present study showed the potential for consumption of additional DHA by lactating mothers in improving the content in HM and the status of their breastfed children. Maternal supplementation with fish-oil or other sources of preformed n-3 LCP is one potential intervention approach in Ethiopia considering the good coverage of breastfeeding (45) and the challenges of securing adequate access to marine food in one of the most populous land-locked country.

This study is limited by the absence of maternal dietary intake data which would have aided in the interpretation of the effect of the studied doses of DHA and EPA on HM concentrations and our understanding of the unexpectedly high AA concentration in HM in this population. Furthermore, we were unable to provide the overall fat content of samples due to laboratory procedures and interference from fatty acids coming from plastic test tubes. On the other hand, our study had a large sample size and relatively low dropout rate compared to similar studies (26).

Conclusions

In conclusion, we demonstrated that fish-oil supplementation of mothers during lactation increased HM DHA and EPA content and improved the status of their breastfed children. However, HM DHA concentrations of lactating mothers remained lower than international norms after supplementation. It is recommended that future studies evaluate different doses of n-3 LCP covering a longer period lactation as well as the impact of potential effect modifiers such as genetic polymorphism, diet, and others.

Abbreviations

AA: Arachidonic acid

CO: Corn-oil

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic acid

FO: Fish-oil

HM: Human milk

LCPs: Long-chain polyunsaturated fatty acids

n-3: Omega-3

n-6: Omega-6

Declarations

Competing interests

The authors declare that they have no competing interests

Funding

The study was funded by the Institutional University Collaboration Programme of the Flemish Interuniversity Council (VLIR-UOS) with Jimma University, Nutrition Third World, the Nutricia Research Foundation (grant 2014-E9), Michiels Fabrieken NV, and Fortitech Inc. The funders had no role in the design, collection, analyses and interpretation of the data or the writing of the manuscript.

Authors' contributions

KPB, LH, PK and MW conceived the study. AA, KPB, MW and LH planned and implemented the study. BDM performed laboratory analysis of fatty acids. AA analyzed the data and wrote the manuscript with support from KPB, LH, PK, CL and GHC. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the mothers who participated in the study.

References

1. Hadley KB, Ryan AS, Forsyth S, Gautier S, Salem N. The essentiality of arachidonic acid in infant development. Vol. 8, *Nutrients*. 2016.
2. Lauritzen L, Hensen H, Jorgensen M, Michaelsen K. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res*. 2001;40:1–94.
3. Bazan N, Reddy T, Bazan H, Birkle D. Metabolism of arachidonic and docosahexaenoic acids in the retina. *Prog Lipid Res*. 1986;25:595–606.
4. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr*. 1992;120:S129–S38.
5. Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. *J Neurochem*. 1998;71:2528–2533.
6. Shulkin M, Pimpin L, Bellinger D, Kranz S, Fawzi W, Duggan C, et al. n–3 Fatty Acid Supplementation in Mothers, Preterm Infants, and Term Infants and Childhood Psychomotor and Visual Development: A Systematic Review and Meta-Analysis. *J Nutr*. 2018;148:409–18.
7. Calder PC. n-3 Fatty acids, inflammation and immunity: new mechanisms to explain old actions. *Proc Nutr Soc*. 2013;72:326–36.
8. Gottrand F. Long-Chain Polyunsaturated Fatty Acids Influence the Immune System of Infants. *J Nutr*. 2008;138:1807–12.
9. Lapillonne A, Clarke SD, Heird WC. Plausible mechanisms for effects of long-chain polyunsaturated fatty acids on growth. *J Pediatr*. 2003;143:S9–16.
10. Lapillonne A, Clarke SD, Heird WC. Polyunsaturated fatty acids and gene expression. *Curr Opin Clin Nutr Metab Care*. 2004;7(2):151–6.
11. Forsyth S, Gautier S, Salem N. Dietary Intakes of Arachidonic Acid and Docosahexaenoic Acid in Early Life - With a Special Focus on Complementary Feeding in Developing Countries. *Ann Nutr Metab*. 2017;70(3):217–27.
12. Michaelsen KF, Dewey KG, Perez-Exposito AB, Nurhasan M, Lauritzen L, Roos N. Food sources and intake of n-6 and n-3 fatty acids in low-income countries with emphasis on infants, young children (6-24 months), and pregnant and lactating women. *Matern Child Nutr*. 2011;7(SUPPL. 2):124–40.

13. Fu Y, Liu X, Zhou B, Jiang AC, Chai L. An updated review of worldwide levels of docosahexaenoic and arachidonic acid in human breast milk by region. *Public Health Nutr.* 2016;19(15):2675–87.
14. Brenna JT, Varamini B, Jensen RG, Diersen-schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr.* 2007;85:1457–64.
15. Yuhas R, Pramuk K, Lien EL. Human milk fatty acid composition from nine countries varies most in DHA. *Lipids.* 2006;41(9):851–8.
16. Koletzko B, Cetin I, Thomas Brenna J, Alvino G, von Berlepsch J, Biesalski HK, et al. Dietary fat intakes for pregnant and lactating women. *Br J Nutr.* 2007;98(5):873–7.
17. Brenna JT, Lapillonne A. Background Paper on Fat and Fatty Acid Requirements during Pregnancy and Lactation. *Ann Nutr Metab.* 2009;55:97–122.
18. Forsyth S, Gautier S, Salem N. Global estimates of dietary intake of docosahexaenoic acid and arachidonic acid in developing and developed countries. *Ann Nutr Metab.* 2016;68(4):258–67.
19. Argaw A, Wondafrash M, Bouckaert KP, Kolsteren P, Lachat C, Belachew T, et al. Effect of n-3 long-chain PUFA supplementation to lactating mothers and their breastfed children on child growth & morbidity: a 2 X 2 factorial randomized controlled trial in rural Ethiopia. *Am J Clin Nutr.* 2018;107(3):454–64.
20. Argaw A, Huybregts L, Wondafrash M, Kolsteren P, Belachew T, Worku BN, et al. 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. *J Nutr.* 2019;149:505–512.
21. Ichihara K, Waku K, Yamaguchi C, Saito K, Shibahara A, Miyatani S, et al. A convenient method for determination of the C20-22 PUFA composition of glycerolipids in blood and breast milk. *Lipids.* 2002;37(0024-4201 (Print)):523–6.
22. Bligh E, Dyer W. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 1959;37(8):911–917.
23. Hall E, Flores S, De Jesús V. Influence of hematocrit and total-spot volume on performance characteristics of dried blood spots for newborn screening. *Int J Neonatal Screen.* 2015;1:69–78.
24. Bailey-Hall E, Nelson E, Ryan A. Validation of a rapid measure of blood PUFA levels in humans. *Lipids.* 2008;43:181–186.
25. Kent J, Mitoulas L, Cregan M, Ramsay D, Doherty D, Hartmann P. Volume and Frequency of Breastfeedings and Fat Content of Breast Milk Throughout the Day. *Pediatrics.* 2006;117(3):387–395.
26. do Amaral Y, Marano D, da Silva L, Guimarães A, Moreira M. Are There Changes in the Fatty Acid Profile of Breast Milk with Supplementation of Omega-3 Sources? A Systematic Review. *Rev Bras Ginecol Obs.* 2017;39:128–41.
27. Fidler N, Sauerwald T, Pohl A, Demmelmair H, Koletzko B. Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. *J Lipid Res.* 2000;41:1376–1383.

28. Makrides M, Neumann M, Gibson R. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. *Eur J Clin Nutr.* 1996;50(7):352–335.
29. Otto S, Houwelingen, AC Van Badart-Smook A, Hornstra G. Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. *Am J Clin Nutr.* 2001;73:1074–1079.
30. van Goor S, Dijck-brouwer D, Hadders-algra M, Doornbos B, Jaap J, Erwich H, et al. Human milk arachidonic acid and docosahexaenoic acid contents increase following supplementation during pregnancy and lactation. *Prostaglandins, Leukot Essent Fat Acids.* 2009;80:65–69.
31. Michalski M, Briard V, Michel F, Tasson F, Poulain P. Size Distribution of Fat Globules in Human Colostrum, Breast Milk, and Infant Formula. *J Dairy Sci.* 2005;88(6):1927–1940.
32. Harzer G, Haug M, Dieterich I, Gentner P. Changing patterns of human milk lipids in the course of the lactation and during the day. *Am J Clin Nutr.* 1983;37:612–621.
33. Dunstan J, Mitoulas L, Dixon G, Doherty D, Hartmann P, Simmer K, et al. The Effects of Fish Oil Supplementation in Pregnancy on Breast Milk Fatty Acid Composition Over the Course of Lactation: A Randomized Controlled Trial. *Pediatr Res.* 2007;62(6):689–694.
34. Barrera C, Valenzuela R, Chamorro R, Bascuñ K, Sandoval J, Sabag N, et al. The Impact of Maternal Diet during Pregnancy and Lactation on the Fatty Acid Composition of Erythrocytes and Breast Milk of Chilean Women. *Nutrients.* 2018;10(839):1–14.
35. Sherry C, Oliver J, Marriage B. Essential Fatty Acids Docosahexaenoic acid supplementation in lactating women increases breast milk and plasma docosahexaenoic acid concentrations and alters infant omega 6 : 3 fatty acid ratio. *Prostaglandins Leukot Essent Fat Acids.* 2015;95:63–69.
36. Gibson RA, Muhlhausler B, Makrides M. Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. *Matern Child Nutr.* 2011;7(SUPPL. 2):17–26.
37. Innis S. Human milk: maternal dietary lipids and infant development. *Proc Nutr Soc.* 2007;66(3):397–404.
38. Prado M Del, Villalpando S, Elizondo A, Demmelmair H, Koletzko B. Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet. *Am J Clin Nutr.* 2001;74:242–7.
39. Martin J, Bougnoux P, Fignon A, Theret V, Antoine J, Lamisse F, et al. Dependence of human milk essential fatty acids on adipose stores during lactation. *Am J Clin Nutr.* 1993;58:653–659.
40. Molto-Puigmartí C, Plat J, Mensink R, Muller A, Jansen E, Zeegers M, et al. FADS1 FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. *Am J Clin Nutr.* 2010;91:1368–1376.
41. Lauritzen L, Brambilla P, Mazzocchi A, Harsløf LBS, Ciappolino V, Agostoni C. DHA Effects in Brain Development and Function. *Nutrients.* 2016;8(6):1–17.
42. Lauritzen L, Carlson SE. Maternal fatty acid status during pregnancy and lactation and relation to newborn and infant status. *Matern Child Nutr.* 2011;7(SUPPL. 2):41–58.

43. Henderson R, Jensen R, Lammi-keefe C, Ferris A, Dardick K. Effect of Fish Oil on the Fatty Acid Composition of Human Milk and Maternal and Infant Erythrocytes. *Lipids*. 1992;27(11):863–869.
44. Jensen C, Maude M, Anderson R, Heird W. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. *Am J Clin Nutr*. 2000;4(71(Suppl)):292S-299S.
45. Central Statistical Agency (CSA) [Ethiopia] and ICF. 2016. Ethiopia Demographic and Health Survey 2016. Addis Ababa, Ethiopia, and Rockville, Maryland, USA: CSA and ICF.

Figures

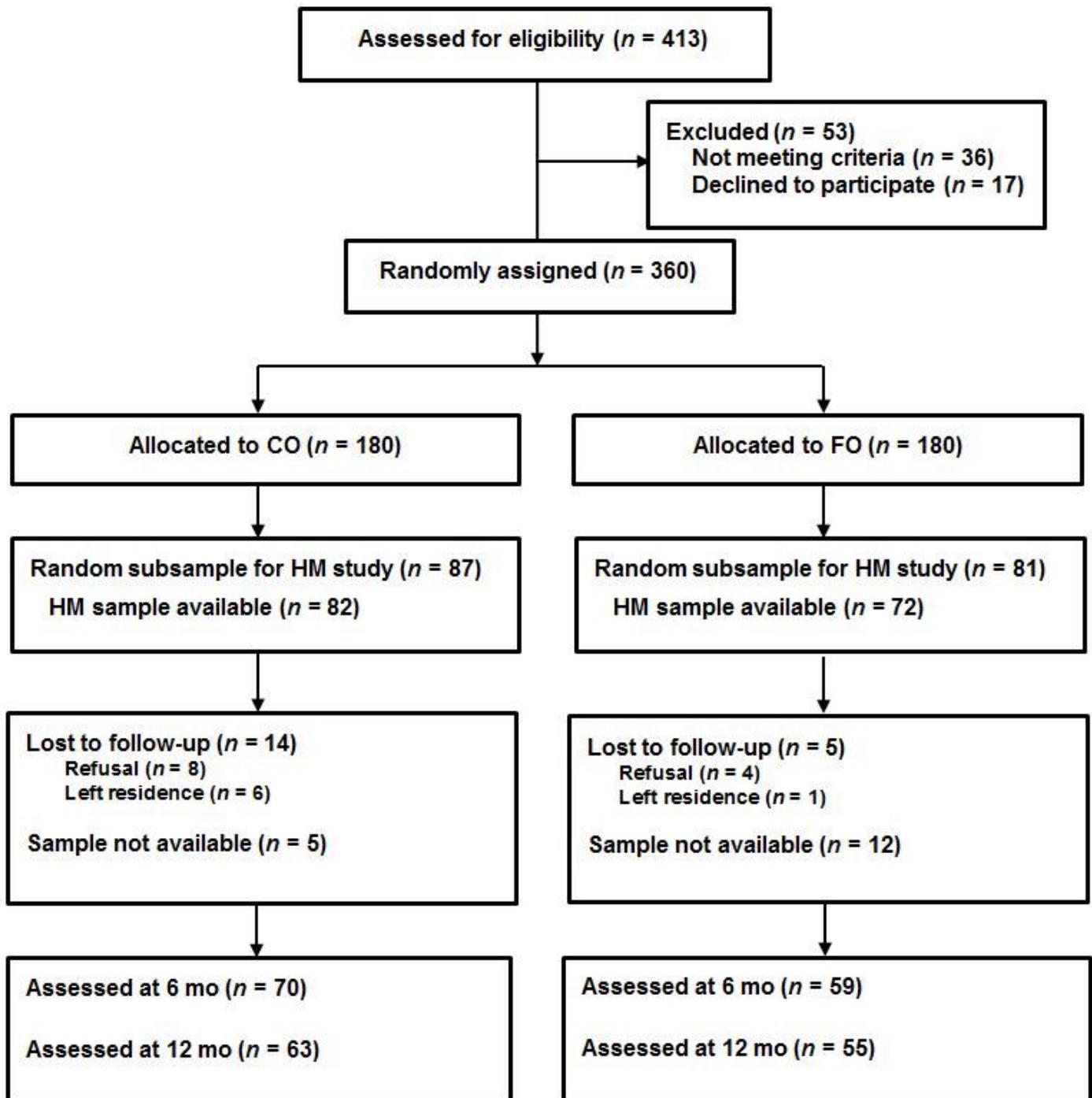


Figure 1

Trial flowchart A random subsample of 168 mothers was drawn from the main trial ($n = 360$) and asked to participate in the HM sub-study. A total of $n = 154$ mothers from whom samples were available at baseline were finally considered for the HM sub-study. CO, control mothers who received a placebo corn-oil capsules; FO, intervention mothers who received fish-oil capsules; HM, human milk.

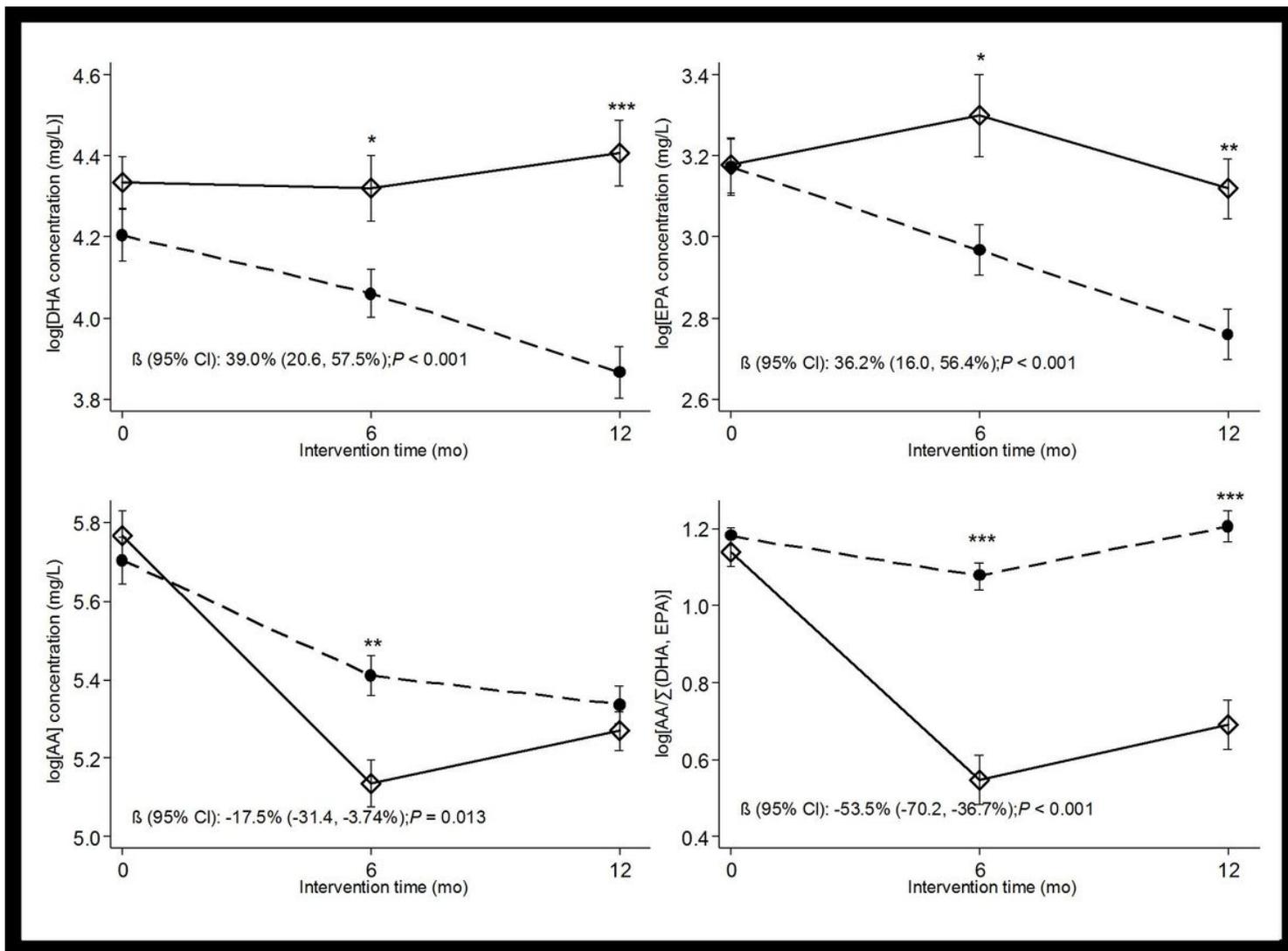


Figure 2

HM LCP concentrations at baseline, midline and endline of intervention in the CO (; n = 82) and FO (; n = 72) groups. Values are expressed as means with their standard error indicated by vertical bars. Group differences in HM LCPs concentration were estimated by fitting a mixed-effects linear model with random intercept mother to account for clustering of the midline and endline measurements. Asterisks (*P < 0.05; **P < 0.01; ***P < 0.001) indicate significant group differences at each measurement-point using a linear regression model. Models were adjusted for baseline LCPs values and additional covariates including household wealth, duration of lactation, breastfeeding frequency, and maternal age, height, parity and occurrence of subsequent pregnancy during follow-up. LCP values were log transformed before analysis and the antilog of coefficients and CIs were used to express group differences as percent of the control value. AA, arachidonic acid; CO, control mothers who received placebo corn-oil capsules; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, intervention mothers who received fish-oil capsules; HM, human milk; LCPs, long-chain PUFAs.

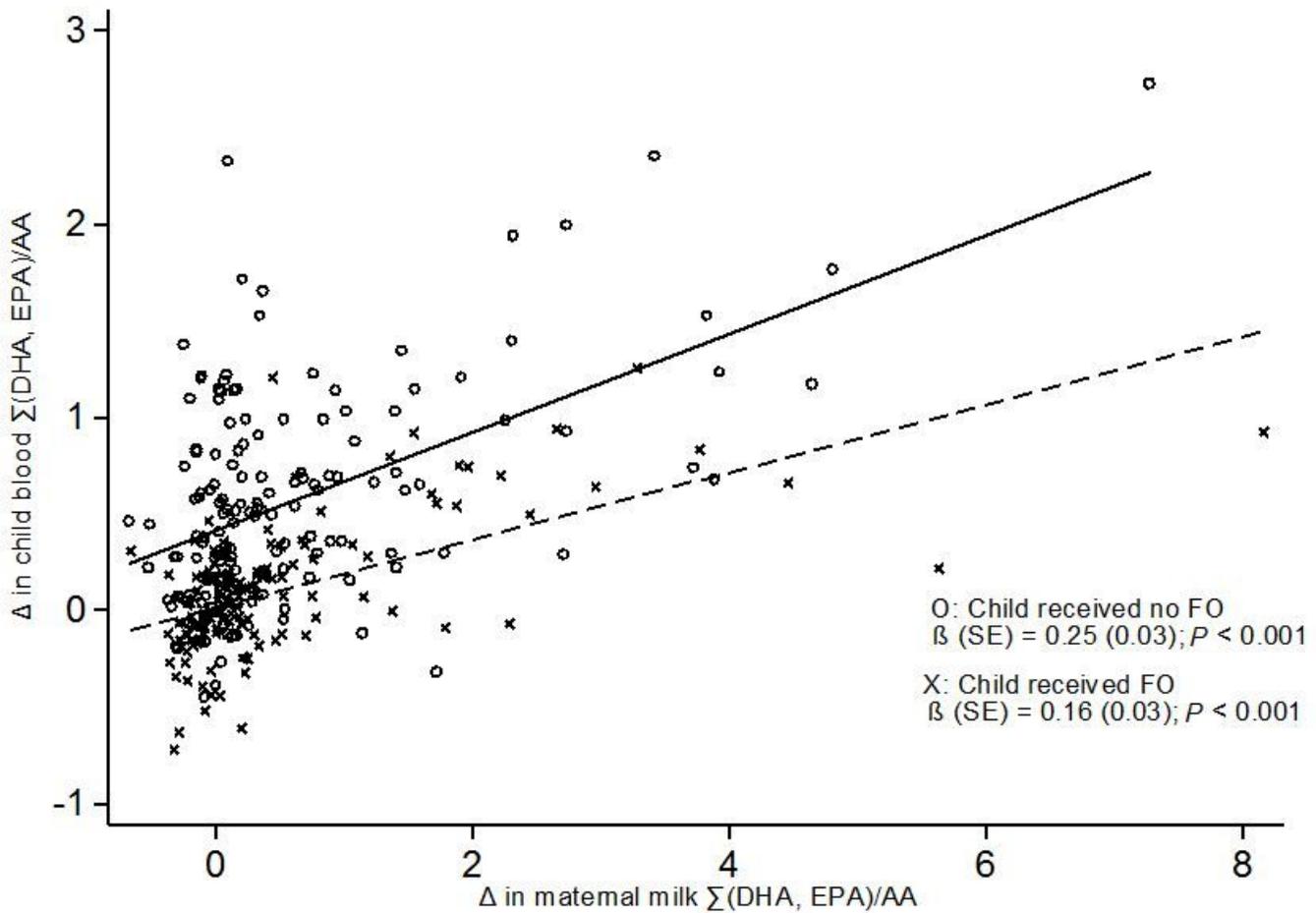


Figure 3

Relationship between the change in HM and child capillary blood (DHA + EPA)/AA ratios following the intervention. Values are expressed as the difference between midline/endline and baseline as a ratio of baseline values. A mixed-effects linear model was fitted to estimate the relationship between HM and child capillary blood ratios, with random intercept mother-child pair to account for clustering of the midline and endline values. The model was adjusted for covariates child age and sex, household wealth, breastfeeding frequency, and maternal age, height, parity and occurrence of subsequent pregnancy during follow-up. Data are presented separately for the group of mother-child pairs where children did not receive (o & solid line ; n = 72) and received (x & dashed line; n = 81) FO as part of the child intervention. AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish-oil; HM, human milk.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement7.docx](#)
- [supplement8.xlsx](#)
- [supplement9.xlsx](#)