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HIGHLIGHTS

- Small amounts of freshwater fish had no impact on infants' n-3 LCPUFA status
- Fish-based foods reduced n-6 PUFA and n-6/n-3 PUFA ratio in non-breastfed infants
- Late cessation of breastfeeding positively correlated with infants' n-3 LCPUFA
- Cambodian infants' FA status was better compared to children in developed high-income countries

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SUMMARY

- n-3 LCPUFA are essential for visual and cognitive development.
- Breast milk and marine fish are recommended sources of n-3 LCPUFA for children.
- Freshwater fish is the dominant source of n-3 LCPUFA in landlocked or riparian regions, but contain lower amounts of n-3 LCPUFA compared to marine fish.
- Complementary food products containing 10-12% ~~percent~~ freshwater fish by dry-weight for nine months yielded a maximum intake of 86.5 mg/day n-3 LCPUFA in ~~six-month-old~~ Cambodian infants.
- Fish-based complementary food had no impact on infants' whole blood n-3 LCPUFA compared to foods without fish.
- The lack of impact of freshwater fish consumption on infant whole blood n-3 LCPUFA status could be due to ~~low content of n-3 LCPUFA and a~~ high intake of n-3 LCPUFA from breast milk and low content of n-3 LCPUFA in the intervention food.

1. INTRODUCTION

~~The~~ n-3 LCPUFA are essential for visual and cognitive functions of infants and children [1], but humans have a limited endogenous conversion of α-linolenic acid (ALA, 18:3 n-3) to n-3 LCPUFA [2]. Breast milk and dietary fish are recommended as key sources of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) for children under 2 years of age [3]. Breast milk

has a relatively high content of n-3 LCFUFA and arachidonic acid (AA, 20:4 n-6) and when the rate of breastfeeding decreases, dietary n-3 LCPUFA plays an important role in maintaining n-3 LCPUFA supply to ensure optimal DHA incorporation into the growing brain.

There are only a few studies on the effect of dietary n-3 LCPUFA in infants and young children in developing countries and poor communities [4]. Cambodia ranks among the poorest countries in the world with high prevalence of undernutrition [5]. Situated in the Mekong River basin, Cambodia has a productive freshwater environment and a variety of several hundred freshwater fish species which is important in the traditional diet [6]. The majority of Cambodian population consume fish at least once a day, making it the most consumed animal source food [7]. National consumption of freshwater fish in Cambodia was more than the consumption of all other animal source food combined, and about five times the consumption of marine fish [8]. The traditional complementary food in Cambodia is a rice porridge (*borbor*) served plain or with a small amount of non-staple foods (vegetables, fish, egg etc). However, a study in 6-12 month-old Cambodian children indicates that overall fish intake among infants is low, even if the frequency may be high showed that (only 3-23 g, with maximum serving frequency of 3 times per week) [9]. The prevalence of breastfeeding in Cambodia is generally high, with a median duration of breastfeeding was 16 months, but less than 40% of 18-23 months old children were still breastfed [5]. Furthermore, low consumption of marine fish [8] possibly limit the breast milk content of n-3 LCPUFA.

While all fish contain some n-3 LCPUFA, fish species from warm freshwater environments generally have considerably less docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3) than cold-water species from marine environments [10-12], making the

nutritional contribution of n-3 LCPUFA from freshwater fish uncertain [10-14]. Without taking into account these differences, Cambodian was estimated to have high intake of n-3 LCPUFA [15, 16]. Due to the important role of n-3 LCPUFA in infant development, it is very relevant from a public health perspective to examine to what extent locally available freshwater fish can improve the n-3 LCPUFA status among infants and young children in riparian regions. There are currently no studies that have investigated the efficacy of small amounts of dietary freshwater fish in improving n-3 LCPUFA status, performed in infants and young children in a developing country with restricted access to marine fish.

Therefore, the aim of the present study was to ~~examine~~ document the general fatty acid status and investigate the impact of daily consumption of small amounts of freshwater fish-based complementary food on whole blood n-3 LCPUFA in Cambodian infants from 6 to 15 months, living in the landlocked and riparian province of Prey Veng. The study is important to provide evidence for dietary recommendations and potential benefits of freshwater fish consumption for fatty acid status of infants and young children in ~~landlocked or riparian regions of developing~~ low-income countries.

~~Breast milk and dietary fish are recommended as key sources of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) for children under 2 years of age [1]. However, fish species from warm freshwater environments generally have less docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5n-3) compared to cold-water species from marine environments [2-4]. Hence, the status of n-3 LCPUFA in infants and children under 2 years of age beyond the breastfeeding period with restricted access to marine food sources is a concern.~~

n-3 LCPUFA are essential for visual and cognitive functions of infants and children [5], but humans have a limited endogenous conversion of α-linolenic acid (ALA, 18:3 n-3) to n-3 LCPUFA [6]. Breast milk has a relatively high content of n-3 LCFUFA and arachidonic acid (AA, 20:4 n-6) and when the rate of breastfeeding decreases, dietary n-3 LCPUFA plays an important role in maintaining n-3 LCPUFA supply to ensure optimal DHA incorporation into the growing brain.

Studies on children's n-3 LCPUFA status and the efficacy of fish consumption as well as fish oil supplementation have mainly been conducted in developed country settings. Breastfeeding aside, children in communities of landlocked or riparian regions, are likely to rely on freshwater fish as the main source of n-3 LCPUFA [9]. The contribution of n-3 LCPUFA from freshwater fish to human diet is uncertain [2-4, 10, 11].

The important role of n-3 LCPUFA in development and the uncertain contribution of freshwater fish as the dominant source of n-3 LCPUFA in landlocked or riparian regions, raises the question about the n-3 LCPUFA status among infants and young children living in such environments beyond the exclusive breastfeeding period. No studies on the efficacy of small amounts of freshwater fish consumption in improving n-3 LCPUFA status have previously been performed in infants and young children in a developing country with restricted access to marine fish.

2. MATERIALS AND METHODS

2.1. *Study design and setting*

This study was part of the 'WinFood study' in Cambodia [17]. The WinFood study was an individually randomized, single-blinded, community-based intervention trial. assessing the

~~impact of four complementary food products on body composition and iron status in Cambodian infants~~. ~~Whole blood fatty acid was a secondary outcome.~~ The study was designed primarily to assess the efficacy of locally developed complementary foods on child growth including body composition, micronutrient status, and gross motor development, in comparison with standard food aid products [18]. Whole blood fatty acid was also analysed as secondary outcome and is the focus of the current sub-study. The study was conducted from March 2011 until March 2012 in seven communes in the Prey Veng province, Cambodia.

Infants were recruited at 6 months (±2 weeks) of age ~~as described in detail elsewhere [12].~~ and unless dropped out, completed at 15 months (±2 weeks) as described in detail elsewhere [17]. Infants with severe acute malnutrition defined as weight-for-length z-score (WLZ) ≤-3 or pitting oedema, clinical signs of vitamin A deficiency, and/or severe anaemia were excluded from the study. Infants with persistent diarrhoea were also excluded from the study and were ~~given~~ offered ORS treatment and re-invited in 2-4 weeks. More details regarding the study participants are available elsewhere [17].

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by The National Ethics Committee for Health Research, Ministry of Health, the Royal Government of Cambodia. This trial was registered at controlled-trials.com as ISRCTN19918531 at http://www.isrctn.com/ISRCTN19918531.

2.2. Intervention food products

The WinFood products in Cambodia were developed with a broad aim of assessing food-based innovations for alleviating childhood malnutrition by utilization of traditional foods.

The WinFood project involved identification of traditional foods with the potential to be used in complementary foods; formulation and optimization of the complementary foods; and palatability and acceptability assessment among mothers and infants [18]. The amount of animal source food added into the formula was limited by the aim to not pronouncedly exceeding the recommended upper level of the safe protein intake for infants and children age up to 1 year old, which was 14.8 gram/day [19]. The amount of total fat in the formula was technologically also limited to maximum 10%. The current study tests the efficacy of the formulated complementary food with respect to their ability to increase n-3 LCPUFA fatty acid status in the infants.

At the baseline visit, the infants were randomized to one of four intervention groups; WinFood (WF) complementary food made of rice with local small freshwater fish species and edible spiders; WinFood-L (WF-L) made of rice with local small freshwater fish of mixed species; CSB+, a fortified standard corn-soy blend product used by the World Food Programme (WFP) with sunflower oil distributed separately to be added at preparation (following standard WFP procedures for CSB+); and CSB++, a standard fortified corn-soy blend product with dried skimmed milk and soya oil, used by WFP.

The intervention foods were distributed in sachets of daily rations, adjusted for age of the child. The sachets had identical WinFood logos with cooking instructions. The intervention lasted for nine months, during which the caregivers were provided with a batch of daily rations of food until the next scheduled follow-up. Local health staff in each village visited the mothers weekly for supervision and support, and could always be consulted for questions.

The fat and fatty acid content of the intervention food were estimated, based on the fat and fatty acid content of the food components obtained from laboratory analyses (for fish and spider) and food composition data [20] (for all other food materials). The maximum fatty acid intake was calculated from the estimated maximum intake of the intervention food. Detailed information on the food composition, fat content and fatty acid composition of the intervention foods is shown in Table 1.

2.3. Intake calculation

Food consumption was estimated from the food sachets returned at the monthly follow-up visit. All caregivers at all visits reported that the distributed foods had been eaten only by the infant and not shared with the household. The empty, partially empty and unopened sachets were collected and weighed to estimate the amount of food consumed every month. We did not collect any information about food waste, thus do not have information about the actual take of the intervention products. Hence, we can only estimate the maximum intake. The total fat content of the intervention foods was obtained from laboratory analyses. The fatty acid contents were calculated from the fatty acid composition of the different food ingredients, which was based on analyses for fish (*Esomus longimanus*, *Paralauba typus* and mixed batches of common indigenous small fish), spider (*Haplopelma* species) and database information (frida.fooddata.dk [20]) for the remaining ingredients. This information was used to calculate the maximum intake of fat and fatty acid. ~~The estimates do not include plate waste and as such are an estimate of maximum intake. The fat and fatty acid content of the intervention foods were obtained from laboratory analyses.~~

The contribution of energy from the ~~consumption of~~ consumed intervention foods to the ~~overall~~ total dietary energy requirement ~~during~~ accumulated for the entire intervention period

was estimated to be 6.8–10.9% (Table 2), based on a total daily energy requirement of 682 kcal/day in the age 6-8 months, 830 kcal/day for 9-11 month-old infants and 1,092 kcal/day for children from 1-2 years of age [21]. ~~The maximum fatty acid intake was calculated from the estimated maximum intake of the intervention food and fatty acid composition of the intervention food.~~

2.4. Blood sampling

Blood samples were collected at baseline and endline. Blood samples were collected by trained local nurses in vacutainers containing heparin and shaken at least eight times to avoid coagulation. Aliquots of blood (500 µL), were pipetted into 1.5 mL-tubes containing 50 µL of 0.1% butylated hydroxytoluene in ethanol for fatty acid analysis. The tubes were quickly covered and stored in a -20°C freezer. After the completion of a sampling round in the field (3-5 days), frozen samples were transported (1-2 hours) to the Department of Fisheries Post-harvest Technologies and Quality Control in Phnom Penh and stored at -20°C until shipment to the laboratory of the University of Waterloo, Canada for analysis. In Canada, the samples were stored at -75°C until immediately before analysis. Storage time in the field prior to arrival in Canada ranged from 4-6 months and total storage period before analysis varied from 9 to 11 months. An assessment of LCPUFA stability during storage and more details about the blood sampling procedures are available in [22].

2.5. Fatty acid analysis

The whole blood fatty acid concentrations were determined in 50 µL of thawed blood that was trans-esterified by addition of 1 mL 14% boron trifluoride in methanol, 300 µL hexane containing 50 µg/mL BHT and 33.33 µg/mL 22:3N-3 ethyl ester (internal standard, Nu-CheckPrep, Elysian, MN, USA), and heated at 95°C on a heating block for 1 h [23, 24]. The

organic and aqueous layers were separated after the addition of 1 mL hexane and 1 mL water, and centrifugation at 1750 *g* for 5 minutes. The upper organic hexane layer, containing the fatty acid methyl esters, was collected, fully dried under a stream of nitrogen gas, reconstituted into 65 µL of hexane and stored in vials until analysis by gas chromatography. More details of the fatty acid analysis of the whole blood method is available in [22].

Fatty acid methyl esters were separated using a Varian 3900 gas chromatograph equipped with a DB-FFAP capillary column of 15 m x 0.10 mm with a 0.10 µm film of nitroterephthalic acid modified polyethylene glycol (J & W Scientific, Agilent Technologies, Mississauga, ON) with hydrogen as the carrier gas [25, 26]. The fatty acid composition data are expressed both quantitatively (concentrations in µg/dL) and qualitatively (relative % of all fatty acids by weight (FA%)).

2.6. Statistical analysis

Anthropometric data were double entered in Epidata version 3.1 (The EpiData Association). All analyses were performed by SPSS (Version 20.0.0, IBM Corp., Kgs. Lyngby, Denmark). Significance was declared for $p<0.05$.

Participants' ~~baseline~~ characteristics and quantitative values for individual fatty acids and fatty acid groups were described using mean (±SD) or count (%). For intake of fatty acids, data were analysed by ~~a~~the Kruskal Wallis non-parametric test. The effect of the intervention on individual fatty acids and fatty acid groups was evaluated by means of analysis of covariance (ANCOVA) models that included adjustment for baseline outcome values, baseline plasma CRP, age (months), sex, age of the child (months) when breastfeeding was terminated, and age of the child (months) when solid foods were introduced, and status of

breastfeeding in the last 24 hours at endline. Model assumptions were assessed visually using residuals. Outcomes were transformed ~~where~~ when needed. Results ~~were~~are reported as unadjusted mean (±SD). Additionally, similar analyses were carried out based on food groups being combined as fish-based (WF and WF-L) and the non-fish-based (CSB+ and CSB++) intervention foods. Likewise, similar analyses stratified according to infants being breastfed or non-breastfed, was carried out. A subgroup analysis comparing the fish-based group with CSB+ and CSB++ was also carried out.

Furthermore, the interaction between treatment and breastfeeding status was investigated by inclusion of the interaction term in the ANCOVA analyses. Subsequently, we conducted a stratified ~~analyses~~ analysis in infants who breastfed in the last 24 hours prior to blood sampling at endline (breastfed group) and those who were not (non-breastfed group~~) were conducted~~. Finally, Pearson correlation coefficients were used for exploring the association between age when breastfeeding was stopped and n-3 LCPUFA status.

3. RESULTS

3.1. Participants' ~~Baseline~~ characteristics

Of the 514 invited infants, 440 (85.6%) were screened and 419 (95.2%) of these met the inclusion criteria and were randomly allocated to one of the four food groups. Blood was successfully sampled from 389 (92.8%) at baseline and 341 (81.4%) at endline, resulting in 315 (75.2%) with a complete pair of blood samples (see flow diagram in Figure 1). The intervention groups were similar in age, anthropometric status, breastfeeding status, social economic status, serum CRP, as well as whole blood fatty acid composition at baseline (Table 2). There was more household in the CSB groups whose primary income was fishery (6 for each CSB++ and CSB+ vs 3 and 2 in WF and WF-L groups, respectively), and more

household in WF group whose primary income was labouring work (18 vs 9, 7 and 15 in WF-L, CSB++ and CSB+ groups, respectively). Furthermore, there were more wasted infants in CSB+ group than in any other groups (7 vs 5, 2, and 4 infants in CSB++, WF and WF-L groups, respectively) and more stunted infants in CSB+ group than in any other groups (22 vs 14, 13 and 13 infants in CSB++, WF and WF-L groups, respectively).

3.2. Consumption of intervention foods

A total of 79 participants did not consume any of the provided food during the 9 months, for various reasons. The monthly consumption of the complementary food during the 9 months of intervention did not differ between the four food groups. Infants in the fish-based groups consumed 67% of the provided food, whereas infants in the non-fish-based group consumed 65%. The mean ± SD of total consumption of fish-based intervention food during the course of 9 months, was 15.1 ± 6.4 kg and that of the non-fish-based foods were 14.6 ± 6.5 kg ($p=0.515$).

3.3. Energy intake from intervention food

The additional supplementation with fat in CSB+ (the sunflower oil was distributed in separate sachet following WFP standard practise) resulted in higher calculated mean ± SD intake of energy from fat in the non-fish-based intervention foods groups (CSB+ and CSB++ pooled) than in the fish-based intervention food groups: 55.8 ± 27.0 vs 44.1 ± 18.9 kcal/day, $p<0.001$). ~~Subsequently~~, assuming the distributed oil was administered as prescribed in the CSB+ group. Consequently, it resulted in higher energy contribution of from fat to the daily energy requirement than in the other intervention groups (Table 2).

3.4. Intake of n-3 LCPUFA from intervention food

The estimated intake of various fatty acids from the intervention foods differed among the food groups (Table 2). There was no or negligible intake of n-3 LCPUFA from the consumption of non-fish-based intervention foods, in comparison to 22.1 ± 11.1 mg/day EPA, 16.8 ± 7.1 mg/day DPA and 47.6 ± 20.5 mg/day DHA, from the fish-based complementary foods. The fish-based and non-fish-based groups also differed in intake of AA from the interventions foods (mean ±SD) of 44.2 ± 22.3 mg/day vs essentially zero.

3.5. Effect of intervention on whole blood *n-3 LCPUFA*

There was no difference in whole blood fatty acid composition between the infants in the WF, WF-L, CSB++ or CSB+ groups after 9 months intervention (Table 4). Furthermore, there was no difference in whole blood fatty acid profiles or n-3 LCPUFA percent, when the fish-based (WF and WF-L) and the non-fish-based (CSB+ and CSB++) intervention foods were combined. Similar results were also found when comparing fish-based group with CSB+ and CSB++ separately (results are not shown in table or figure).

3.6. Effect of intervention on whole blood PUFA stratified by endline breastfeeding status

Breastfeeding status decreased from 98.3% of the infants being breastfed at baseline to 77.9% at the end of the 9 months intervention. Those who were not breastfed at endline, had on average stopped breastfeeding at mean (± SD) age of 13.1 ± 2.8 mo. Both the breastfed infants and those who were no longer breastfed at endline, had started receiving solid foods from 5.3 mo of age (5.3 ± 1.5 vs 5.3 ± 1.3 mo).

The fatty acid composition at endline differed significantly between the infants who were still breastfed and those who had stopped, except for ALA and linoleic acid (LA, 18:2n-6) (Table 5). The percentage of whole blood PUFA, n-6 and n-3 PUFA and specifically LCPUFA was

higher among breastfed infants and the ratio of n-6 to n-3 PUFA was lower. There was an interaction between complementary food received, and breastfeeding for the n-6/n-3 PUFA ratio (p=0.026), corresponding to a lower level in the fish-based intervention groups compared to the non-fish-based groups (7.1 ± 1.2 vs 7.9 ± 1.8) among the subsample of infants who had stopped breastfeeding. There was also an interaction (p=0.003) between breastfeeding and intervention products for n-6 PUFA, which among the breastfed infants tended to be lower in the non-fish-based groups compared to the fish-based groups (26.6 ± 2.4 vs 27.0 ± 2.1 FA%, p=0.069), but conversely tended to be lowest in the fish-based group among the non-breastfed infants (24.2 ± 3.5 vs 25.4 ± 3.8 FA%, p=0.092).

3.7. Association between age when breastfeeding stopped and n-3 LCPUFA status

Age of child when breastfeeding stopped (months) was strongly correlated with endline DHA (r=0.489, p<0.001) and with the change from baseline to endline in the percentage of whole blood DHA (r=0.277, p<0.001). It also correlated with relative percentages of EPA and DPA at endline (r=0.288, p<0.001 and r=0.125, p=0.021 respectively), and changes in EPA and DPA from baseline to endline (r=0.145, p=0.010 and r=0.122, p=0.030 respectively). Intake of EPA, DPA and DHA ~~was~~were not correlated with the changes or the endline FA% of the corresponding fatty acids.

4. DISCUSSION AND CONCLUSIONS

4.1. Discussion

The results of our study showed no overall effect of consumption of complementary foods containing small amounts of freshwater fish for nine months on whole blood fatty acid composition in Cambodian infants. The lack of effect of freshwater fish consumption on n-3 LCPUFA status was unexpected. Exploratory analysis indicated that the effect of the

intervention foods was modified by breastfeeding and that the fish-based complementary foods reduced n-6 PUFA and the n-6/n-3 PUFA ratio in infants who ~~were not breastfed~~ stopped breastfeeding at endline.

Furthermore, bivariate correlation showed no association between n-3 LCPUFA status ~~of the infants~~ and intake of n-3 LCPUFA, but age at cessation of breastfeeding was strongly correlated with endline whole blood n-3 LCPUFA. ~~This indicates~~ These findings suggest ~~this results indicate~~ that prolonged breastfeeding played a more important role for the n-3 LCPUFA status of the infants, than the n-3 LCPUFA intake from the intervention foods.

Breastfeeding is nearly universal in Cambodia [5], and the majority of the infants in the current study were breastfed during the intervention period. The exclusive breastfeeding rate was higher in the study area than in the national data. More than 87% of the children were still exclusively breastfed at 5 months old, and the median duration of exclusive breastfeeding was 6 months (compared to 50.9% and 4 months in the overall population) [5] and 78% of the children in this study was still partially breastfed at the end of the intervention. A recent study on fatty acid composition of breast milk from Cambodian mothers has been reported to be higher in DHA (0.40%) and AA (0.68% [30], compared to the world wide average content of 0.32 FA% DHA and 0.47 FA% AA [31]. Assuming the breast milk intake of the Cambodian infants was ranging between 516-875 ml/day [32], daily breastfeeding alone contributed to an intake of 89-151 mg/day of n-3 LCPUFA, which is around 1-1.5 times more than the maximum contribution of n-3 LCPUFA from the fish-based intervention food (87 mg/day).

Breastfeeding has been associated with higher whole blood EPA and DHA as well as higher AA and total PUFA and a lower n-6/n-3 PUFA ratio, compared to non-breastfed infants [33],

and this is in agreement with what we found in the current study. Breastfeeding was found to modify the effect of the intervention, as the ratio of n-6/n-3 PUFA was lower after intervention with the fish-based product than after intervention with the non-fish-based in the sub-group of non-breastfed infants only. This indicates that the high breastfeeding rate could have blurred the effect of the intervention. This is supported by a randomized study in which consumption of both DHA-enriched eggs (180 mg/day DHA) and regular eggs (40 mg/day DHA) for 6 months improved erythrocyte DHA among ~~6 months-old~~ non-breastfed infants, whereas only the enriched eggs improved DHA in the breastfed infants [34]. Another study on neonates also reported a stronger influence of breast milk on infant erythrocyte DHA status, than six -months supplementation with fish -oil capsules which supplied 250-280 mg/day of DHA, which was nearly three times that of the breast milk [35]. The supplementation resulted in the erythrocyte DHA which was only 0.6 FA% higher than in the placebo group [29].

While the primary outcomes reported from this study indicated that small fish may be an affordable alternative to cow milk in complementary food products [17], for this secondary outcome, the intervention foods formulation was not designed to meet the n-3 LCPUFA recommendation. The WinFood product formulation, was based on balancing protein intake from Cambodian animal source foods and micronutrients. The low content of n-3 LCPUFA in WF and WF-L is ~~due~~ the result of ~~to~~; 1) technologically limited fat content in the products, 2) the limitation of how much animal source food can be added to provide the desired level of total protein, and 3) combined with the relatively low content of n-3 LCPUFA in the freshwater fish. With a total fat content of only 10% and the addition of tropical freshwater fish to the WF and WF-L products that gave rise to an n-3 LCPUFA content of around 1.6 FA%, thus resulting in an estimated daily intake of n-3 LCPUFA of 87 mg/day. Intakes of n-3 LCPUFA in the same range did not result in any change in the on blood fatty acid

composition. An observational study in pre-school South African children from urban and rural areas, did not detect any difference in erythrocyte n-3 LCPUFA despite a significantly higher intake of EPA (20 mg/day) and DHA (32 mg/day) in the children from the urban compared to the rural areas (1 and 2 mg/day, respectively) [27]. Supplementation of school-age (6-10 years) children in Australia and Indonesia with a daily dose of 88 mg of DHA and 20 mg of EPA for 12 months did result in higher plasma DHA in both countries, but did not affect plasma EPA in the Indonesian children [28].

Whole blood DHA in the ~~breastfed~~ Cambodian infants at the beginning of the study was relatively high, compared to their peers from ~~developed countries~~ high-income countries, even for infants who were no longer breastfed at endline. At 15 months of age, the whole blood fatty acid composition of infants in this study was similar to that observed in ~~Cambodian infants in~~ a study by Agostoni et al. [36] with a breastfeeding rate of 99% (online supplementary table 1). In both studies, Cambodian infants had higher DHA, AA, and total PUFA as well as an increased ratio of DHA to ALA, compared to Italian or Danish infants of similar age. ~~Even the Cambodian infants in this study who were no longer breastfed at endline, had high DHA, EPA, and n-6/n-3 PUFA ratio compared to Danish and Italian infants.~~ The good n-3 LCPUFA status of the Cambodian infants could indicate that n-3 LCPUFA-rich complementary foods may not be of great importance to ensure a good n-3 LCPUFA status in this population. This could be due to the extended breastfeeding practice, and or, could also be due to differences in the overall diet in the Cambodian settings relative to that in Europe, e.g. the overall intake of fat. As freshwater fish remains a significant dietary source in this population, it is worth investigating in future studies if the contribution of n-3 LCPUFA from freshwater fish to the Cambodian infants is more efficient when channelled through their mothers' diet.

The lack of an overall effect of the freshwater fish-based complementary foods on n-3 LCPUFA in these mostly breastfed infants could be due to the low supply of n-3 LCPUFA from the fish-based intervention foods. The low content of n-3 LCPUFA in WF and WF-L is due to a technologically limited fat content in the products, combined with the relatively low content of n-3 LCPUFA in the freshwater fish. The fat content in the fish-based intervention food was only around 10%. With addition of the tropical freshwater fish, the n-3 LCPUFA content in the WF and WF-L product was around 1.6 FA% giving rise to an estimated daily intake of n-3 LCPUFA from the WinFood fish-based products of 86.5 mg/day, which is equivalent to the intake from 6 g of raw wild Atlantic salmon, 37 g of cod [20].

Other studies have also reported an absence of an effect of similar intakes of n-3 LCPUFA on blood fatty acid composition. No difference in erythrocyte n-3 LCPUFA was observed between 3.6 year-old South African children from urban and rural areas, despite a significantly higher intake of EPA (20 mg/day) and DHA (32 mg/day) in the children of the urban compared to the rural areas (1 and 2 mg/day, respectively) [27]. Supplementation of school-age (6-10 years) children in Australia and Indonesia with a daily dose of 88 mg of DHA and 20 mg of EPA for 12 months did result in higher plasma DHA in both countries, but did not affect plasma EPA in Indonesian children [28]. supplementation of 6 month-old infants with 180 vs 40 mg/d of DHA from eggs for 6 months only resulted in a difference in erythrocyte DHA of 0.6 FA% [29].

This is supported by a study that found strong correlation between infant erythrocytes DHA and breastmilk DHA even in the presence of infant supplementation with a high doses of n-3 LCPUFA [23]. Breastfeeding is nearly universal in Cambodia, with 96% breastfeeding of the 0-2 year-old children, and a median duration of breastfeeding of 18.8 months in rural area

~~[24]. The majority of the infants in the current study were breastfed during the intervention period.~~

~~Breastfeeding was associated with higher whole blood EPA and DHA as well as higher AA and total PUFA and a lower n-6/n-3 PUFA ratio compared to the non-breastfed infants [25]. Furthermore, breastfeeding was found to modify the effect of the intervention. Among the sub-group of non-breastfed infants, the infants in fish-based intervention groups had a lower ratio of n-6/n-3 PUFA compared to the infants in the CSB food groups. This indicates that the high breastfeeding rate could have blurred the effect of the intervention. This is supported by a randomized study in which consumption of both DHA-enriched eggs (180 mg/day DHA) and regular eggs (40 mg/day DHA) for 6 months improved erythrocyte DHA among 6 months-old non-breastfed infants, whereas only the enriched eggs improved DHA in the breastfed infants[26].~~

4.2. Strengths and limitations

To our knowledge, this study is the first randomized intervention study analysing the impact of freshwater fish consumption on n-3 LCPUFA status of infants. The result showed lack of impact on n-3 LCPUFA status from small amounts of freshwater fish in processed complementary food products, in infants with a high frequency of breastfeeding. ~~The result provides the first evidence on the absence of n-3 LCPUFA benefit from small amounts of freshwater fish in complementary foods for infant and young children.~~ The results support the importance of continued emphasis on the benefits of continuing breastfeeding in dietary recommendations for infants and young children. ~~The result can contribute to dietary recommendation for infants and young children.~~ The validity of the data ~~results~~ in this study, are supported by its similarity with previously reported whole blood fatty acid composition of

Cambodian infants [36] differences in fatty acid composition of breastfed compared to non-breastfed [37], and differences in fatty acid composition of boys and girls (online supplementary table 2) [38, 39]. ~~Furthermore, the observed endline values of whole blood DHA+EPA closely match predicted value based on the daily intake of EPA+DHA and the equation by Patterson et al. (actual values for the fish-based and non-fish-based groups 3.43±0.79 and 3.41±0.80 FA%, respectively, vs predicted values 2.93±0.10 vs 2.70±0.03 FA%, respectively). With 300 infants, we should have a power of around 0.75 to detect the predicted difference (0.23 FA% - i.e. around 0.3 SD), so the fact that we do not see this difference could indicate that we overestimated the intake.~~

~~Although, there are some limitations in our study~~ Unfortunately, we ~~posit that they do~~ were not ~~diminish~~able to assess the ~~validity~~ actual intake of the ~~results. The design is limited by~~ intervention products and thus, do not know the ~~lack~~ exact n-3 LCPUFA intake. However, the observed endline values of ~~direct assessment of fatty acid intake from other~~ whole blood DHA+EPA closely match predicted value based on the estimated daily intake of EPA+DHA from the intervention food and breast milk [30, 32] and the equation by Patterson et al., [40]. ~~breast milk consumption, but the use of randomization should have ensured that consumptions was homogeneous in the fish-based and non-fish-based groups.~~ Our study could have benefited from the use of erythrocyte fatty acid measurement, which ~~best~~ better reflects ~~a~~ the long-term ~~accumulation of LCPUFA.~~n-3 LCPUFA intake and thus could have given a more precise measure of intake during the intervention. Nevertheless, whole blood fatty acid sampling was practically more feasible under the field condition and provides a balanced picture of PUFA intakes especially in groups ~~[32]. Group~~ [41].

The study design is limited by being a secondary outcome of an intervention in which the intervention foods were not formulated to fulfill the n-3 LCPUFA requirements, and ~~we did not make direct assessment of~~ the fatty acid intake from other foods and breast milk consumption was not directly assessed. However, the strength of the randomization should have ensured that consumptions were homogeneous in the fish-based and non-fish-based groups. Shelf life was not measured, but preclusion was taken by producing the food within one to three months before distribution, to assure that all intervention foods were consumed within six months of production (or changed with the products from new batch). This diminish the risk for contamination or degradation.

Additionally, differences in number of infants who were stunted, wasted and whose family income was generated from fishery were relatively small and thus, would not be expected to have an impact on the results of the study. ~~However, a~~ Apart from the differences in fish in the complementary foods, three of the products (CSB++ and CSB+, and WF-L) were also ~~provided micronutrient, which~~ fortified with micronutrients. Some of these, might have improved the infants' ~~fatty acid~~n-3 LCPUFA status, and reduced the effect of n-3 LCPUFA intake from fish-based intervention foods, as micronutrient supplementation has been shown to affect ~~n-3 LCPUFA~~ PUFA status ~~[21, 27]~~[28, 36] by a yet unknown mechanism.

4.3. Conclusions

In conclusion, daily consumption of complementary ~~foods~~ food products with 10-12% local freshwater fish for 9 months by largely breastfed infants resulted in an estimated maximal intake of ~~up to 86.5~~87 mg/day of n-3 LCPUFA, ~~additional~~ in addition to the intake from breast milk and other sources. The inclusion of the small amounts of freshwater fish in the complementary food had no significant effect on whole blood n-3 LCPUFA ~~in this population~~

~~of largely breastfed infants. The~~ , but the n-6/n-3 PUFA ratio was reduced among the non-breastfed infants. The overall whole blood PUFA profile of the Cambodian infants in this study was comparable to or better than infants in ~~more affluent~~ high-income countries, presumably due to the high rate of prolonged breastfeeding. ~~Thus,~~ This indicates that supplementation with n-3 LCPUFA ~~in this population~~ may not be necessary as long as the breastfeeding rate is high~~. Further~~, but studies of the effect of freshwater fish on n-3 LCPUFA status in communities with lower breastfeeding rate could clarify the amount of fish needed to achieve potential ~~developmental benefits.~~ benefits. It is noted that breastfeeding practices were not compromised by the intervention and despite of the lack of pronounced effect on n-3 LCPUFA status of largely breastfed children, early introduction of complementary food with local fish will contribute to food diversification and benefits beyond the breastfeeding stage.

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Table 1. Intervention foods content per 100 g dry weight[1]

	WF	WF-L	CSB++	CSB+
Energy (kcal)	474	428	458	482
Carbohydrate (g)	79.9	73.7	73.6	69.9
Protein[2] (g)	15.4	12.6	16.8	14.6
Fat[3] (g)	9.8	8.7	9.6	14.5
Fat contributed from (g)[3]:				
Rice, white, milled	1.26	1.15	-	-
Fish, *Esomus longimanus*	1.22	-	-	-
Fish, *Paralaubuca typus*	2.27	-	-	-
Spider, *Haplopelma species*	0.24	-	-	-
Mixed small fish species	-	2.71	-	-
Soya oil	4.80	4.80	3.00	-
Sunflower oil	-	-	-	8.50
Maize (white or yellow)	-	-	1.83	2.05
Dehulled soya (flour)	-	-	4.68	-
Whole soya	-	-	-	3.96
Skimmed milk powder	-	-	0.12	-
Fatty acids[4] (g)				
SFA	2.45	2.08	1.38	1.68
MUFA	2.37	1.93	2.26	3.31
PUFA	3.96	3.85	5.25	8.41
n-6 PUFA	3.32	3.19	4.67	8.11
LA (18:2n-6)	3.14	3.06	4.67	8.11
AA (20:4n-6)	0.11	0.07	0.00	0.00
n-3 PUFA	0.63	0.64	0.56	0.31
ALA (18:3n-3)	0.44	0.47	0.56	0.31
EPA (20:5n-3)	0.06	0.03	0.00	0.00
DPA (22:5n-3)	0.03	0.03	0.00	0.00
DHA (22:6n-3)	0.08	0.10	0.00	0.00

[1]Food groups: WF, WinFood; WF-L, WinFood-L; CSB++, corn-soy blend plus plus; CSB+, corn-soy blend plus included distributed oil

[2]Refer to Skau et al., (2015) [17]

[3]Calculated from the fat contributed from each ingredient used in the product composition

[4]Calculated from the fatty acid composition of the different food ingredients, which was based on analyses for fish (*Esomus longimanus*, *Paralauba typus* and mixed batches of small fish), spider (*Haplopelma* species) and database information (frida.fooddata.dk [20]) for the rest of the ingredients. Fat content in CSB+ includes the supplied oil to be added at the time of consumption.

TABLE 2. Estimated maximum intake of fat and fatty acid from intervention food and the overall contribution of energy from the intervention foods and from the fat in these relative to total daily energy requirement and estimated requirement from complementary food[1,2,3]

	WF	WF-L	CSB++	CSB+	P^4	Fish-based	Non-Fish-based	P^4
	(n=85)	(n=88)	(n=83)	(n=84)		(n=173)	(n=167)	
Fatty acid and fat intake (g/day)								
SFA	1.25 ± 0.58	1.13 ± 0.44	0.70 ± 0.32	0.87 ± 0.40	<0.001	1.19 ± 0.51	0.79 ± 0.37	<0.001
MUFA	1.21 ± 0.56	1.04 ± 0.41	1.15 ± 0.53	1.68 ± 0.73	<0.001	1.13 ± 0.49	1.42 ± 0.69	<0.001
PUFA	2.03 ± 0.94	2.08 ± 0.81	2.68 ± 1.23	4.26 ± 1.87	<0.001	2.06 ± 0.87	3.47 ± 1.77	<0.001
n-6 PUFA	1.70 ± 0.78	1.73 ± 0.67	2.38 ± 1.09	4.11 ± 1.80	<0.001	1.71 ± 0.73	3.25 ± 1.72	<0.001
LA (18:2n-6)	1.61 ± 0.74	1.66 ± 0.65	2.38 ± 1.09	4.10 ± 1.81	<0.001	1.63 ± 0.69	3.25 ± 1.72	<0.001
AA (20:4n-6)	0.05 ± 0.03	0.03 ± 0.01	0.00	0.00	<0.001	0.04 ± 0.02	0.00	<0.001
n-3 PUFA	0.32 ± 0.14	0.34 ± 0.13	0.29 ± 0.13	0.16 ± 0.08	<0.001	0.34 ± 0.14	0.22 ± 0.13	<0.001
ALA (18:3n-3)	0.22 ± 0.10	0.25 ± 0.10	0.29 ± 0.13	0.16 ± 0.07	<0.001	0.24 ± 0.10	0.22 ± 0.12	0.092
EPA (20:5n-3)	0.03 ± 0.01	0.02 ± 0.01	0.00	0.00	<0.001	0.02 ± 0.01	0.00	<0.001
DPA (22:5n-3)	0.02 ± 0.01	0.02 ± 0.01	0.00	0.00	<0.001	0.02 ± 0.01	0.00	<0.001
DHA (22:6n-3)	0.04 ± 0.02	0.05 ± 0.02	0.00	0.00	<0.001	0.05 ± 0.02	0.00	<0.001
Total fat	5.0 ± 2.3	4.7 ± 1.8	4.9 ± 2.3	7.4 ± 3.2	<0.001	4.9 ± 2.1	6.2 ± 3.0	<0.001
Energy from intervention food fat relative to the daily energy requierement[5] (%)	6.32 ± 23.80	5.8 ± 2.2	6.12 ± 2.76	9.32 ± 34.90	<0.001	6.0 ± 2.56	7.7 ± 3.7	<0.001
Energy from intervention foods relative	34.9 ± 15.8	33.0 ± 12.4	33.4 ± 14.8	35.5 ± 15.1	0.294	33.9 ± 14.1	34.4 ± 14.9	0.620

to the daily energy requierement[5] (%)								
Energy from intervention food fat relative to the energy requirement from complementary food[5] (%)	14.2 ± 6.2	13.0 ± 4.7	13.8 ± 5.8	21.0 ± 8.6	<0.001	13.6 ± 5.5	17.4 ± 8.1	<0.001
Energy from intervention foods relative to the requirement from complementary food[5] (%)	79.2 ± 34.8	74.2 ± 26.5	75.5 ± 31.8	80.4 ± 32.8	0.252	76.7 ± 30.9	78.0 ± 32.3	0.658

[1]Values are mean ± SD for 9 months intervention study, based on the exact number of days of participation from baseline to endline for each child. Food groups: WF, WinFood; WF-L, WinFood-L; CSB++, corn-soy-blend plus plus; CSB+, corn-soy-blend plus including the distributed oil.

[2]Compliance data were collected every month for 340 of the 358 infants who completed the study (came to both baseline and endline). Compliance was based on amount of food returned to the food distributor every month. The amount of food retained by the participants represents the maximum intake since any wasted food after preparation was not registered.

[3]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

[4]*P* values for difference between groups based on Kruskal-Wallis test.

[5]Daily energy requirement was set at 682 kcal/day, 830 kcal/day and 1092 kcal/day for infants at age groups 6-8, 9-11 months and children age 12-23 months, respectively. Daily energy required from complementary food was set at 269 kcal/day, 451 kcal/day, and 746 kcal/day for infants at age groups 6-8, 9-11 months and children age 12-23 months, respectively [21].

Table 3. Characteristics of study participants by food group[1,2]

	WF	WF-L	CSB++	CSB+
	(n=106)	(n=104)	(n=103)	(n=106)
Boys, n (%)	56 (52.8)	54 (51.9)	54 (52.4)	56 (52.8)
Age, mo	5.79 ± 0.50	5.95 ± 0.59	5.87 ± 0.53	5.90 ± 0.59
Anthropometry				
Weight, kg	6.89 ± 0.90	6.89 ± 0.84	6.75 ± 0.94	6.82 ± 0.82
Length, cm	64.4 ± 2.5	64.6 ± 2.3	64.0 ± 2.7	64.4 ± 2.4
Weight-for-length z score	-0.3 ± 1.0	-0.4 ± 0.9	-0.4 ± 1.0	-0.4 ± 0.9
Infants with z score <-2, n (%)	2 (1.88)	4 (3.85)	7 (6.80)	5 (4.72)
Length-for-age z score	-0.9 ± 1.0	-0.9 ± 1.0	-1.1 ± 1.1	-1.0 ± 0.9
Infants with z score , <-2, n (%)	13 (12.3)	13 (12.5)	22 (21.4)	14 (13.2)
Breastfeeding status				
Age when stop breastfeeding (%)	14.95 ± 2.32	15.38 ± 1.91	15.40 ± 1.94	15.48 ± 1.47
Age when introduced to solid food, mo	5.19 ±1.54	5.31 ± 1.44	5.25 ± 1.55	5.29 ± 1.46

Children who were still breastfed at baseline, n (%)	102 (97.1)	101 (98.1)	101 (98.1)	106 (100)
Children who were still breastfed at endline, n (%)	59 (69.4)	74 (80.4)	70 (79.5)	75 (81.5)
Biological indicators, n (%)				
Haemoglobin, g/L	10.77 ± 0.99	10.66 ± 0.89	10.80 ± 0.86	10.77 ± 0.92
CRP median, interquartile range (mg/L)	9.70 (36.97)	9.39 (43.24)	9.46 (20.03)	7.11 ± 29.85
Infants with CRP < 5 mg/L	36 (35.0)	44 (43.1)	34 (34.0)	37 (37.4)
Infants with CRP ≥ 5 mg/L	67 (65.1)	58 (56.9)	66 (66.0)	62 (62.6)
Compliance				
Total compliance (kg/9 mo intervention)	14.7 ± 6.8	14.7 ± 6.9	13.8 ± 7.3	13.4 ± 7.4
Total compliance rate (% of distributed food)	65.2 ± 30.0	65.2 ± 30.5	61.2 ± 32.6	59.5 ± 32.9
Treat water before drinking, n (%)				
Boil or filter	82 (77.4)	73 (70.2)	80 (77.7)	76 (71.7)
No treatment	22 (20.8)	27 (26.0)	22 (21.4)	27 (25.5)
Toilet facilities, n (%)				
Flush toilet or pit latrine	24 (22.6)	26 (25.0)	22 (21.4)	19 (17.9)
No toilet/in the nature	80 (75.5)	77 (74.0)	80 (77.7)	84 (79.3)
Household primary income, n (%)				
Labour	18 (17.0)	9 (8.7)	7 (6.8)	15 (14.2)
Fishery	3 (2.8)	2 (1.9)	6 (5.8)	6 (5.7)
Farming (incl. livestock owning)	60 (56.6)	63 (60.6)	66 (64.1)	63 (59.4)
Economic possessions, n (%)				

TV	68 (64.2)	72 (69.2)	71 (68.9)	81 (76.4)
Mobile phones	61 (57.6)	63 (60.6)	66 (64.1)	75 (70.8)
Bicycle	78 (73.6)	75 (72.1)	80 (77.7)	74 (69.8)
Motorcycle	46 (43.4)	48 (46.2)	50 (48.5)	53 (50.0)
Livestock	78 (73.6)	77 (74.0)	85 (82.5)	87 (82.1)
Whole blood fatty acid composition (% of fatty acids)[3,4]				
SFA	45.53 ± 1.85	45.79 ± 1.83	45.45 ± 1.82	45.34 ± 2.06
MUFA	24.05 ± 2.53	23.95 ± 2.48	24.03 ± 2.59	24.19 ± 2.39
PUFA	26.73 ± 2.82	26.51 ± 2.53	26.71 ± 2.58	26.73 ± 2.05
n-6 PUFA	23.10 ± 2.74	22.85 ± 2.27	23.03 ± 2.43	23.13 ± 1.85
n-3 PUFA	3.62 ± 0.69	3.66 ± 0.60	3.68 ± 0.64	3.60 ± 0.63
EPA (20:5n-3)	0.21 ± 0.06	0.22 ± 0.07	0.23 ± 0.08	0.21 ± 0.07
DPA (22:5n-3)	0.53 ± 0.09	0.52 ± 0.10	0.53 ± 0.10	0.51 ± 0.09
DHA (22:6n-3)	2.71 ± 0.61	2.73 ± 0.52	2.73 ± 0.55	2.69 ± 0.53
Total fat (µg/dL)	354 ± 91	350 ± 87	353 ± 95	341 ± 83

[2]Values are mean ± SD or n (%). Food groups: WF, WinFood; WF-L, WinFood-L; CSB++, corn-soy-blend plus plus; CSB+, corn-soy-blend plus with inclusion of the separately distributed oil

[3]Out of 419 infants who participated in the study, blood was not taken from 30 infants at baseline, so the baseline data is based on n=389 (WF=98, WF-L=97, CSB++=95, CSB+=99).

[3]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, docosahexaenoic acid

[4]The listed n are the number of infants who entered the study, information for all variables was not always complete, so the given data was based on available data.

TABLE 4. Whole blood fatty acid composition and total fatty acids after 9 months of intervention[1,2,3]

	WF	WF-L	CSB++	CSB+	*P*	Fish-based	Non-fish-based	*P*
	(n=73)	(n=80)	(n=79)	(n=76)		(n=153)	(n=155)	
SFA (FA%)	43.80 ± 2.01	43.84 ± 1.50	43.64 ± 1.59	43.94 ± 1.95	0.888	43.82 ± 1.75	43.79 ± 1.78	0.936
MUFA (FA%)	22.25 ± 3.50	21.80 ± 3.31	21.76 ± 3.20	22.10 ± 2.79	0.575	22.02 ± 3.40	21.93 ± 3.00	0.608
PUFA (FA%)	30.35 ± 3.33	30.75 ± 3.18	30.91 ± 3.08	30.33 ± 3.08	0.433	30.55 ± 3.25	30.62 ± 3.08	0.482
n-6 PUFA (FA%)	26.12 ± 2.92	26.37 ± 2.70	26.61 ± 2.73	26.05 ± 2.70	0.463	26.25 ± 2.80	26.34 ± 2.72	0.608
LA (18:2n-6) (FA%)	14.02 ± 2.89	14.00 ± 2.41	14.20 ± 2.57	13.79 ± 2.41	0.805	14.01 ± 2.64	14.00 ± 2.49	0.729
AA (20:4n-6) (FA%)	9.12 ± 1.86	9.38 ± 1.60	9.50 ± 1.85	9.33 ± 1.70	0.695	9.26 ± 1.73	9.42 ± 1.78	0.915
n-3 PUFA (FA%)	4.23 ± 0.87	4.38 ± 0.87	4.30 ± 0.90	4.28 ± 0.83	0.689	4.31 ± 0.87	4.29 ± 0.87	0.259
ALA (18:3n-3) (FA%)	0.18 ± 0.11	0.17 ± 0.08	0.18 ± 0.08	0.16 ± 0.05	0.649[4]	0.18 ± 0.10	0.17 ± 0.07	0.629[4]
EPA (20:5n-3) (FA%)	0.28 ± 0.10	0.29 ± 0.10	0.31 ± 0.12	0.29 ± 0.10	0.927[5]	0.29 ± 0.10	0.30 ± 0.11	0.634[5]
DPA (22:5n-3) (FA%)	0.67 ± 0.13	0.68 ± 0.12	0.69 ± 0.13	0.66 ± 0.11	0.543	0.67 ± 0.13	0.68 ± 0.12	0.822
DHA (22:6n-3) (FA%)	3.08 ± 0.73	3.21 ± 0.70	3.09 ± 0.75	3.13 ± 0.70	0.539	3.15 ± 0.72	3.11 ± 0.72	0.142

LCPUFA (FA%)	15.78 ± 2.78	16.21 ± 2.38	16.17 ± 2.77	16.03 ± 2.56	0.777	16.01 ± 2.58	16.10 ± 2.66	0.570
n-6/n-3 PUFA	6.39 ± 1.25	6.21 ± 1.09	6.46 ± 1.55	6.30 ± 1.26	0.493	6.29 ± 1.17	6.38 ± 1.41	0.301[5]
n-3 LCPUFA (%)	25.55 ± 2.72	25.84 ± 3.05	25.34 ± 3.03	25.56 ± 2.62	0.542	25.70 ± 2.89	25.44 ± 2.83	0.278
Total fatty acid (μg/L)	334 ± 63	333 ± 52	338 ± 56	339 ± 68	0.927	335 ± 58	338 ± 62	0.696

[1]Values are mean ± SD. *P* values for difference between groups from ANCOVA of the endline values with baseline values, sex, age, age when stop breastfeeding, age when starting complementary food, breastfeeding status in the last 24 hours at endline, and baseline CRP values as covariates

[2]Of the 419 infants enrolled in the study, complete data for endline whole blood fat and covariates were available from 308 infants

[3] FA%, % of all fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCPUFA, long-chain unsaturated fatty acid (≥20C and ≥3 double bonds); n-3 LCPUFA, % of n-3 LCPUFA among total LCPUFA

[4]P-value is based on analysis of log-transformed data

[5]P-value is based on analysis of square root transformed data

TABLE 5. Whole blood fatty acid composition and total fatty acids after 9 months of intervention with food with and without fish segregated by breastfeeding status[1,2, 3]

	All respondents			Breastfed at endline			Not breastfed at endline			
	Breastfed	Non-breastfed	*P*	Fish-based	Non-fish-based	*P*	Fish-based	Non-fish-based	*P*	*P*[6] *interaction*
	n=239	n=69		n=114	n=125		n=39	n=30		
SFA (FA%)	44.04 ± 1.68	42.97 ± 1.79	0.007	44.09 ± 1.61	44.00 ± 1.75	0.891	43.03 ± 1.92	42.89 ± 1.64	0.726	0.677
MUFA (FA%)	21.07 ± 2.49	25.11 ± 3.41	<0.001	20.78 ± 2.36	21.33 ± 2.59	0.128	25.64 ± 3.39	24.42 ± 3.36	0.111	0.003
PUFA (FA%)	31.30 ± 2.51	28.13 ± 3.88	<0.001	31.55 ± 2.37	31.07 ± 2.63	0.071	27.64 ± 3.71	28.76 ± 4.07	0.140	0.005
n-6 PUFA (FA%)	26.75 ± 2.26	24.71 ± 3.63	<0.001	26.96 ± 2.13	26.56 ± 2.36	0.069	24.18 ± 3.47	25.40 ± 3.78	0.092	0.003
LA (18:2n-6) (FA%)	14.07 ± 2.03	13.79 ± 3.90	0.185	14.24 ± 1.85	13.91 ± 2.17	0.075	13.34 ± 4.13	14.37 ± 3.56	0.125	0.010
AA (20:4n-6) (FA%)	9.81 ± 1.53	7.70 ± 1.44	<0.001	9.82 ± 1.44	9.80 ± 1.62	0.790	7.61 ± 1.43	7.80 ± 1.48	0.656	0.570
n-3 PUFA (FA%)	4.55 ± 0.74	3.42 ± 0.68	<0.001	4.60 ± 0.74	4.51 ± 0.73	0.440	3.46 ± 0.62	3.36 ± 0.76	0.557	0.748

ALA (18:3n-3) (FA%)	0.17 ± 0.06	0.21 ± 0.13	0.475[4]	0.17 ± 0.06	0.17 ± 0.06	0.726[4]	0.21 ± 0.16	0.21 ± 0.08	0.787	0.023[4]
EPA (20:5n-3) (FA%)	0.32 ± 0.10	0.22 ± 0.07	<0.001[5]	0.31 ± 0.10	0.32 ± 0.11	0.788[5]	0.21 ± 0.06	0.23 ± 0.08	0.119	0.355[5]
DPA (22:5n-3) (FA%)	0.69 ± 0.12	0.61 ± 0.11	<0.001	0.70 ± 0.12	0.69 ± 0.13	0.590	0.60 ± 0.12	0.62 ± 0.10	0.479	0.446
DHA (22:6n-3) (FA%)	3.35 ± 0.58	2.36 ± 0.61	<0.001	3.40 ± 0.58	3.31 ± 0.58	0.374	2.41 ± 0.55	2.29 ± 0.68	0.296	0.982
LCPUFA (FA%)	16.73 ± 2.28	13.70 ± 2.35	<0.001	16.81 ± 2.16	16.66 ± 2.40	0.563	13.65 ± 2.27	13.78 ± 2.48	0.876	0.575
n-6/n-3 PUFA	6.02 ± 1.02	7.44 ± 1.52	<0.001	6.01 ± 1.02	6.03 ± 1.04	0.757	7.12 ± 1.21	7.85 ± 1.79	0.026	0.026
n-3 LCPUFA (%)	26.19 ± 2.50	23.42 ± 3.01	<0.001	26.32 ± 2.63	26.08 ± 2.37	0.839	23.89 ± 2.89	22.81 ± 3.11	0.139	0.418
Total fatty acid (μg/L)	338 ± 59	332 ± 62	0.658	337 ± 58	339 ± 60	0.941	328 ± 56	337 ± 70	0.625	0.964

[1]Values are mean ± SD. *P* values for difference between groups from ANCOVA of the endline values, with baseline values, sex, age, age when stop breastfeeding, age when starting complementary food, and baseline CRP values as

covariates.

[2]Breastfeeding in the last 24 hours was successfully measured at endline in 357 infants, of whom 278 were breastfed, and 79 were not breastfed in the last 24 hours. The final number of respondents with covariate information was 306; hereof 237 who were breastfed and 69 who were not breastfed in the last 24 hours at endline.
[3]FA%, % of all fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCPUFA, long-chain unsaturated fatty acid (≥20C and ≥3 double bonds); n-3 LCPUFA, % of n-3 LCPUFA among total LCPUFA.

[4]Data were inverse square root transformed to create normal distribution for the statistical analysis.

[5]Data were square root transformed to create normal distribution for the statistical analysis.

[6]*P* for interaction between breastfeeding and fish vs non-fish-based groups.

Figure 1. CONSORT Flow Diagram