Long-chain PUFA supplementation in rural African infants: a randomized controlled trial of effects on gut integrity, growth, and cognitive development[1–3]

Liandré F van der Merwe, Sophie E Moore, Anthony J Fulford, Katherine E Halliday, Saikou Drammeh, Stephen Young, and Andrew M Prentice

ABSTRACT

Background: Intestinal damage and malabsorption caused by chronic environmental enteropathy are associated with growth faltering seen in infants in less-developed countries. Evidence has suggested that supplementary omega-3 (n−3) long-chain PUFAs (LC-PUFAs) might ameliorate this damage by reducing gastrointestinal inflammation. LC-PUFA supplementation may also benefit cognitive development.

Objective: We tested whether early n−3 LC-PUFA supplementation improves infant intestinal integrity, growth, and cognitive function.

Design: A randomized, double-blind, controlled trial [200 mg DHA and 300 mg EPA or 2 mL olive oil/d for 6 mo] was conducted in a population of 172 rural Gambian infants aged 3–9 mo. The primary endpoints were anthropometric measures and gut integrity [assessed by using urinary lactulose:mannitol ratios (LMRs)]. Plasma fatty acid status, intestinal mucosal inflammation (fecal calprotectin), daily morbidity, and cognitive development (2-step means-end test and an attention assessment) were secondary endpoints.

Results: PUFA supplementation resulted in a significant increase in plasma n−3 LC-PUFA concentrations ($P < 0.001$ for both DHA and EPA) and midupper arm circumference (MUAC) (effect size: 0.31 *z* scores; 95% CI: 0.06, 0.56; $P = 0.017$) at 9 mo of age. At 12 mo, MUAC remained greater in the intervention group, and we observed significant increases in skinfold thicknesses ($P \leq 0.022$ for all). No other significant differences between treatment groups were detected for growth or LMRs at 9 mo or for secondary outcomes.

Conclusions: Fish-oil supplementation successfully increased plasma n−3 fatty acid status. However, in young, breastfed Gambian infants, the intervention failed to improve linear growth, intestinal integrity, morbidity, or selected measures of cognitive development. The trial was registered at www.isrctn.org as ISRCTN66645725. *Am J Clin Nutr* 2013;97:45–57.

INTRODUCTION

Chronic environmental enteropathy, which is characterized by intestinal villous atrophy, crypt hyperplasia, and inflammatory cell invasion of the lamina propria, affects many children living in developing countries (1–7). Repeated exposure to a wide variety of pathogenic organisms and allergens that are due to the ingestion of contaminated weaning foods and water are thought to initiate this persistent inflammation of the gut, which leads to intestinal damage and malabsorption (7–12). Mucosal injuries that result from inflammatory responses are slow or resistant to healing and repair and leave the gut vulnerable to additional damage (9, 13). This decreased gut integrity, which is shown by markedly and consistently raised lactulose:mannitol ratios (LMRs)[4] in the dual-sugar permeability test (3, 4, 11), begins in Gambian infants at ∼3 mo of age (when weaning foods are first introduced) and is associated with the faltering in both height and weight seen in these rural African children (3, 9). There is evidence that supplementary n−3 long-chain PUFAs (LC-PUFAs) might reduce or delay this damage by reducing gastrointestinal inflammation (14–19), but this has not been tested in an African population.

Meta-analyses of the effects of n−3 PUFA interventions on growth outcomes have been published for high-income countries and concluded that there is no substantive evidence of a benefit (20–23). However, the evidence base in respect to infants living in low-income countries, where growth faltering is of much greater concern, is exceptionally weak. We could not find any published or registered trials in low- to middle-income countries that investigated the effects of LC-PUFA supplementation in infants on growth or immune modulation.

We performed a double-blind, randomized, controlled, parallel-group trial that was designed to test the hypothesis that supplementary n−3 LC-PUFAs would reduce intestinal damage caused by chronic enteropathy and reduce the associated growth faltering

[1] From the Medical Research Council (MRC) International Nutrition Group (LFvdM, AJF, SEM, and AMP) and Faculty of Infectious and Tropical Diseases (KEH), London School of Hygiene and Tropical Medicine, London, United Kingdom; the MRC Keneba, Keneba, The Gambia (LFvdM, AJF, SEM, AMP, and SD); and the MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, United Kingdom (SY).

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[3] Address reprint requests and correspondence to AM Prentice, Medical Research Council International Nutrition Group, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom. E-mail: andrew.prentice@lshtm.ac.uk.

[4] Abbreviations used: AA, arachidonic acid; FA, fatty acid; LC-PUFA, long-chain PUFA; LMR, lactulose:mannitol ratio; MRC, Medical Research Council; MUAC, midupper arm circumference.

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in 3–9-mo-old Gambian infants living in the rural area of West Kiang. Supplementation was started at 3 mo of age in an attempt to prime the intestinal mucosa and delay or prevent the initiation of events that lead to gut damage.

One of the consequences of increased gut permeability is the translocation of antigenic macromolecules, which stimulate and perpetuate local and systemic inflammatory responses (9). To test whether n−3 LC-PUFA supplementation is associated with reduced infant systemic inflammation (either via immune-modulation or by reducing gut leakiness), concentrations of acute-phase proteins plasma C-reactive protein, α-1-glycoprotein, and albumin were also assessed. In addition, stool calprotectin was measured as a marker of intestinal inflammation. General morbidities experienced by infants (eg, diarrhea, vomiting, and fever) were measured to examine treatment effects in terms of clinical wellness, and these data contributed to safety monitoring.

In addition, the trial allowed investigations into the effect of n−3 LC-PUFA supplementation on developmental outcomes. Evidence has suggested that cognitive function in children is affected by nutritional and health status, particularly during the rapid-growth phase in the first 2 y of life (24–27), and stunting during infancy has been related to poor cognitive function in late childhood (28, 29). Gambian infants may suffer developmental insults in early life, considering the high rates of stunting and infections they suffer. LC-PUFAs play a central role in the normal development and functioning of the brain and central nervous system, and associations between infant DHA status and neurodevelopmental outcomes have been shown in several studies (30–36). Infant LC-PUFA–supplementation studies have shown mixed results in high-income countries (37), but to our knowledge, such research in a low-income country setting such as rural Gambia is lacking.

SUBJECTS AND METHODS

Subjects

The study was carried out in the West Kiang region of The Gambia from May 2007 to October 2008. The Gambian climate is tropical with 2 main seasons that consist of a hot, rainy season (June to October) and a cooler, dry season (November to May). West Kiang is 145 km inland from the capital and spans an area of 80 km^2. Keneba is the largest village in this rural area, where subsistence farming (primarily rice and groundnuts) predominates. A combination of in utero growth retardation, poor-quality and frequently contaminated weaning foods, and high levels of infection cause moderately severe growth faltering in almost all children with an onset at ~3 mo postpartum. All infants aged 3 mo who were living in the 16 largest villages of West Kiang and not enrolled in any other study were eligible to take part in the current study. In total, 220 infants were assessed for eligibility, of whom 183 infants were randomly assigned (**Figure 1**). Infants with severe congenital abnormalities that could affect growth and development, infants from multiple births, and infants with known HIV infection were ineligible for participation.

Potential subjects were identified from the West Kiang Demographic Surveillance System (http://www.ing.mrc.ac.uk/research_areas/west_kiang_dss.aspx). Subjects were automatically allocated a study number by the database system on recruitment. Each study number had previously been randomly assigned to one of 4 treatment codes (4 rather than 2 codes were used to promote blinding) represented by 4 simple pictures on the supplements so that infants were allocated to either the n−3 LC-PUFA or the control group in a 1:1 ratio. The trial statistician implemented a permuted block random assignment (block size = 16), which ensured a uniform distribution of treatments across the seasons of birth. After mothers had given their consent, and their infants had been recruited, a fieldworker issued them with a card printed with the appropriate picture. Mothers were asked to bring their picture-coded cards when their infants were brought to be administered the supplement. Fieldworkers were also given a treatment booklet from which to crosscheck for any card swapping. All infants in the study stayed on the same treatment allocations throughout the duration of treatment. Participants, staff, and investigators remained blinded to treatment assignment throughout the duration of the trial.

Intervention and study procedures

Supplementation started at 3 mo of age and ended at 9 mo of age when all outcome measurements were made apart from cognitive function (assessed at 12 mo of age). Field assistants administered the supplement at a central meeting point in each of the villages each day. Sterile, graded pastettes were used to squeeze the oil into the side of the mouth of each infant. Mothers were asked to breastfeed their infants immediately after the oil had been given. Field assistants who administered the dose recorded daily compliance.

The intervention group received 2 mL of highly purified fish oil, which supplied 200 mg DHA and 300 mg EPA/d. The control group was given the same volume of olive oil. Both oils were supplied by Nordic Naturals Inc and contained 1.25% lemon oil for blinding and 0.5% rosemary extract and *d*-α-tocopherol as an antioxidant. On the advice of the trial monitor, the usual tocopherol concentration of 30 IU/5 mL was reduced to 5 IU/5 mL as appropriate to this age group. The dosage of 500 mg combined DHA plus EPA/d was designed to achieve a substantial increase in plasma n−3 PUFA to both eliminate any existing deficiencies and elicit a therapeutic response.

A morbidity questionnaire was administered to mothers or caretakers on a daily basis to assess diarrhea, vomiting, cough, fever, and (for safety monitoring) possible abnormal bleeding that might have been related to clotting interference. If a mother felt that her infant was in need of medical attention, she was free to take her infant to the Medical Research Council (MRC) clinic in Keneba to be seen by a doctor or request a visit by a nurse. If the infant was seen by a nurse or doctor, the diagnosis and treatment details were recorded.

To reduce intraindividual variance and estimate the reliability of outcome variables, primary endpoints were taken in triplicate (on separate days) at baseline and endpoint visits, and the median value was used for analyses. Mothers were asked to collect a sample of their infant's stool into a pot provided the evening before they were due to come to Keneba for the clinic visit and to continue the collection during the clinic visit if no stool was passed the previous evening. Stools were thoroughly homogenized before freeze drying to a constant weight. Mothers expressed ~5 mL breast milk from each breast before their infants received their first feed at the clinic and again immediately after they had fed their infants. The 4 breast-milk samples were pooled by mixing together 1 mL of each sample, and an aliquot of this pooled sample was stored at −70°C until analysis. An experienced nurse

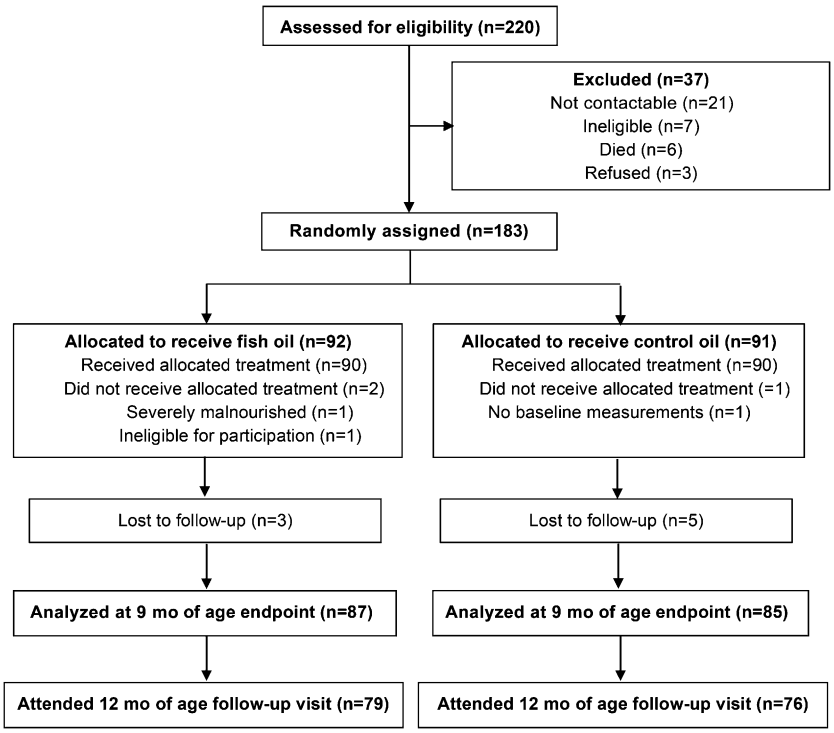

FIGURE 1. Subject flowchart summarized according to Consolidated Standards of Reporting Trials that shows the number of subjects randomly assigned, lost to follow-up, and analyzed by treatment arm.

drew 2 mL venous blood from infants at baseline and endpoint into anticoagulant-coated tubes. At this age, it is not possible to obtain fasted samples. Each month, a basic weaning-food questionnaire was administered to the mother that asked about which foods the infant had been weaned onto the previous month.

Primary endpoints

Anthropometric measures at 3 and 9 mo were performed (by LFvdM). Infant lengths and weights were measured by using a Harpenden Infantometer length board (Holtain Ltd) and electronic baby scale (model 336; Seca), to a precision of 0.1 cm and 0.01 kg, respectively. Left-side triceps, biceps, and subscapular skinfold thicknesses were measured with a skinfold-thickness caliper (Holtain Tanner/Whitehouse) to 2-mm precision, and midupper arm circumference (MUAC) was measured by using a paper measuring tape to a precision of 0.1cm. Head circumference, as a proxy for brain size, was measured to the nearest 0.1 cm with a stretch-proof measuring tape (Model CTM08l Chasmors Ltd) around the maximum circumference of the head (forehead to occiput). Measures for which reference data were available were expressed as *z* scores on the basis of WHO Growth Reference Data (38, 39). Anthropometric measures were repeated at 12 mo by a fieldworker.

Intestinal absorptive capacity and permeability were assessed by using the dual-sugar permeability test (4). Infants were given a 2 mL/kg body weight dose of sugar solution that contained 400 mg lactulose (Lactulose Solution BP; Sandoz Ltd) and 100 mg mannitol (Sigma-Aldrich Co) per 2 mL H_2O. The recovery of the monosaccharide mannitol provides a measure of passive intestinal absorption and is reduced by villous atrophy. Paracellular uptake of the nondigestible disaccharide lactulose is a marker of intestinal leakiness. Therefore, intestinal permeability is measured as the urinary lactulose concentration divided by the urinary mannitol concentration. In addition, the ratio of urinary lactulose:lactose is measured as an indication of lactase activity. By measuring the total urine volume passed over 5 h postdose (Hollister U-bag; Abbot Laboratories), the percentage of recoveries of the 2 probes was calculated as a reflection of the amounts taken up by the passive and paracellular intestinal routes. Urine samples were stored with 2–3 drops of chlorhexidinegluconate (5% wt:vol) as a bacteriostatic agent.

Cognitive development at 12 mo

At 12 mo of age (±7 d), infants were brought in to the MRC Keneba field station for their final follow-up visit, during which cognitive development was tested by using a 2-step means-end problem-solving test (Willatts' Infant Planning Test) (40–42) and a single-object task attention assessment (toddler attention assessment) (43). The 12-mo time point for assessments was determined in a pilot study that evaluated the frequency distribution of test scores in 75 West Kiang infants aged 10–12 mo.

On the morning of the test, mothers were asked to ensure that their infant had been fed before the 15-min test. For standardization, the 2-step Infant Planning Test was performed first and was followed by the single-object task toddler assessment. If an infant did not respond during the Infant Planning Test, the attention assessment was completed first, and a second attempt at the planning test was made thereafter. The assessment was completed in a restricted-access room with few distractions. For both tests, the mother was asked to sit on a chair with her infant on her lap facing a table placed in front of her. She was asked not to help, encourage, or prompt her child by either words or actions and not to

encourage the infant's eye contact when he or she looked up at the mother. A Gambian fieldworker (SD) carried out all tests. The tests were filmed and saved for later scoring.

The aim of the Willatts' 2-step Infant Planning Test is to present a challenge to an infant and observe whether the infant is capable of planning and executing a solution. The challenge is to retrieve a toy, which is either concealed or out of reach. The infant needs to manipulate an intermediary object (cloth or cover) to retrieve the goal, and the intentionality which the child uses to retrieve this goal (toy) is assessed. This problem-solving test is simple to perform and has proven sensitive to nutritional interventions in infants (40, 41). It has been successfully used in high-income countries and also in middle- and low-income countries (44, 45). The test was performed and scored as described by Willatts et al (46). For the first outcome measure, trial behavior scores were averaged to give a mean total intention score. Each trial in which there was a score of $\geq$1 for all 3 behaviors was considered to be an intentional solution. The number of trials in which the child showed some sign of intention became the second outcome measure of the total intentional solution score.

By conducting this test at 12 mo of age, it was possible to combine it with the toddler attention assessment. This test involves a free-play task, whereby the toddler's attention to a complex toy is assessed. Infant habituation, which is a decrease in attention to a repeatedly presented stimulus, is considered a basic tool for the assessment of cognition or processing speed in infancy. The test was performed by using previously described methods and equipment (43). VirtualDub Mpeg-2, FccHandler software (version 1.6.19, SourceForge.net), which allows frame-by-frame playback viewing, was used for scoring. The 2 outcome measurements were as follows: *1*) the mean length of looks at the toy (or mean look duration = the total looking time divided by the number of looks at the toy) and *2*) the inattention rate (the number of looks away from the toy per minute).

A graduate student (KEH) who trained in the Willatts Laboratory scored all cognitive tests. Because the scoring of the Infant Planning Test relies in part on subjective interpretation, cross-validation was achieved by using a 30% sample of Infant Planning Tests scored by a second observer (LFvdM) who also trained in the Willatts Laboratory.

Laboratory methods

Urinary lactulose, mannitol, and lactose concentrations were measured at MRC Keneba by using a 96-well microplate enzymatic assay that was based on previously published methods (47–50). All samples were measured in duplicate. The average intraassay percentage CV, which was calculated across all replicates run during sample analysis, was 3.01% (urine samples) and 3.97% (standards) for the lactulose assay and 1.89% (samples) and 2.36% (standards) for the mannitol assay. The repeatability of the LMR within individual subjects at each time point gave an intraclass correlation coefficient of 0.26 and an estimated reliability of an individual mean of 0.49.

Plasma and breast-milk samples were flushed with nitrogen to minimize the oxidation of volatile PUFAs before storage at -70°C. Samples were later transported to MRC Human Nutrition Research, Cambridge, United Kingdom. To measure plasma and breast-milk fatty acid (FA) concentrations, total lipid extracts were prepared from 200 μL thawed plasma or homogenized breast milk by using an adaptation of the method of Folch (51), and FA methyl esters were prepared by using acid-catalyzed esterification. A BPX70 column [70% cyanopropylpolysilphenylene-siloxane (SGE)], with polarity designed specifically for FA methyl ester analysis, was used for gas chromatography separation. Elution profiles for each sample were obtained, which consisted of 38 peaks. Each of the FA components was quantified against a calibration range of external standards of known concentration (ng/μL) by using linear regression.

Plasma C-reactive protein was measured by using a commercial colorimetric immunoassay (DimensionCardioPhase high-sensitivity C-reactive protein method; Siemens Healthcare Diagnostics Ltd). α-1-Glycoprotein was measured with an immunoturbidimetric specific reaction by using a commercial kit (Sentinel Diagnostics). The Flex reagent cartridge (Siemens Healthcare Diagnostics Ltd) was used for the measurement of plasma albumin.

Fecal calprotectin concentrations were measured at MRC Keneba by using a commercial ELISA kit (Phical; CALPRO AS). The kit is used primarily for the determination of disease activity and monitoring the response to treatment in adult patients with inflammatory bowel disease and patients with colorectal cancers. The protocol was adapted for use in this population of infants and on freeze-dried stool. To enable the comparison with reference values, results were expressed per kilogram of wet stool.

Data handling and statistical analyses

A central database with specific access entry forms and automatic range checks was created for the trial. All data were double entered, and real-time checks were performed to enable a rapid resolution of queries.

Sample-size calculations were based on growth measurements of 1621 Gambian children and, subsequently, the first 25% collected urine samples. The main analysis was an intention-to-treat analysis. Multiple and negative binomial regression analyses, where appropriate, were used to test the effect of treatment on all primary and secondary endpoints. All endpoints for which baseline values were collected were fitted as covariates. In a subsequent analysis, breast-milk DHA and EPA, sex, season of birth, age of treatment commencement, and, only for cognitive development outcomes, the highest level of maternal schooling were added as extra covariates to the regression models. These variables were identified as potential variance inflators and/or effect modifiers. To look at the possibility of dose effects, an additional analysis that fitted the number of doses of treatment, with compliance controlled for, in the regression models were done for all primary and secondary endpoints. Compliance was measured as the percentage of doses taken compared with the doses offered during each infant's supplementation period. With adjustment for the compliance and number of doses, the effect of treatment as it was received in each individual case was measured on the various outcomes. A linear regression model was used to test the relation between each outcome at endpoint with the various FAs at 9 mo. We used the statistical package Stata (version 10; StataCorp LP) throughout. Log transformations were used to both normalize the distribution and stabilize the variance of skewed variables. For the main LC-PUFAs, we analyzed relative percentage and absolute concentration differences

between study groups separately. For other FAs and combinations, we used absolute concentrations. Sex differences between cognitive development test scores were assessed by using regression analysis. For the assessment of test validity, correlations between scores of a second observer who scored a sample of the Infant Planning Test and those of the first observer were examined by using Pearson's product-moment correlation coefficients. Bias-corrected 95% CIs for correlation coefficients were estimated by using the bootstrap approach. The bias between markers was tested by using a paired *t* test.

Ethics, governance, and registration

The trial observed MRC UK Good Clinical Practice guidelines, the current version of the Declaration of Helsinki, and applicable local ethical and legal requirements. Ethics approval was obtained from the London School of Hygiene and Tropical Medicine and the joint Gambian Government/MRC ethics boards. The Gambian National Nutrition Agency gave their written approval to carry out the study, and informed assent was given by the village elders. Written informed consent was provided by the parents or caretaker of each child. Independent trial and safety monitors were appointed to monitor and supervise the progress and safety of the trial throughout and ensure the study abided by MRC UK Good Clinical Practice standards. The trial was registered as ISRCTN66645725.

RESULTS

Ninety-four percent of the 180 infants who received treatment completed the follow-up visit at 9 mo of age so that data sets were

TABLE 1
Baseline characteristics of study participants[1]

Characteristics	Fish-oil group	Control group	Differences[2]
Male sex [*n* (%)]	49 (56)	50 (59)	−3[3]
Age (d)[4]	92.3 ± 4.25[5]	93.2 ± 4.22	−0.10
Highest level of education of mothers (*n*)	68	68	—
Arabic school (*n*)			
0–2 y	26	19	7
3–6 y	28	26	2
7–11 y	4	4	0
English school (*n*)			
1–4 y	4	9	−5
5–9 y	6	10	−4
Anthropometric indexes (*n*)	87	85	—
Weight (kg)	5.86 ± 0.84	5.75 ± 0.83	0.11
Length (cm)	60.1 ± 2.20	60.0 ± 25.6	0.10
HC (cm)	40.1 ± 1.24	40.0 ± 1.29	0.10
MUAC (cm)	13.2 ± 1.15	13.0 ± 1.12	0.22
Knee-heel length (cm)	16.1 ± 0.75	16.0 ± 0.90	0.10
Biceps skinfold thickness (mm)	6.84 ± 1.24	6.72 ± 1.38	0.12
Triceps skinfold thickness (mm)	8.84 ± 1.62	8.60 ± 1.71	0.24
Subscapular skinfold thickness (mm)	8.24 ± 1.50	8.21 ± 1.79	0.03
Plasma FAs (ng/μL)	(*n* = 85)	(*n* = 83)	
DHA	70.2 ± 23.8	76.4 ± 23.2	−6.20
EPA	23.0 (14.5, 35.5)[6]	24.3 (16.1, 40.4)	−1.30
AA	125 ± 42.5	133 ± 39.9	−8.00
Percentage of total FAs			
DHA	3.75 ± 0.89	4.00 ± 0.95	−0.25
EPA	1.15 (0.81, 1.66)	1.31 (0.82, 2.03)	−0.16
AA	6.66 ± 1.41	6.90 ± 1.51	−0.24
Acute-phase proteins (*n*)	80	78	—
Plasma albumin (mg/L)	33.0 ± 3.01	33.0 ± 2.61	0.00
α-Acid glycoprotein (g/L)	0.95 (0.77, 0.11)	0.88 (0.69, 0.12)	0.07
C-reactive protein (mg/L)	3.02 (2.23, 5.31)	2.91 (2.22, 4.67)	0.11
Gut integrity (*n*)	85	82	—
Urinary LMR	0.15 (0.10, 0.22)	0.15 (0.12, 0.20)	0.00
Lactulose:lactose ratio	0.75 (0.53, 1.10)	0.72 (0.50, 0.98)	0.03
Lactulose percentage recovery	0.26 (0.19, 0.45)	0.24 (0.16, 0.34)	0.02
Mannitol percentage recovery	7.02 ± 3.46	6.21 ± 2.94	0.81
Intestinal inflammation	82	83	—
Calprotectin wet weight (mg/kg)	1029 (627, 1534)	1058 (610, 1639)	−29

[1] AA, arachidonic acid; FA, fatty acid; HC, head circumference; LMR, lactulose-mannitol ratio; MUAC, midupper arm circumference.
[2] Fish-oil group mean or median minus control group mean or median.
[3] Percentage.
[4] Age on the first day of supplementation.
[5] Mean ± SD (all such values).
[6] Median; 25th, 75th percentiles in parentheses (all such values).

available for 172 infants (87 infants in the fish-oil group and 85 infants in the control group) from which growth and other outcomes could be analyzed (Figure 1). Baseline characteristics of infants were distributed evenly with no large differences observed between study groups (**Table 1**). Compliance rates ≥90% were measured for 85% of infants (74 of 87 infants) in the fish-oil group and 88% of infants (75 of 85 infants) in the control group. The remaining 23 infants all showed compliance rates >75%. Of infants treated, 155 infants (90%) came for their outcome measurements at 12 mo of age. Eight infants from the fish-oil group and 9 infants from the control group were lost to follow-up because they moved away from the area.

Effect of supplementation on PUFA status

Fish-oil supplementation resulted in a significant increase in the percentage of DHA and EPA in plasma total lipids, which confirmed the tissue uptake of n−3 LC-PUFAs in fish-oil–supplemented infants (**Table 2**). Arachidonic acid (AA) and total n−6 FA concentrations did not differ significantly between fish-oil and control groups. *See* Table 1 under "Supplemental data" in the online issue for other FAs measured.

When compliance and the number of doses received were fitted to the regression model, we observed similar results with some evidence of an interaction between compliance and treatment on DHA and EPA concentrations ($P = 0.029$ and $P = 0.026$, respectively).

Scatter plots of plasma DHA and EPA at 9 against 3 mo by treatment group are shown in **Figure 2**. Elevated n−3 LC-PUFAs at 9 mo in the fish-oil compared with control groups can be observed, although the effect was subtle for DHA. DHA concentrations at 9 mo remained correlated with those at 3 mo in the control group ($r = 0.44$). This result was less clear in the fish-oil group ($r = 0.17$).

The relative percentages of DHA (0.77 ± 0.392%), EPA (0.27%; 25th, 75th percentiles: 0.16, 0.44), and AA (0.49 ± 0.11) in the milk of mothers at baseline did not differ significantly from those measured at the 9-mo follow-up visit, whereas 18:3n−6 and 20:3n−6 significantly increased (to 0.11 ± 0.04% and 0.34 ± 0.10% at the endpoint, respectively). *See* Table 2 under "Supplemental data" in the online issue for an overview of all breast-milk FAs measured.

Primary endpoints

Growth

With the use of intention-to-treat analysis, a statistically significant larger MUAC z score (effect size: 0.31 z scores; 95% CI: 0.06, 0.56 z scores; $P = 0.017$) was detected in the fish-oil

TABLE 2
Plasma fatty acid endpoints by treatment groups[1]

Endpoint	Fish-oil group (n = 86)	Control group (n = 83)	Effect size (95% CI)[2]	P[2]
Main LC-PUFAs (percentage of total FA)				
DHA	4.87 ± 1.01[3]	4.44 ± 0.81	0.53 (0.28, 0.79)	<0.001
EPA	2.13 [1.66, 2.95][4]	1.34 [0.94, 1.72]	1.65 (1.45, 1.88)[5]	<0.001
AA	7.28 ± 1.52	7.25 ± 1.35	0.09 (−0.33, 0.51)	0.680
Main LC-PUFAs (ng/μL)				
DHA	70.0 ± 20.6	65.4 ± 16.3	5.54 (−0.03, 11.2)	0.051
EPA	31.1 [22.8, 43.7]	19.3 [14.3, 25.0]	1.61 (1.38, 1.84)[5]	<0.001
AA	104 ± 29.0	107 ± 27.6	−2.38 (−10.8, 6.05)	0.578
Other FAs and FA indicators (ng/μL)				
Saturated (8:0–18:0)[6]	516 ± 150	541 ± 137	−27.0 (−70.8, 16.7)	0.224
Saturated (20:0–24:0)[7]	11.6 ± 2.79	11.8 ± 3.60	−0.17 (−1.17, 0.83)	0.739
Monounsaturated[8]	350 ± 99.7	373 ± 106	−23.4 (−54.4, 7.67)	0.139
18:3n−3	4.86 ± 2.87	4.76 ± 2.16	0.05 (−0.73, 0.84)	0.893
22:5n−3	13.2 ± 4.64	11.9 ± 3.82	1.32 (0.00, 2.64)	0.051
18:2n−6c	317 ± 93.8	322 ± 91.9	−4.40 (−33.0, 24.2)	0.761
20:3n−6	17.1 ± 5.80	19.1 ± 6.49	−1.93 (−3.67, −0.10)	0.039
Total n−3 FAs (ng/μL)	123; 41	104; 28	20 (9, 30)	<0.001
Total n−6 FAs (ng/μL)	440; 118	452; 119	−10 (−46, 27)	0.595
22:5n−6	2.59 ± 1.00	3.32 ± 1.24	−0.76 (−1.07, −0.44)	<0.001
Essential and nonessential PUFAs[9]	1.88 ± 0.39	1.75 ± 0.34	0.14 (0.02, 0.25)	0.018
DHA:22:5n−6 ratio	28.4 ± 7.34	21.5 ± 7.31	6.85 (4.64, 9.05)	<0.001
AA:EPA + DHA ratio	1.05 ± 0.26	1.28 ± 0.31	−0.24(−0.32, −0.16)	<0.001

[1] AA, arachidonic acid; FA, fatty acid; LC-PUFA, long-chain PUFA.
[2] General least-squares regression analysis with the dependent variable and treatment entered and baseline controlled for.
[3] Mean ± SD (all such values).
[4] Median; 25th, 75th percentiles in brackets (all such values)
[5] Because data were log transformed, effect sizes and CIs are expressed as the antilog of calculated CIs and regression coefficients, which indicate the ratio of the geometric mean of fish-oil to control groups.
[6] Includes 18:0, 17:0, 16:0, 15:0, 14:0, 13:0, 12:0, 11:0, 10:0, and 8:0.
[7] Includes 24:0, 23:0, 22:0, 21:0, and 20:0.
[8] Includes 14:1, 15:1, 16:1, 17:1, 18:1n9c, 20:1, 22:1n−9, and 24:1.
[9] Ratio of n−6 + n−3 to n−9 unsaturated PUFAs. Ordinarily, n−7 PUFAs should be included in the ratio as nonessential FAs, but data for these were unavailable.

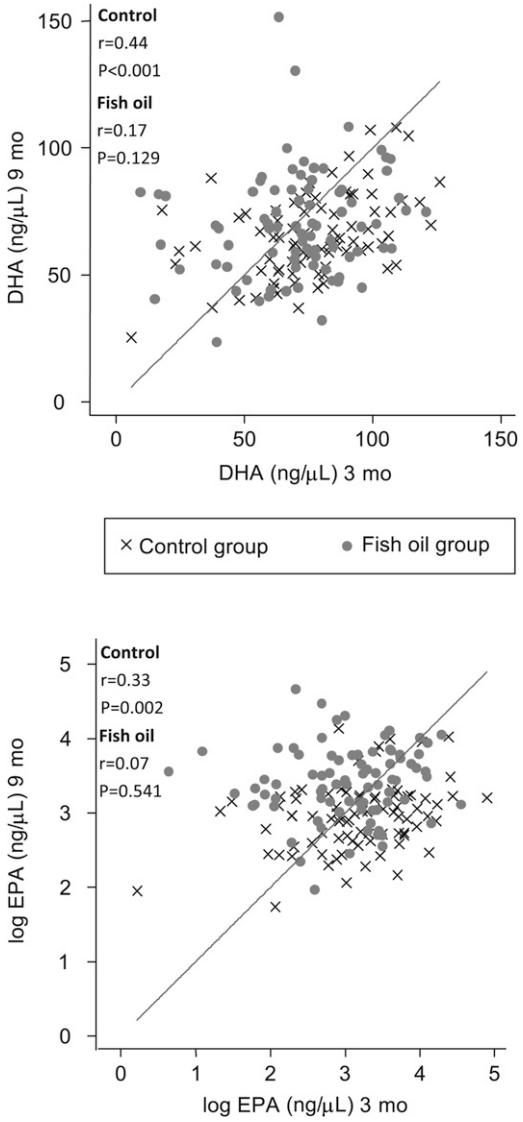

FIGURE 2. Plasma DHA and EPA scattered at 9 compared with 3 mo by treatment group (n = 168).

group (**Table 3**). A small significant difference for triceps skinfold-thickness–for-age was also detected (P = 0.048). Linear growth showed evidence of a benefit with a substantial effect size of 0.79 z scores. However, CIs were wide, and the difference was not significant (95% CI: −0.27, 0.90; P = 0.084). This difference had largely disappeared by 12 mo. *See* Tables 3 and 4 under "Supplemental data" in the online issue for a presentation of anthropometric measures at 9 and 12 mo of age.

When subjects were remeasured at 12 mo of age, the significant difference in MUAC measurements between groups was maintained. With respect to other proxy measures of infant adiposity, skinfold-thickness measurements were also all significantly larger in the fish-oil group than in the control group at this age, although BMI-for-age z scores did not differ significantly (**Table 4**).

Gut permeability

No group differences between LMRs or percentage recoveries of lactulose and mannitol were evident. The lactulose:lactose ratio was also investigated, and again, there was no evidence of an effect of treatment on gut integrity (Table 3). In comparison with European references for healthy children (52, 53), only 5% of infants had a normal LMR <0.07. Similar results emerged with respect to the significance of treatment effects when we repeated the analyses on all primary endpoints, with our chosen covariates and for compliance controlled for.

There was no significant interaction between season, breast-milk DHA, or breast-milk EPA and treatment on growth, the LMR, or lactulose and mannitol recoveries ($P > 0.09$ for all). A significant interaction between compliance and treatment (P = 0.012) was shown for LMR. However, the CI included 1.0, and the effect size was trivial (1.01). There were no compliance-treatment interactions for lactulose or mannitol recovery.

Secondary endpoints

Acute-phase proteins

PUFA supplementation did not reduce the relatively high degree of systemic inflammation in infants (**Table 5**).

Fecal calprotectin

Although overall fecal calprotectin concentrations were very high, there were no significant differences in the average or proportion of elevated calprotectin concentrations between infants in the 2 treatment groups. More than 85% of infants had calprotectin concentrations higher than a European reference for infants at 3 mo of age (54) (Table 5).

Morbidity

With the use of negative binomial regression analyses, we showed evidence of no treatment effect when inspecting group differences in rates of illness, fever, respiratory complaints, vomiting, or nurse visits/doctor visits, referrals in infants. Although the difference was not significant, diarrhea rates were far higher (roughly 25%) in the control group than in the fish-oil group (**Table 6**).

The logarithm of the number of visits was controlled for in each analysis because the number of times a particular complaint was reported increased with the number of times that the infant was visited but possibly not in a straightforward, multiplicative way. The results when adjusted for our chosen covariates and compliance were similar, and no significant interactions between compliance and treatment group on morbidity outcomes were observed. The most common complaints were cough and respiratory conditions followed by fever.

We finally investigated whether group differences existed for the number of severe episodes of illnesses subjects experienced. The number of episodes of illness that lasted for ≥3 d was compared between groups by using a 2-sample t test with equal variances, but no significant differences in severity (as judged by duration) were shown for any of the morbidities assessed ($P > 0.255$ for all). A total of 9 serious adverse events occurred, all of which were reported to the trial safety monitor. Four of these events were in the treatment group, and 6 events were in the control group.

Cognitive development at 12 mo

On both the Willatts' Infant Planning and attention tests, we observed no significant difference in performance between the 2 treatment groups (**Table 7**). There were no significant differences

TABLE 3
Effect of n−3 LC-PUFA supplementation on primary endpoints at 9 mo by treatment group[1]

Endpoints	Fish-oil group (n = 87)	Control group (n = 85)	Effect size (95% CI)	P
Growth (anthropometric z scores)[2,3]				
Weight-for-age	−0.90 ± 1.09[4]	−1.20 ± 1.28	0.15 (−0.08, 0.38)	0.208
Weight-for-length	−0.57 ± 0.99	−0.76 ± 1.10	0.12 (−0.14, 0.38)	0.377
Length-for-age	−0.79 ± 1.02	−1.07 ± 1.30	0.79 (−0.27, 0.90)	0.084
HC-for-age	−0.52 ± 0.84	−0.64 ± 1.06	−0.01 (−0.18, 0.17)	0.954
MUAC-for-age	−0.27 ± 0.92	−0.66 ± 1.18	0.31 (0.06, 0.56)	0.017
BMI-for-age	−0.60 ± 0.99	−0.79 ± 1.12	0.08 (−0.16, 0.33)	0.503
Triceps skinfold thickness–for-age	−0.01 ± 0.85	−0.30 ± 1.09	0.27 (0.00, 0.55)	0.048
Subscapular skinfold thickness–for-age	0.41 ± 1.12	0.27 ± 1.29	0.16 (−0.16, 0.48)	0.326
Gut integrity (n)	86	81	—	—
Mannitol percentage recovery	4.22 ± 2.48	4.51 ± 2.04	−0.34 (−1.04; 0.35)	0.332
LMR[5,6]	0.22 [0.14, 0.37][7]	0.22 [0.15, 0.29]	0.96 (0.87, 1.07)[8]	0.507
Lactulose:lactose ratio[5,9]	0.50 [0.30, 0.91]	0.64 [0.42, 0.98]	0.79 (0.60, 1.05)[8]	0.110
Lactulose percentage recovery[5]	0.21 [0.11, 0.38]	0.23 [0.15, 0.32]	0.91 (0.72, 1.15)[8]	0.434

[1] HC, head circumference; LC-PUFA, long-chain PUFA; LMR, lactulose:mannitol ratio; MUAC, midupper arm circumference.
[2] General least-squares regression analysis with the dependent variable and treatment entered and baseline controlled for. Median growth measurements of each individual were used.
[3] With the use of WHO reference curves (38, 39).
[4] Mean ± SD (all such values).
[5] Data were log transformed.
[6] Regression analysis fitting (log)lactulose on (log)mannitol with baseline values controlled for.
[7] Median; 25th, 75th percentiles in brackets (all such values).
[8] For log-transformed data, effect sizes and CIs are expressed as the antilog of calculated CIs and regression coefficients, which indicate the ratio of geometric means of fish-oil to control groups.
[9] Regression analysis fitting (log)lactulose on (log)lactose with baseline values controlled for.

detected between intention scores or solutions, inattention rates, or mean look times. In an attempt to retrieve the toy, infants pulled the cloth off the table an equal number of times in control and fish-oil groups (P = 0.918), which indicated that n−3 LCP supplementation had no effect on motor control (55). Sixteen percent of infants were unable to show any form of intention for the retrieval of the toy whatsoever, and only 2% of infants were able to obtain the maximum intention score. In the infant planning test only, we showed sex differences between test scores. The average total intentional solution score was significantly higher in boys than in girls (average increase: 1.61 points; 95% CI: 0.35, 3.86 points; P = 0.012), and for intentional solutions, we observed a weak statistical difference (P = 0.067). There were no sex differences in attention test scores. Interobserver reliability was high on both the total intention (r = 0.97; bias-corrected 95% CI: 0.936, 0.989) and intentional solution scores (r = 0.92; bias-corrected 95% CI: 0.821, 0.961), and there was no significant evidence of a bias between markers (P = 0.121 and P = 0.097, respectively).

TABLE 4
Anthropometric indexes at 12 mo of age by treatment group with baseline measures controlled for[1]

Endpoint	Fish-oil group (n = 79)	Control group (n = 76)	Effect size (95% CI)[2]	P
Anthropometric z scores[3]				
Weight-for-age	−1.09 ± 1.13[4]	−1.41 ± 1.21	0.11 (−0.13, 0.35)	0.357
Weight-for-length	−0.79 ± 1.10	−1.03 ± 0.97	0.17 (−0.09, 0.43)	0.207
Length-for-age	-0.1.00 ± 1.11	−1.30 ± 1.32	0.08 (−0.15, 0.32)	0.489
HC-for-age	−0.61 ± 0.87	−0.72 ± 1.02	−0.04 (−0.24, 0.16)	0.713
MUAC-for-age	−0.20 ± 0.94	−0.64 ± 0.98	0.33 (0.09, 0.57)	0.008
BMI-for-age	−0.69 ± 1.12	−0.90 ± 0.94	0.09 (−0.15, 0.34)	0.459
Triceps skinfold thickness–for-age	0.19 ± 0.79	−0.15 ± 0.82	0.31 (0.06, 0.55)	0.014
Subscapular skinfold thickness–for-age	0.23 ± 1.05	−0.14 ± 1.02	0.36 (0.06, 0.67)	0.019

[1] HC, head circumference; MUAC, midupper arm circumference.
[2] General least-squares regression analysis with the dependent variable and treatment entered and baseline controlled for.
[3] With the use of WHO reference curves (38, 39).
[4] Mean ± SD (all such values).

TABLE 5
Secondary endpoints, by treatment group[1]

Endpoint	Fish-oil group	Control group	Effect size (95% CI)	*P*
Acute-phase proteins (*n*)	86	83	—	—
Plasma albumin (mg/L)	35.3 ± 3.1[2]	35.3 ± 2.8	0.07 (−0.83, 0.96)[3]	0.880[3]
AGP (g/L)	1.01 [0.85, 1.34][4]	1.03 [0.82, 1.36]	0.97 (0.88, 1.08)[3,5]	0.596[3]
CRP (mg/L)	4.7 [3.1, 8.2]	4.7 [3.2, 7.9]	0.98 (0.81, 1.19)[3,5]	0.838[3]
Intestinal inflammation (*n*)	76	69	—	—
Calprotectin dry weight (mg/kg)	78.0 [43, 120]	66.5 [46, 138]	0.90 (0.86, 1.42)[3,5]	0.427[3]
Calprotectin wet weight (mg/kg)	647 [357, 996]	552 [382, 1145]	—	—
Concentrations out of normal range				
Plasma albumin[6]	5/86 [0.77, 10.9][7]	3/83 [−0.49, 7.71]	—	0.933[8]
AGP[6]	44/86 [40.4, 61.9]	43/83 [40.8, 62.8]	—	0.501[8]
CRP[6]	39/86 [34.6, 56.1]	36/83 [32.5, 54.3]	—	0.796[8]
Calprotectin[9]	70/79 [81.4, 95.8]	62/74 [75.2, 92.4]	—	0.386[8]

[1] AGP, α1-acid glycoprotein; CRP, C-reactive protein.
[2] Mean ± SD (all such values).
[3] General least-squares regression analysis with the dependent variable and treatment entered and baseline controlled for.
[4] Median; 25th, 75th percentiles in brackets (all such values).
[5] Because data were log transformed, effect sizes and CIs are expressed as the antilog of calculated CIs and regression coefficients, which indicated the ratio of the geometric mean of fish-oil to control groups.
[6] With the use of standard adult cutoff ranges from healthy individuals (CRP ≥5 mg/L, AGP ≥1 g/L, and albumin ≤30 mg/L).
[7] Proportion abnormal; 95% CI in brackets (percentage abnormal) (all such values).
[8] Group differences were analyzed by using Pearson's chi-squared test.
[9] With the use of the European healthy reference value [calprotectin ≤263 mg/kg (54)].

Associated covariates

Weaning

Only 2.68% of mothers reported exclusively breastfeeding their infants by the time they had reached 6 mo of age. Solid foods were reportedly introduced in 31.5% of 3-mo-old infants, and all infants were eating solid foods by 9 mo of age. In respect of rich sources of preformed LC-PUFAs, 14% of infants had had some form of egg in their diet by 6 mo. Roughly the same number of infants had shared the family bowl, including dishes that contained small amounts of dried fish. By 9 mo of age, roughly 80% of infants had had some egg yolk or some form of fish. The most common complementary food fed, by far, was a rice-based porridge. Other frequently fed foods were bread, margarine, mayonnaise, bananas, and cooking oil.

Maternal schooling

Education data were available for 94% of mothers whose infants completed the cognitive testing, and of those infants, 75% of them received some form of formal schooling. There were no significant differences in the education level between mothers in the 2 treatment groups, and no associations were shown between the education levels of mothers and the performance of their infants on any of the tests.

DISCUSSION

To our knowledge, this was the first publicly registered or published study to investigate the effects of n−3 LC-PUFA supplementation during infancy on gut integrity, growth, and cognitive development in infants from a low-income country.

TABLE 6
Morbidity endpoints by treatment group

Morbidity rates	Fish-oil group (*n* = 87)	Control group (*n* = 85)	Effect size (95% CI)[1]	*P*[1]
Unwell, any complaint[2]	3603[3]	3088	0.13 (−0.08, 0.34)	0.238
Diarrhea[4]	474	605	−0.27 (−0.61, 0.06)	0.110
Fever[4]	1479	1297	0.11 (−0.12, 0.34)	0.338
Respiratory complaints[4]	1645	1503	0.06 (−0.2, 0.32)	0.670
Nurse or doctor visits[4]	186	161	0.13 (−0.10, 0.36)	0.268
Vomiting[4]	251	268	−0.12 (−0.56, 0.03)	0.570
Abnormal bleeds/other complaints[4,5]	12	17	—	—

[1] Negative binomial regression by using the number of visits an infant was reported ill or with a symptom as a negative binomial variable with log(number of visits) controlled for.
[2] Number of visits at which one or more symptoms were recorded.
[3] Total recorded (all such values).
[4] Number of visits at which any infant was reported to have had this complaint.
[5] Too few observations to make a meaningful statistical comparison.

TABLE 7
Cognitive development endpoints by treatment group[1]

Endpoint	Fish-oil group	Control group	Effect size (95% CI)	*P*
2-step Infant Planning Test (*n*)	73	65	—	—
Average total intention score	4.63 ± 3.77[2]	4.45 ± 3.71	0.20 (−1.10, 1.46)[3]	0.759[3]
Average intentional solutions	0.44 ± 0.52	0.43 ± 0.54	0.01 (−0.17, 0.19)[3]	0.871[3]
Toddler attention assessment (*n*)	74	69	—	—
Inattention rate	2.38 [1.70, 2.91][4]	2.37 [1.70, 2.91]	0.98 (0.82, 1.81)[3,5]	0.868[3]
Mean look duration	23.8 [19.5, 33.9]	23.2 [17.2, 35,5]	1.01 (0.86, 1.20)[3,5]	0.884[3]

[1] Assessments could not be made for the Infant Planning Test in 17 cases (6 cases in the fish-oil group and 11 cases in the control group) and for the attention test in 12 cases (5 cases in the fish-oil group and 7 cases in the control group) because the children were disturbed or crying or the video recording malfunctioned.

[2] Mean ± SD (all such values).

[3] General least-squares regression analysis with the dependent variable and treatment entered.

[4] Median; 25th, 75th percentiles in brackets (all such values).

[5] Because data were log transformed, effect sizes and CIs are expressed as the antilog of calculated CIs and regression coefficients, which indicate the ratio of the geometric mean of fish-oil to control groups.

The intervention successfully increased infant n−3 plasma FA status. Supplementation also resulted in a 0.3-cm increase in MUAC after 6 mo of intervention. At the 12-mo follow-up (ie, 3 mo after the end of the intervention) significant increases in MUAC and all 3 skinfold-thickness measurements were detected in the intervention group. Apart from a substantial but nonsignificant increase in the length of 0.79 *z* scores in the fish-oil group at 9 mo ($P = 0.08$), no additional effects on growth were detected. There were no cross-sectional associations between outcome variables and PUFA status; neither plasma nor breast milk PUFA at 3 or 9 mo predicted urinary LMR, lactulose or mannitol percentage recoveries, fecal calprotectin concentrations, or cognitive-function scores. Therefore, results of the trial provided insufficient evidence to support the primary hypotheses that dietary n−3 LC-PUFA supplementation improves the growth performance of rural African infants and/or protects mucosal epithelial integrity even at the very large dose used. Also, the dietary n−3 LC-PUFA supplement did not lead to reduced degrees of intestinal and systemic inflammation or reduced rates of morbidity in these rural African infants.

A possible explanation for why the intervention achieved such a modest impact is that the PUFA analysis of breast milk showed that the average mother's milk was at the higher end of the range in EPA and DHA compared with that in breast milk of women in most other populations (56, 57). Therefore, the majority of infants aged 3–9 mo were ensured diets that supplied adequate amounts of n−3 FAs. It is likely that even at 9 mo of age, when weaning foods contribute to a large part of the infant diet, breast milk supplied most infants with substantial amounts of preformed LC-PUFAs even when it was consumed in smaller volumes. Together, DHA and EPA accounted for ∼1% of total FAs and were measured (by applying reliable, validated methods) at concentrations that matched those in populations who consume a large amount of fish, such as in the Philippines. Brenna et al (56) measured the breast milk in 2472 women from 30 different countries, and, in comparison with these results, breast-milk DHA concentrations in Gambian women were shown to be considerably high. Brenna et al (56) showed an average relative DHA concentration of 0.32 ± 0.22% (compared with the concentration of 0.77 ± 0.39% shown in our sample of women). Yuhas et al (57) showed average EPA concentrations of 9 different populations worldwide to range from 0.07 ± 0.07% to 0.26 ± 0.14%, whereas the Gambian mean was even higher than this range (0.36 ± 0.29%). However, average breast-milk AA concentrations in Gambian women were similar to averages globally (0.49 ± 0.11% compared with 0.47 ± 0.13%, respectively). The reasons for these high PUFA levels are not certain. The Gambian diet is not considered to supply large quantities of n−3 LC-PUFAs, although precursor n−3 FAs are regularly consumed via green leafy vegetables. The conversion efficiency may be particularly high in these women, possibly because of polymorphisms of *FADS1* and *FADS2* (FA desaturase) gene clusters, but this relation remains untested.

Our data suggested that fish-oil administration may have a delayed effect on body fat as shown by the sustained effect on MUAC and significant increases in skinfold thicknesses of the fish-oil group seen at 12 but not at 9 mo. This is not the first time that such an effect has been observed. In a study that investigated breastfed infants whose mothers were supplemented with fish oil during lactation, an increased subscapular skinfold thickness was also detected in infants of supplemented women but only at follow-up 2 y after the intervention was ceased (58–60). Thus, the impact of early n−3 LC-PUFA supplementation may change over time, which suggests the possibility of a (possibly epigenetically mediated) memory effect.

The Willatts' Infant Planning Test was chosen because it has proven sensitive to past nutritional interventions in infants (46, 55) and has been successfully used in low-income countries (44, 45) where it has, for example, allowed the detection of differences between groups of infants aged 7 mo after a 2-mo psychosocial intervention (44). In contrast, LCPs in infant development, by using standard generalized tests, have rarely shown any benefit. DHA is important to cognitive processes supported by frontal regions of the brain (61–66), where functions that integrate and control attention and response components with long-term and working memory take place. Studies on look duration in human and primate infants have shown associations between n−3 FAs or DHA and accelerated attention maturity (41, 46, 67, 68). DHA has also been shown to improve synaptic efficiency (69) and transmission speed (70), thereby theoretically aiding in information-processing efficiency. Therefore, tests that measure frontal region processes, such as attention and problem solving,

may be more appropriate when examining n−3 LCP in relation to cognitive development.

Gambian infants scored poorly on the problem-solving test compared with younger (9-mo-old) infants in Denmark (71), the United States (72), and the United Kingdom (46). (It was not possible to make valid comparisons with other reported studies that used the 1- or 3-step test.) Apart from one study in Bangladesh (45), the sex differences we observed were not seen in other studies. In rural Gambia, sex distinctions appeared to be particularly pronounced, possibly because of differences in maternal attention, and marked differences in sex roles are the norm. However, a difference in cognitive development first needs to be verified before inferences about its causes and consequences can be made.

Strengths of this study

Because growth faltering is highly resistant to energy or micronutrient supplementation [as shown in numerous studies (73–78)] and so many interventions have failed to improve the growth and gut integrity of Gambian infants (76, 79, 80), it was hoped that n−3 LC-PUFA supplementation would provide some beneficial effect. Despite the supplementation of young infants with a very large dose of n−3–rich fish oil, administration of supplements under direct observation, achievement of very high compliance rates, and the demonstration of significant shifts in plasma PUFA profiles at 9 mo, remarkably little effect was observed on tested outcomes. Aided in part by the use of triplicate measures on alternate days for primary outcomes at baseline and 9 mo, 95% CIs calculated for most endpoints were acceptably narrow, which provided reasonable certainty that the study was adequately powered to detect clinically meaningful effects. This statement was less true for the lactulose-mannitol sugar permeability test for which, as indicated by the low intraclass correlation coefficient, a high amount of error (mostly attributable to the lactulose assay) was introduced during measurement. This result may have partly been ascribed to our use of a field-adapted method, the low reliability of which has, for the first time to our knowledge, been shown.

Weaknesses of the study

Because of the high concentrations of n−3 PUFAs in breast milk and the very high rates of breastfeeding in this community, it may be argued that the intervention was targeted at infants who were already replete with n−3 LC-PUFAs. Nevertheless, the size of the dose was large enough to still further increase plasma n−3 LC-PUFA concentrations and warrant possible associated benefits. We had also speculated that high concentrations of n−3 PUFAs might have a topical effect on gut mucosae. It is possible that a delayed intervention directed at children receiving a lower (or absent) proportion of breast milk in their diet, and, hence, likely to be deficient in all PUFAs (81), would have shown a greater benefit. However, our early intervention was intentionally predicated on the assumption that it would be better to attempt to block the initial development of enteropathy rather than attempt to redress the fully established gut damage, which is slow to repair.

Growth faltering and environmental enteropathy is the result of a complex set of mechanisms that involve varied physiologic and environmental interactions. Although environmental enteropathy appears to be clinically asymptomatic, its effect on growth is imputed to be large, and its full effect on the child cannot be known until an effective intervention to ameliorate or prevent the condition is shown. Thus, we believe that the problem should continue to be explored for the benefit of future generations in The Gambia and elsewhere.

In conclusion, n−3 LC-PUFAs remain critical to human health and development, but breast-fed infants in rural Gambia are ensured an adequate supply of preformed LC-PUFAs by mother's milk, which in the present sample proved considerably rich in these FAs. A better understanding of the cause and pathogenesis of environmental enteropathy is recommended for assisting in the development of effective interventions. Etiological assumptions should be confirmed or refuted and the mucosal immune response studied in further depth. The identification of specific gut pathogens that lead to gut damage and consequent immunoprophylactic approaches that target these and established pathogens are recommended. The increased power of analytic methods for the study of the human microbiome may facilitate such exploration. The importance of attempts to address the worldwide problems of poor hygiene and sanitation, which lead to enteric infections in the first instance, should not be undermined, and their priority should be considered high.

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The authors' responsibilities were as follows—LFvdM and AMP: conceived and designed the study with input from SEM and AJF and wrote the manuscript with input from all authors; LFvdM: conducted the study with the support of SEM and SD; AJF: was responsible for statistical aspects of the study; KH: led the cognitive development part of the study and scored all videos; SD: performed all cognitive tests; SY: was responsible for FA analyses; and all authors: read and approved the final manuscript. LFvdM is currently employed by Danone Research, Netherlands. None of the authors had a conflict of interest.

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