Journal of Pediatric Gastroenterology and Nutrition
44:136–142 © 2007 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

Double-blind, Placebo-controlled Trial Comparing Effects of Supplementation of Two Micronutrient Sprinkles on Fatty Acid Status in Cambodian Infants

*Carlo Agostoni, *Marcello Giovannini, *Debora Sala, *Michele Usuelli, *Luca Livio, *Gaia Francescato, *Marta Braga, *Enrica Riva, †Antonella Martiello, †Claudio Colombo, †Franca Marangoni, and †Claudio Galli

*Department of Pediatrics, San Paolo Hospital, and †Department of Pharmacological Sciences, University of Milan, Italy

ABSTRACT

Background: Infants in developing countries require early dietary interventions to prevent nutritional deficiencies, above all protein, energy, iron and zinc. To what extent these interventions may affect the fatty acid (FA) status is still unknown.

Objectives: To examine and compare the effects of 2 micronutrient "sprinkles" supplementations (iron 12.5 mg + folic acid 150 μg, iron/folate and iron 12.5 mg + folic acid 150 μg + zinc 5 mg + vitamins A, C and D_3, mineral/micronutrient [MMN]) versus placebo on the FA status of Cambodian infants.

Methods: A total of 204 infants age 6 mo and living in Kompong Chhnang Province, Cambodia, were randomly assigned to receive daily supplementation of MMN (n = 68) and iron/folate (n = 68) or placebo (n = 68) for a 12-mo period in powder form as sprinkles. At the end of the intervention period, FAs in the range of 16 to 24 C were determined in blood drops absorbed on a strip collected from 182 subjects, and values among the 3 intervention subgroups and those of 21 Italian 18-mo-old, normal-growing infants as the reference group were compared.

Results: At the end of the supplementation trial, higher levels of the 2 essential FAs (EFAs) (linoleic acid, 18:2n-6, and α-linolenic acid, 18:3n-3) were found in the MMN group. No differences occurred for the major longer chain derivatives of both EFAs arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3). In MMN supplemented Cambodians, blood levels of linoleic acid approached those of Italian infants, and in addition their α-linolenic acid levels were improved. Cambodian infants, mostly still breast-fed through the second year of life, showed significantly higher levels of long-chain derivatives of both the n-6 and the n-3 series compared with Italians.

Conclusions: Supplementation with iron, folic acid, zinc and vitamins was associated with an increase of linoleic acid and α-linolenic acid levels in Cambodian infants versus placebo, without significant changes in the concentrations of their longer chain derivatives, resulting in a FA status closer to Italian counterparts for the essential polyunsaturated FA levels. The iron/folate–treated infants showed no differences compared with the other 2 groups. Studies are needed to differentiate the potential effects of the supplemented micronutrients on the FA status. ***JPGN 44:136–142, 2007.***

Key Words: Essential fatty acids—Linoleic acid—Micronutrient supplementation—Iron—Zinc.

INTRODUCTION

A large proportion of infants and young children living in developing countries is exposed to several nutritional deficiencies, first of all, energy, protein, iron and zinc. Although an adequate supply of energy and protein is mainly based on the improvements of both quality and availability of local staple foods, iron and zinc may be provided through ad hoc preparations to prevent early nutritional deficiencies because complementary feeding in developing countries is scarce in animal foods, which are the main sources of both minerals (1). Therefore, iron supplementation has been given major attention with the aim to prevent or to treat iron deficiency anaemia and its deleterious consequences on infant development (2). Schedules and preparations for optimal iron supplementations have been the object of several investigations and discussions in the last decade (3–6). Zlotkin et al. (7) recently found that daily sprinkle supplementations (based on microencapsulated ferrous fumarate in powder

Received February 20, 2006; accepted August 14, 2006.

Address correspondence and reprint requests to Carlo Agostoni, Department of Paediatrics, San Paolo Hospital, University of Milan, 8 Via A di Rudinì, 20142 Milan, Italy. (e-mail: agostoc@tin.it/carlo.agostoni@unimi.it).

form plus folic acid) are efficacious in treating anaemia among infants ages 6 to 18 mo, with limited side effects.

The prevention of zinc deficiency is also considered of great relevance to optimise growth and development (8). The administration of iron or zinc in fact appears to be a way to improve not only iron and zinc status but also relevant pathophysiological outcomes such as growth, development and reduction of morbidity and inflammatory responses (9–12). Although supplementing iron plus zinc alone may result in competitive mechanisms on micronutrient use (13), further addition of vitamins could improve at least the use of iron (14,15) in addition to improving the general metabolic conditions. A multimicronutrient supplementation may be effective in raising blood hemoglobin levels and the individual micronutrient status, even improving the growth of stunted children (16).

Among the indicators of the individual health status in infancy and childhood, the analysis of the fatty acid (FA) pattern is gaining consideration because associations have been found with major functional indexes such as anthropometric parameters and developmental performance and with markers of disease (17,18). The most relevant FA, in relation to physiological functions, are the polyunsaturated FA (PUFA) of both metabolic series (ie, the 2 18 C precursors: essential FA [EFA] linoleic acid [LA; 18:2n-6] and α-linolenic acid [ALA; 18:3n-3]), together with their longer-chain derivatives long-chain PUFA (LC-PUFA), arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6 n-3), respectively. Several investigators have reported abnormal levels of EFA or LC-PUFA in malnourished populations, with large biochemical differences between the various geographic locations (19). Limited data are available on the effects of interventions based on dietary fats concerning either general nutritional rehabilitation (20) or specific supplementations (21), and more research is consequently needed (19). Because the assessment of the overall effects of current interventions, including micronutrient supplementations, is a primary goal in developing countries, we have assessed the FA status in Cambodian infants at the end of a double-blind, placebo-controlled trial comparing 2 different combinations of micronutrients daily delivered as sprinkles whose effects on growth, anaemia and iron deficiency have been reported previously (22).

PATIENTS AND METHODS

Study Design, Location and Subjects

The study was designed as a double-blind, placebo-controlled comparative longitudinal trial carried out in infants age 6 mo at recruitment living in Tuk Phos district, Kompong Chhnang Province, Cambodia. In this geographic area, people depend on farming, and the principal crop and complementary food is rice, which is low in bioavailable iron.

TABLE 1. *Nutrient composition of sprinkles' lots*

Sprinkles	Composition	DRI
MMN		
Fe (iron II fumarate)	12.5 mg	7 mg
Zn gluconate	5 mg	3 mg
Vitamin C	50 mg	15 mg
Vitamin A	300 μg	300 μg
Vitamin D_3	7.5 μg	5 μg
Folic acid	150 μg	150 μg
Potato maltodextrins	SQ to 1 g	–
Iron/folate		
Fe (iron II fumarate)	12.5 mg	7 mg
Folic acid	150 μg	150 μg
Potato maltodextrins	SQ to 1 g	–
Placebo		
Potato maltodextrins	1 g	–

DRI, Dietary Reference Intakes (20) per day for children ages 1–3 y; MMN, multimineral and multivitamin; SQ, standard quantity to reach 1 g of final product.

A total of 204 infants were recruited according to the following eligibility criteria: born between January and July 2003, age 6 mo ± 7 d at recruitment. Exclusion criterion was severe anaemia (hemoglobin [Hb] < 70 g/L). Infants were recruited in 28 villages randomly selected to homogeneously represent the studied geographic area and randomly (same chance) assigned to receive either folic acid + iron + multimin- multimineral and multivitamin (MMN) or folic acid + iron (iron/folate) or placebo as sprinkles (powder form). Sprinkles were mixed and packaged by Heinz Co (Toronto, Canada) and shipped by air to Phnom Penh. Treatments or placebo were administered daily for a 12-mo period. Details on study protocol, randomization, monitoring and assessments are reported elsewhere (22), whereas the nutrient composition of intervention sprinkles lots is reported in Table 1. The doses of single nutrients were in agreement with international recommendations, and recent values for Dietary Reference Intakes are reported for comparison (23). Immunization, vitamin A capsules and antielminthic (Mebendazole) coverage were also provided to all infants according to the guidelines of the Cambodian Ministry of Health (24).

FA Measurements

Blood samples were taken at 9 h ± 30 min at the end of the intervention period as 2-mL venous blood and immediately delivered to the Institut Pasteur du Cambodge to be analysed for markers of iron status, as detailed elsewhere (22). For the measurement of the FA status, a drop of blood was absorbed on a strip of chromatographic paper containing the antioxidant BHT following the method described and validated by Marangoni et al. (25). The sample-containing strips were immediately stored at 4°C in individual cellophane envelopes with airtight closure until they were shipped to the analytical laboratory in Italy, where they were processed for direct FA methylation without lipid extraction as described previously (25) and accordingly modified from a published methodology (26). The FA methyl ester preparation was dissolved in 50 μL hexane and 1/50 of the sample was injected in a gas chromatograph equipped with a 30-m capillary column, programmed

temperature vaporization injector, flame ionization detector and a dedicated data system. Temperature programming was from 170° to 205°C with a 5°C/min increment and after 5-min isotherm to 220°C at the same rate. Peaks were identified by the use of pure reference compounds. The following individual FA and FA classes were considered: 16:0 and 18:0 and total saturated FA; 16:1, 18:1n-9, 20:1 and total monounsaturated FA; 20:3n-9, 18:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6 and total n-6 FA; 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3 and total n-3 FA; total PUFA, the product/precursor ratios in the 2 metabolic series (20:4n-6/18:2n-6, 20:4n-6/20:3n-6, 22:6n-3/18:3n-3) and the total n-3/n-6 ratio. However, only values for major individual FA are reported.

Infants and young children living in developing countries have a low EFA status, notably concerning n-6 series FAs, when compared for age- and sex-matched healthy controls (24). To better evaluate the effects of the supplementations on Cambodian infants' FA status, we considered reference values from a matched group. Blood drops were taken with the same method from 21 healthy Italian infants undergoing blood sampling for a balance of health upon request of their pediatricians at the Day-Hospital of the Department of Pediatrics at San Paolo Hospital, Milan. Although they were comparable with Cambodian counterparts for age and sex distribution, their weight-for-age and length-for-age *z* scores were lower (Table 2), with underweight 11.5%, stunting 27% and wasting 4.7% prevalence, respectively (22). Samples were taken at 8 AM after an overnight fast, and parents gave their informed consent.

Dietary and Health Assessments

At baseline, demographic, nutritional and health information about the infants were collected. Health operators visited the infants at home at 1-week intervals through a 12-mo period starting from baseline. At each weekly visit, Cambodian mothers were interviewed concerning the health status of the infant, adherence to the prescribed supplementation schedules (including information on the daily meal patterns, in particular the frequency per week of foods of animal origin) and adverse events, including diarrhea, constipation and discomfort. Acceptance of sprinkles was evaluated by a questionnaire, including questions that assessed whether the infant refused the treatment and whether sprinkles changed the color, taste or texture of the complementary food, as reported (22). Finally, monitors collected the 1-dose sprinkle sachets that mothers had been instructed to set aside after use to check compliance. Monitors weekly updated the individual infants' schedules that were finally collected at the Cooperation and Development Onlus office in Phnom Penh. As far as reference Italian infants, mothers were administered a 24-h dietary recall after blood sampling and were asked about breastfeeding practices.

TABLE 2. *Characteristics of 191 Cambodian and 21 Italian infants sampled for FA analysis**

	Cambodians	Italians
Age, mo (range)	18 (18–19)	18 (17–19)
Males/females	100/91	10/11
WA *z* score	−1.14 (−1.25, −1.03)	0.14 (−0.34, 0.62)
LA *z* score	−1.55 (−1.67, −1.43)	0.06 (−0.46, 0.58)
WL *z* score	−0.37 (−0.48, −0.26)	0.12 (−0.36, 0.60)

WA indicates weight-for-age; LA, length-for-age; WL, weight-for-length.

* Mean, 95% CI, when not differently indicated.

Sample Size and Statistical Analysis

Because the intervention was based on iron supplementation, the sample size was determined to detect a ≥5-g/L difference in mean haemoglobin at the end of the study period between any of the treatment groups, and 68 infants needed to be recruited in each group. No data are available in large infant and children populations on FA profiles, a type of assessment that has been possible by applying the new analytical strategy. Based on the average levels of LA in plasma total lipids in a normal healthy population of 105 Italian children (27), the sample size of Cambodian infants included in each intervention group would allow for detecting a 10% between-group difference of LA as statistically significant ($P < 0.05$), with 90% power.

In defining the size of the Italian reference sample, we estimated that we would enroll 10% of the original whole sample of Cambodian infants. With this sample size, a 20% difference in LA levels (SD 3%) between the Cambodian and the Italian group could have been detected as statistically significant ($P < 0.05$), with 80% power.

Descriptive data are given as mean, standard deviation or percentage. The effect of treatments on FA profiles was assessed with analysis of variance, including treatment as a fixed factor. Bonferroni's correction was used in calculating the significance of post hoc multiple comparisons. A significance level of 0.05 was used, and all of the statistical tests were 2-tailed. Statistical analysis was performed with SPSS version 12.0 (SPSS, Inc, Chicago, IL).

Ethics

The project was coordinated with the Cambodian Ministry of Health and received the approval of the National Ethical Committee of the Cambodian Ministry of Health. Parents were informed about the procedures and purpose of the study and gave written informed consent. The Departmental Ethical Committee of San Paolo Hospital agreed on the portion of the study design involving Italian infants.

RESULTS

A total of 214 potentially eligible infants were initially contacted. One of them exhibited haemoglobin <70 g/L and was excluded from the study, whereas 9 families refused to participate. Therefore, a total of 204 (95.4%) infants (109 males, 95 females) were recruited and randomised into 3 groups of treatments. Final assessment was completed on 191 (93.6) of them. Figure 1 shows the flow diagram of the trial, according to the CONSORT statement (28). During the supplementation period, the groups were similar for weight, length and weight-for-length parameters, but the growth patterns were significantly lower than international reference standards in each group.

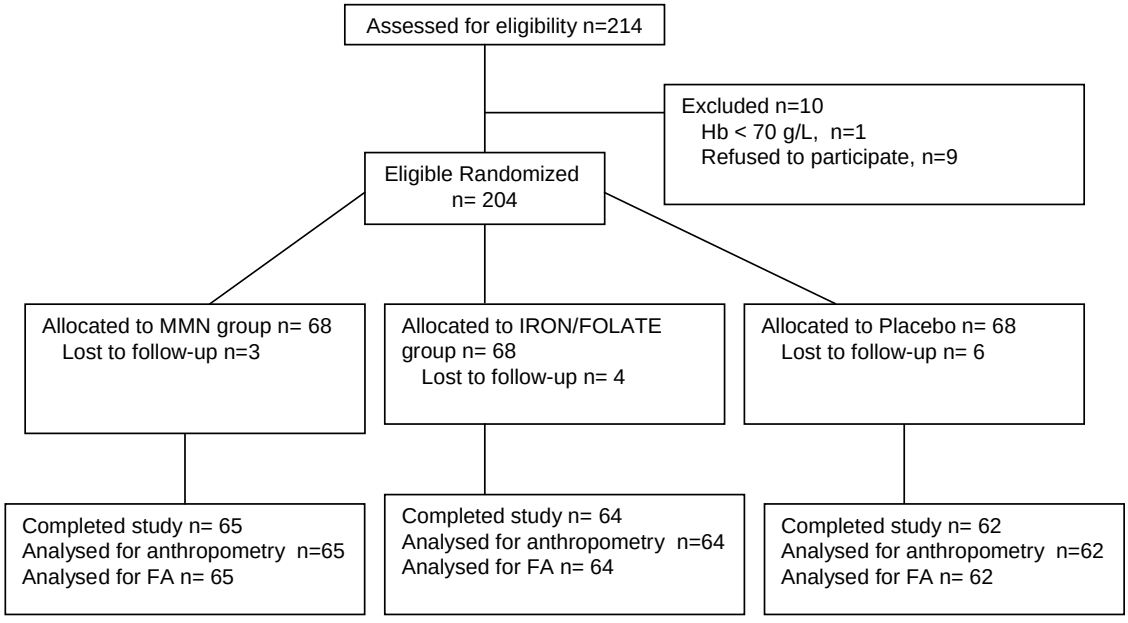

FIG. 1. Flow diagram of subject progress through study.

FA Status

The comparison of the FA profiles of whole blood lipids of all of the Cambodian and Italian infants at the end of the trial (Table 3) reveals, as expected, striking differences. In Cambodian infants the levels of palmitic acid, total saturates and oleic acids are lower, whereas those of stearic acid are higher. Also, levels of total PUFA are higher in the Cambodians, with selected differences for the 2 series. In the n-6, LA levels were lower, but those of arachidonic acid (AA) were higher, and in the n-3, although levels of α-linolenic acid (ALA; C18:3 n-3) are similar between the 2 groups, those of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are significantly higher in Cambodians. Within Cambodian infants (Table 4), significant differences are shown in the MMN group but not in the iron/folate group, compared with those who were given placebo, such as a reduction of 18:0 and increments of the 2 18 C EFAs LA and ALA of 10% and 45%, respectively. The corresponding FA classes (ie, saturates, total PUFA and total n-6 and n-3 FA) were not modified. No change occurred in the percentage values of LC-PUFA. The concentrations of LA in the MMN group became closer but were still significantly lower compared with those of Italian infants ($P = 0.024$), whereas the Cambodian levels of ALA were not different from the Italian. In Table 4, the levels of haemoglobin and haematocrit found at the end of the supplementation in the 3 groups are reported for a better evaluation of the effects on FA status.

TABLE 3. *Blood FA patterns of Cambodian vs Italian infants*

FA*	Cambodian (n = 191), mean ± SD	Italian (n = 21), mean ± SD	*P*
16:0	23.64 ± 2.78	31.47 ± 2.58	0.000
18:0	10.62 ± 1.35	9.58 ± 0.84	0.001
18:1n-9	17.51 ± 2.87	23.73 ± 2.21	0.000
20:3n-9	0.12 ± 0.22	0.11 ± 0.06	0.821
18:2n-6	14.57 ± 3.24	17.56 ± 3.02	0.000
20:4n-6	9.65 ± 1.96	6.79 ± 0.80	0.000
18:3n-3	0.24 ± 0.20	0.23 ± 0.06	0.792
20:5n-3	0.43 ± 0.24	0.17 ± 0.06	0.000
22:6n-3	3.72 ± 1.03	1.30 ± 0.87	0.000
Saturated FA	39.54 ± 2.88	43.76 ± 2.44	0.000
Monounsaturated FA	27.68 ± 3.79	27.58 ± 2.22	0.901
Polyunsaturated FA	32.77 ± 4.28	28.65 ± 3.12	0.000
n3/n6 ratio	0.20 ± 0.04	0.08 ± 0.03	0.000
ALA/LA ratio	0.016 ± 0.011	0.013 ± 0.004	0.282
AA/LA ratio	0.69 ± 0.21	0.40 ± 0.11	0.000
DHA/ALA ratio	20.41 ± 12.13	5.59 ± 3.14	0.000

* FA as percent of total blood FA.

In consideration of dietary assessments, all but 2 of the Cambodian infants were still breast-fed at least twice per day at 18 mo; none were supplemented with formulas. Most of their energy and protein intakes besides human milk came from local cereal staple foods (*bobor*, the most common local preparation, is made of rice) and small servings of fish, meat or eggs were supplied 1 to 2 times per week on average. Consumptions of minimal amounts of seed oils were reported in a minority of infants. All of the Italian infants followed mixed diets in line with local dietary habits, providing whole cow's milk and animal foods every day. All of the Italian infants stopped breastfeeding between 3 and 6 mo of age and were

TABLE 4. *FA patterns and blood parameters (haemoglobin, g/L; haematocrit, %) in Cambodian infants subdivided per dietary supplement*

FA*	MMN (n = 65), mean ± SD	Iron/folate (n = 64), mean ± SD	Placebo (n = 62), mean ± SD	*P*
16:0	23.54 ± 3.56	23.47 ± 2.43	23.93 ± 2.14	0.610
18:0	10.33 ± 1.62[a]	10.63 ± 1.22	10.92 ± 1.10[b]	0.048
18:1n-9	17.56 ± 3.11	17.53 ± 2.75	17.44 ± 2.77	0.972
20:3n-9	0.13 ± 0.24	0.14 ± 0.29	0.10 ± 0.07	0.584
18:2n-6	15.36 ± 4.02[a]	14.45 ± 2.90	13.87 ± 2.45[b]	0.032
20:4n-6	9.48 ± 2.41	9.60 ± 1.70	9.88 ± 1.68	0.503
18:3n-3	0.29 ± 0.31[a]	0.22 ± 0.10	0.20 ± 0.12[b]	0.037
20:5n-3	0.40 ± 0.22	0.42 ± 0.22	0.46 ± 0.27	0.383
22:6n-3	3.55 ± 1.26	3.79 ± 0.90	3.82 ± 0.86	0.265
Saturated FA	39.18 ± 3.40	39.43 ± 2.81	40.04 ± 2.88	0.227
Monounsaturated FA	27.65 ± 3.95	27.74 ± 4.30	27.64 ± 3.05	0.986
Polyunsaturated FA	33.16 ± 5.12	32.81 ± 4.36	32.30 ± 3.09	0.530
n-3/n-6 ratio	0.19 ± 0.05	0.21 ± 0.03	0.21 ± 0.04	0.060
ALA/LA ratio	0.017 ± 0.015	0.015 ± 0.007	0.015 ± 0.008	0.424
AA/LA ratio	0.65 ± 0.23a[a]	0.68 ± 0.16	0.74 ± 0.23[b]	0.046
DHA/ALA ratio	18.52 ± 14.10	19.28 ± 8.57	23.56 ± 12.58	0.041
Blood parameters				
Haemoglobin	107.6 ± 11.0[a]	109.3 ± 11.7[a]	99.7 ± 10.3[b]	<0.0001
Haematocrit	35.0 ± 2.7[a]	35.4 ± 2.8[a]	33.6 ± 2.5[b]	<0.0001

Different superscripts ([a,b]) indicate significant between-group differences at Bonferroni's post hoc analysis.
* FA as percent of total blood FA.

given a standard follow-up formula up to 9 (minimum) to 12 (maximum) mo of age.

DISCUSSION

We report for the first time changes in the FA status of infants with poor nutritional condition following dietary supplementation with a multimicronutrient preparation and comparative data between Cambodian and Italian infants. Significant differences are observed between FA profiles in the Cambodian and Italian infants, wherein the dietary supplementation with MMN in Cambodians resulted in higher LA and ALA levels, balanced by a reduction in 18:0, compared with the placebo group. Changes were statistically significant, although at this stage, their biological relevance cannot be established conclusively. It is of interest, however, that only 1 type of treatment (MMN) was able to modify blood EFA levels, even if no differences at the end of the trial were present between the 2 supplemented groups, MMN and iron/folate. The differences in LA, ALA and 18:0 were found out of 9 comparisons between the most relevant individual FA (Table 4). On the basis of the above considerations, we propose that although some chance differences could be expected when dealing with many comparisons, our results are biologically plausible because they concern 18 C compounds, with an increase of both 18 C EFA on the one hand, consistent with a parallel decrease of the saturated 18 C compound on the other hand. The trend for 18 C was partly independent from the changes of the haemoglobin and haematocrit values, which were significantly different from the placebo in both supplemented groups, with the highest levels found within the iron/folate group. Finally, the between-group significant difference, even if rather limited, was still evident after using conservative Bonferroni's post hoc analysis.

As a first general remark concerning the differences between Cambodian and Italian infants, Cambodians (as a whole group) have lower levels of LA, comparable ALA levels and higher levels of LC-PUFA in blood when compared with their Italian counterparts. They do not show the classic biochemical signs of EFA deficiency—higher levels of 20:3n-9 and low levels of 20:4n-6 in the blood (19). Because Cambodian infants, according to the dietary assessments, were still breast-fed and supplied with minimal amounts of fats from complementary foods, it is plausible that their blood FA profiles were mostly affected by the ingestion of maternal milk. Human milk is a constant source of highly available LC-PUFA (29), possibly contributing to the striking differences in AA and DHA levels found between Cambodian and Italian infants. Although the AA content of human milk is stable and does not vary widely throughout the world, the content of DHA is more closely connected to the environmental and dietary background of mothers (30) but is in any case present also in vegetarian and vegan mothers' milk (31). Because the composition of human milk from developing countries is highly variable, depending on dietary habits (32), this issue should be explored further in studies from different areas. Low DHA levels have been in fact reported in Pakistani infants in relation to the low DHA content in maternal milk (33), whereas in well-nourished Nigerian children, higher proportions of both n-6 and n-3 FA were reported, possibly in connection with more adequate dietary conditions (34). Differences in blood FA profiles, especially for LC-PUFA, may also be attributed to lower

triglyceride and cholesterol esters and higher phospholipid (usually higher in LC-PUFA) levels in the blood of Cambodian infants when compared with Italian infants.

As to the possible mechanisms of the effects on blood EFA levels in the MMN group, limited information is available in the literature on the relationships between dietary micronutrients and circulating EFA. In essence, vitamins with antioxidant activity may protect FA, especially PUFA, from catabolic processes (35,36), and folate administration has increased the levels of all of the n-3 PUFA, including ALA, in rats (37). Zinc deficiency during experimental pregnancies increased the oxidation of linoleate and decreased the synthesis or transport of PUFA to the foetus, and these effects appeared to be specific for zinc depletion (38). Similarly, in acrodermatitis enteropathica, a congenital autosomic recessive disease characterized by zinc malabsorption, zinc supplementation leads to quick clinical improvement and increments in LA and AA levels (39). In this exploratory study, we cannot exclude the possibility that any of the added supplemented nutrients could have had partial additive roles in the modification of the EFA status, according to the major effects observed in the MMN group versus placebo and the lack of differences of the MMN versus the iron/folate group. The lack of increment in the levels of LC-PUFA, in spite of a suggested enhancing effect of iron and zinc on LC-PUFA synthesis (19), may be related to the already elevated levels of AA and DHA, as derived from maternal milk, and result in a sort of product downregulation of LC-PUFA synthesis (40). Alternatively, a threshold level of LA may be needed to activate the biosynthetic enzymes, or a more complete, time-intensive recovery from specific nutritional deficiencies may be required to allow for the full activation of the metabolic pathways.

As for the biological meaning of the increase in EFA levels after MMN treatments, at present, we can only speculate. In fact, there is a huge lack of data from comparable studies in developing countries. Cystic fibrosis (CF), in which poor nutrition is common (41), may point the way, and abnormalities of EFA metabolism are now believed to be primary events and not just a consequence of fat malabsorption (42). In patients with CF, LA levels in plasma are ≈80% of those found in healthy individuals (43,44), and improvement in LA may have relevant functional effects on growth (45). Indeed, patients with CF who increase their LA levels are more likely to be responders to a program of recovering from malnutrition and growth faltering compared with those who do not recover (46). Therefore, it may be that positive functional effects of improved LA status could become evident after the institution of an adequate energy intake.

In conclusion, compared with the placebo group, supplementation with MMN in Cambodian infants was associated with an increase of blood LA and ALA levels (without significant differences in their longer-chain derivatives), up to levels that are closer to those displayed by well-nourished and normal-growing Italian counterparts. No differences were found between the MMN and the iron/folate groups. Significant decline was observed for growth parameters in any group through the study, as shown by anthropometric parameters at 18 mo, suggesting that mostly cereal-based complementary foods together with human milk are inadequate sources of energy and protein to support normal growth in endemic malnutrition during the first 2 y of life and that specific dietary interventions should consequently be planned (47). However, micronutrient supplementations may positively affect general metabolic condition beyond the simple improvement of the mineral body status, as indicated by the present observations on circulating FA, with possible long-term effects on growth and health, which deserve further investigation.

Acknowledgments: The authors acknowledge the financial assistance of the Canadian International Development Agency-Health and Nutrition Initiatives Fund. This project was developed as a partnership between the University of Milan, Department of Pediatrics at San Paolo Hospital, and CESVI (Cooperation and Development Onlus, Bergamo, Italy) and is based on the project ''Influence of Micronutrients Supplementation in Mortality, Morbidity and Growth Pattern in Rural Cambodian Infants in the First 2 Years of Life'' (HNIF/2003/02-090). In August 2002, a Memorandum of Understanding was signed between CESVI and the Ministry of Health, Cambodia. We acknowledge our local partners Institut Pasteur du Cambodge, Phnom Penh, and the Provincial Health Department of Kompong Chhnang. A special thanks to Tat Sovann, MD, Medical Officer, CESVI-Cooperation and Development, Cambodia.

REFERENCES

1. Yip R, Ramakrishnan U. Experiences and challenges in developing countries. *J Nutr* 2002;132 (Suppl):827S–30S.
2. Iron Deficiency Anaemia: Assessment, Prevention and Control. A Guide for Programme Managers (WHO/NHD/01.3). Geneva: World Health Organization, 2001.
3. Schultink W, Gross R. Use of daily compared with weekly iron supplementation: apples and pears. *Am J Clin Nutr* 1999;69: 739–42.
4. Beard JL. Weekly iron intervention: the case for intermittent iron supplementation. *Am J Clin Nutr* 1998;68:209–12.
5. Hurrell RF. Preventing iron deficiency through food fortification. *Nutr Rev* 1997;55:210–22.
6. Nestel P, Alnwick D, eds. Iron/Multi-micronutrient Supplements for Young Children. Summary and Conclusions of a Consultation Held at UNICEF Copenhagen, Denmark, August 19–20, 1996. Washington, DC: International Life Sciences Institute, 1997.
7. Zlotkin S, Arthur P, Antwi KY, et al. Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods. *Am J Clin Nutr* 2001;74:791–5.
8. Salgueiro MJ, Zubillaga MB, Lysionek AE, et al. The role of zinc in the growth and development of children. *Nutrition* 2002;18: 510–9.

9. Roy SK, Tomkins AM, Haider R, et al. Impact of zinc supplementation on subsequent growth and morbidity in Bangladeshi children with acute diarrhoea. *Eur J Clin Nutr* 1999;53:529–34.
10. Bhutta ZA, Black RE, Brown KH, et al. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. *J Pediatr* 1999;135: 689–97.
11. Lind T, Lönnerdal B, Stenlund H, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *Am J Cl Nutr* 2004;80:729–36.
12. Brooks WA, Santosham M, Naheed A, et al. Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: randomised controlled trial. *Lancet* 2005;366:999–1004.
13. Lind T, Lönnerdal B, Stenlund H, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. *Am J Clin Nutr* 2003;77:883–90.
14. Nhien NV, Lien DK, Trung NQ, et al. Approaches to improve iron bioavailability from complementary foods. *J Nutr* 2003;133: 1560S–2.
15. Kolsteren P, Rahman SR, Hilderbrand K, et al. Treatment for iron deficiency anaemia with a combined supplementation of iron, vitamin A and zinc in women of Dinajpur, Bangladesh. *Eur J Clin Nutr* 1999;53:102–6.
16. Thu BD, Schultink W, Dillon D, et al. Effect of daily and weekly micronutrient supplementation on micronutrient deficiencies and growth in young Vietnamese children. *Am J Clin Nutr* 1999;69:80–6.
17. Forsyth JS, Carlson SE. Long-chain polyunsaturated FA in infant nutrition: effects on infant development. *Curr Opin Clin Nutr Metab Care* 2001;4:123–6.
18. Innis SM. Polyunsaturated FA in human milk: an essential role in infant development. *Adv Exp Med Biol* 2004;554:27–43.
19. Smit EN, Muskiet EAJ, Boersma ER. The possible role of EFA in the pathophysiology of malnutrition: a review. *Prostaglandins Leukot Essent Fatty Acids* 2004;71:241–50.
20. Smit EN, Oelen EA, Seerat E, et al. Fish oil supplementation improves docosahexaenoic acid status of malnourished infants. *Arch Dis Child* 2000;82:366–9.
21. Koletzko B, Abiodun PO, Laryea MD, et al. FA composition of plasma lipids in Nigerian children with protein-energy malnutrition. *Eur J Pediatr* 1986;145:109–15.
22. Giovannini M, Sala D, Usuelli M, et al. Double blind placebo controlled trial comparing effects of supplementation with two different combinations of micronutrients delivered as sprinkles on growth, anaemia and iron deficiency in Cambodian infants. *J Pediatr Gastroenterol Nutr* 2006;42:306–12.
23. USDA Food and Nutrition Information Center. Dietary Guidance. Dietary Reference Intakes. DRI tables Available at: http://fnic.nal.usda.gov/nal_display/index.php?info_center=4&tax_level=2&tax_subject=256&topic_id=1342&placement_default=0. Accessed November 22, 2006.
24. Guidelines for Outreach Services from Health Centers. Phnom Penh: Ministry of Health, Cambodia 2001.
25. Marangoni F, Colombo C, Galli C. A method for the direct evaluation of the FA status in a drop of blood from a fingertip in humans: applicability to nutritional and epidemiological studies. *Anal Biochem* 2004;326:267–72.
26. Marangoni F, Angeli MT, Colli S, et al. Changes of n-3 and n-6 FA in plasma and circulating cells of normal subjects, after prolonged administration of 20:5 (EPA) and 22:6 (DHA) ethyl esters and prolonged washout. *Biochim Biophys Acta* 1993;1210: 55–62.
27. Scaglioni S, Verduci E, Agostoni C, et al. Dietary habits and plasma FA levels in a population of Italian children: is there any relationship? *Prostaglandins Leukot Essent Fatty Acids* 2004;71: 91–5.
28. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet* 2001;357:1191–4.
29. Marangoni F, Agostoni C, Lammardo AM, et al. Polyunsaturated FA concentrations in human hindmilk are stable throughout 12-month lactation and provide a sustained intake to the infant during exclusive breastfeeding. An Italian study. *Br J Nutr* 2000;84:103–9.
30. Agostoni C. LC-PUFA content in human milk: is it always optimal? *Acta Paediatr* 2005;94:1532–7.
31. Sanders TA, Reddy S. The influence of a vegetarian diet on the FA composition of human milk and the EFA status of the infant. *J Pediatr* 1992;120:S71–7.
32. Koletzko B, Thiel I, Abiodun PO. The FA composition of human milk in Europe and Africa. *J Pediatr* 1992;120:S62–70.
33. Smit EN, Oelen EA, Seerat E, et al. Breast milk docosahexaenoic acid (DHA) correlates with DHA status of malnourished infants. *Arch Dis Child* 2000;82:493–4.
34. Koletzko B, Abiodun PO, Laryea MD, et al. Comparison of FA composition of plasma lipid fractions in well-nourished Nigerian and German infants and toddlers. *J Pediatr Gastroenterol Nutr* 1986;5:581–5.
35. Wood LG, Fitzgerald DA, Lee AK, et al. Improved antioxidant and FA status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function. *Am J Clin Nutr* 2003;77:150–9.
36. Kardinaal AF, Aro A, Kark JD, et al. Association between beta-carotene and acute myocardial infarction depends on polyunsaturated FA status. The EURAMIC Study. European Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast. *Arterioscler Thromb Vasc Biol* 1995;15:726–32.
37. Pita ML, Delgado MJ. Folate administration increases n-3 polyunsaturated FA in rat plasma and tissue lipids. *Thromb Haemost* 2000;84:420–3.
38. Yang J, Cunnane SC. Quantitative measurements of dietary and [1-14C] linoleate metabolism in pregnant rats: specific influence of moderate zinc depletion independent of food intake. *Can J Physiol Pharmacol* 1994;72:1180–5.
39. Koletzko B, Bretschneider A, Bremer HJ. FA composition of plasma lipids in acrodermatitis enteropathica before and after zinc supplementation. *Eur J Pediatr* 1985;143:310–4.
40. Gomez Dumm IN, de Alaniz MJ, Brenner RR. Effect of dietary fatty acids on delta 5 desaturase activity and biosynthesis of arachidonic acid in rat liver microsomes. *Lipids* 1983;18:781–8.
41. Poustie VJ, Russell JE, Watling RM, et al. Oral protein energy supplements for children with cystic fibrosis: CALICO multicentre randomised controlled trial. *BMJ* 2006;332:632–6.
42. Strandvik B. Fatty acid metabolism in cystic fibrosis. *N Engl J Med* 2004;350:605–7.
43. Strandvik B, Gronowitz E, Enlund F, et al. Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis. *J Pediatr* 2001;139:650–5.
44. Freedman SD, Blanco PG, Zaman MM, et al. Association of cystic fibrosis with abnormalities in fatty acid metabolism. *N Engl J Med* 2004;350:560–9.
45. van Egmond AW, Kosorok MR, Koscik R, et al. Effect of linoleic acid intake on growth of infants with cystic fibrosis. *Am J Clin Nutr* 1996;63:746–52.
46. Shoff SM, Ahn HY, Davis L, et al. Temporal associations among energy intake, plasma linoleic acid, and growth improvement in response to treatment initiation after diagnosis of cystic fibrosis. *Pediatrics* 2006;117:391–400.
47. Rivera JA, Habicht JP. Effect of supplementary feeding on the prevention of mild-to-moderate wasting in conditions of endemic malnutrition in Guatemala. *Bull WHO* 2002;80: 926–32.