European Journal of Clinical Nutrition (2009) 63, 355–368
 0954-3007/09 $32.00
www.nature.com/ejcn

ORIGINAL ARTICLE

# Efficacy and safety of twice-weekly administration of three RDAs of iron and folic acid with and without complement of 14 essential micronutrients at one or two RDAs: a placebo-controlled intervention trial in anemic Cambodian infants 6 to 24 months of age

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**Objective:** To determine the differential efficacy and safety of twice-weekly administration of 3 RDAs of iron and folic acid, with and without a complement of 2 RDAs of 11, and 1 RDA of 3 additional essential micronutrients as compared to a placebo control (PlbCON) given as foodLETs.
**Subjects/Methods:** A total of 250 children aged 6–24 months were enrolled after recruitment by village health workers; 19 of them dropped out during the trial. Children were assigned to one of three treatment arms and followed for 20.5 weeks; 41 supervised twice-weekly dosings of 30 mg of iron plus folic acid, either with or without accompanying micronutrients or placebo were given as foodLETs, a tool for ready-to-eat fortification in infant food. Initial and final measurements of anthropometry and blood biomarkers for hematological, iron stores and inflammatory status, as well as for abnormal hemoglobin (Hb), were obtained. Symptoms of listlessness, vomiting, watery stools and acute respiratory infections were monitored weekly.
**Results:** Iron-containing supplements increased Hb concentrations significantly ($P<0.0001$) and virtually eradicated any IDA, as compared to no change in hematological status in the PlbCON group ($P=0.011$). Iron stores, as reflected by ferritin, increased significantly with iron-containing treatments ($P<0.0001$). Responses were as effective in individuals with HbE as in those with exclusively HbA phenotypes. Watery stools ($P=0.002$) and listlessness ($P=0.001$) were significantly more frequent in those receiving iron and folic acid alone than in the PlbCON group. In contrast, acute respiratory infections ($P=0.014$) and listlessness ($P=0.001$) were significantly less frequent in those receiving the multiple micronutrient formulation than in the PlbCON group.
**Conclusions:** Supplementation of micronutrients along with iron and folic acid mitigates the excess morbidity of iron-folate alone, without reducing its efficacy in correcting anemia and building iron stores. FoodLETs are a suitable vehicle to provide micronutrient supplementation to infants.
*European Journal of Clinical Nutrition* (2009) **63,** 355–368; doi:10.1038/sj.ejcn.1602930; published online 31 October 2007

**Keywords:** anemia; iron; multi-micronutrients; infant nutrition; hemoglobinopathies; excess morbidity

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Received 25 May 2006; revised 3 May 2007; accepted 11 September 2007; published online 31 October 2007

## Introduction

Iron deficiency and anemia are almost inevitable in the course of human infancy. The highest concentrations of iron reserves are found shortly after birth (Yip, 2001). With the doubling of infant size, the expansion of the red cell mass over the first 6 months of life and low iron concentration of maternal milk, the original iron stores become exhausted (Allen, 2001). Moreover, iron in complementary foods to

which most infants are weaned has a poor biological availability (Dallman *et al*., 1981; Brown *et al*., 1998). However, there is increased awareness that a large fraction of anemias in children is not related to iron-deficiency (Allen *et al*., 2000; Asobayire *et al*., 2001; Zlotkin *et al*., 2001; Schümann *et al*., 2005), but possibly to deficiencies in other micronutrients, such as folate (Institute of Medicine, 1998), vitamins B12 (Herbert, 1973), B2 (Powers, 2003), vitamin A (Semba and Bloem 2002) or copper (Hoffman *et al*., 1988). Moreover, there are adverse consequences for infant development from iron-deficiency anemia, such as cognitive deficits and a compromised host-defense immunity (Chandra and Kumari, 1994; Bhaskaram, 2002; Algarin *et al*., 2003; Failla, 2003).

There are, however, consequences to the administration of concentrated forms of iron, such as fortificant iron in prophylactic settings or iron supplements for anemia therapy. Iron is the major intracellular oxidant (Fang *et al*., 2002) and is recognized as a gastric irritant in doses above 40 mg for children (Institute of Medicine, 2001). The literature is divided as to whether fortificant iron aggravates the risk of diarrhea. Moreover, supplementation of young children with iron in Indonesia showed a tendency to impair the growth of children without evidence of iron deficiency, although not among subjects with initial unmet iron needs (Idjradinata *et al*., 1994), and 3 mg $kg^{-1}$ of daily iron dosages retarded linear growth of iron-replete Indian toddlers, whereas 6 mg $kg^{-1}$ doses had no growth effect on anemic compatriots (Majumdar *et al*., 2003).

Iron is most often administered on a daily basis either alone or with accompanying folic acid. In this regard, two new paradigms have emerged into public health consideration. First, because a large fraction of anemias in infants is not due to iron deficiency but possibly due to lack of other micronutrients (Allen *et al*., 2000), and because deficiency of iron presents some interesting interactions, for example, with zinc (Solomons, 1986; Kordas and Stoltzfus, 2004), vitamin A (Bloem, 1995) and riboflavin (Powers, 1995), a changing philosophical trend in public health is looking away from single-nutrient paradigms to multiple micronutrients (Thu *et al*., 1999; Gross, 2001; Allen and Shrimpton, 2005). Second, due to issues of cost, tedium and, in the case of iron, intestinal adaptation, the administration of micronutrients with a weekly or semi-weekly periodicity, has been proposed (Viteri, 1997; Schultink and Gross, 1999) and explored in intervention trials (Schultink *et al*., 1995; Angeles-Agdeppa *et al*., 1997; Thu *et al*., 1999).

Since both maximal efficacy and the security and safety are legitimate considerations, these two paradigms were addressed in the study reported here, in which iron with folic acid alone or as a multi-nutrient combination was compared in a regimen involving semi-weekly administration. The goal was to determine whether addition of the micronutrient combination would influence the impact of iron plus folic acid on iron-deficiency anemia (IDA) or non-iron-deficiency anemia (non-IDA). Moreover, responses to iron and micronutrients in populations with a high prevalence of hemoglobinopathies could be examined in this Cambodian setting. Any positive or adverse effects of the trace minerals and vitamins on health, growth and safety aspects were also of interest. We present here the findings from such an intervention trial conducted among anemic Cambodian children, 6–24 months of age. The objective was to determine the best combination of efficacy and safety in intermittent-day dosing of iron for the alleviation of anemia.

## Subjects and methods

*Population and subjects*

We chose 13 out of the 50 villages in Kampot Province, where GTZ has been working since 1998 and where trained volunteers were available as village health workers. A list of all children, aged 6–24 months as of 1 September 2003, was developed for each village. Village population numbers varied from 665 to 1494 inhabitants, who live in cabins scattered in rice fields. Demographic variables are as follows: 141 boys (56.4%) and 109 girls (43.6%) were included in the study; 111 children (44.4%) were below 12 months of age and 139 (55.6%) were 12 months old or older.

*Study design and treatment assignments*

The study was designed as a doubly masked, placebo-controlled, longitudinal intervention trial. To avoid possible mix-up of supplements, only one intervention was assigned to each specific village. A computerized randomization program assigned five villages to each intervention group and three villages to the placebo control (PlbCON) group. FoodLETs containing iron-folate (FeFOL), iron-multi-micronutrients (FeMMN) or placebo were differently coded by the manufacturer and were allocated to the respective villages by the local study supervisor. The assignment to the different intervention arms was equally distributed in Kampot province (Figure 1). No differences in demographic or sociological stratification, sources of income or food intake pattern were present among the villages in the 60 $km^2$ area in which the study was performed. Therefore, for primary statistical treatment, the enrolled children were regarded as a reproducible sample for a homogenous population. To maintain conservative conclusions, additional adjustment was made for possible cluster effects. On average, 20 children were randomly selected from the list in each village and two volunteers per village were responsible for 10 children each. Exclusion criteria included acute malnutrition below 80% W/H, severe anemia with Hb $< 70$ g $l^{-1}$, any severe diseases, iron-supplementation from other sources or lack of parental approval for participation. The protocol was designed in accordance with the Cambodian Ministry of Health (MoH) and approved by the National Ethical Committee of the Cambodian MoH.

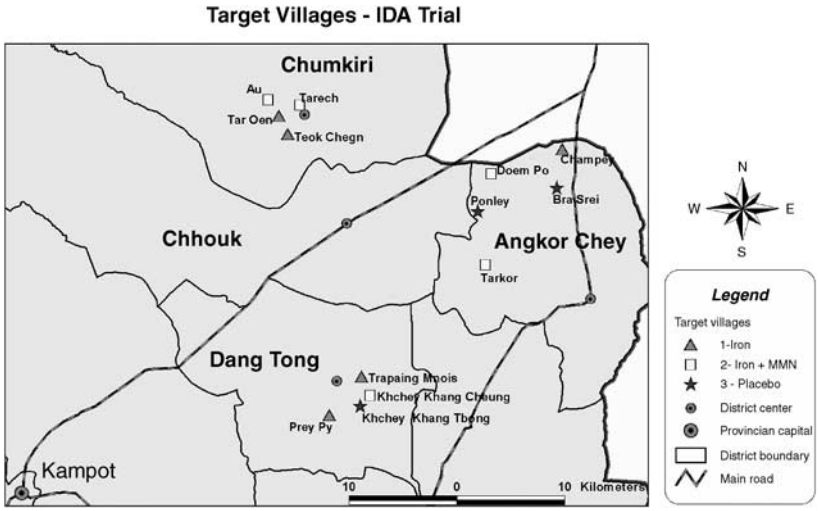


**Figure 1** Map of target villages: The chosen villages were randomly attributed to treatment and formed no clusters for any of the three treatment arms.

*Sample size considerations*

Sample size estimation was based on our results of earlier studies of children of comparable age from Guatemala City (Dewey *et al*., 1997; Schümann *et al*., 2005). We argued that sample sizes of 30 and 100 children per cohort would suffice to detect a difference in hemoglobin increases of 12.5 or 5%, respectively, at a significance level of 5% with a power of 80% (two-sided testing). In consequence, 100 children were allocated to each of the two verum interventions to enable detection of a difference in effect of 5% between them. Only 50 children were allocated to the placebo control group, as we expected a difference in effect in excess of 12% in comparison to the two verum groups.

*Nutrient supplement intervention*

The daily recommendation by the US Food and Nutrition Board is 11 mg Fe at 7–12 months and 7 mg Fe at 1–3 years of age (Institute of Medicine, 2001). Accordingly, a supplementation of 10 mg Fe per day as ferrous sulfate seemed closest to this recommendation for the age interval of 6–24 months. The recommendation for folic acid is 100 $\mu$g day$^{-1}$. Within the concept of a twice-weekly intervention regime, a total of 30 mg of elemental iron and 300 $\mu$g of folic acid were included for each of the 41 semi-weekly dosings of the intervention trial. This group was called 'FeFOL.'

The group called 'FeMMN' provided 14 essential micronutrients in addition to the aforementioned three RDAs of iron and folic acid. The amounts of micronutrients contained in each dosing are shown in the lower panel of Table 1. For micronutrients with high toxic potential, such as vitamin A and Se, only one RDA was supplemented. Zn supplementation was reduced to one RDA to avoid interaction with iron absorption (Solomons and Jacob, 1981; Kordas and Stoltzfus, 2004). On reanalysis by the manufacturer after 18 months, the micronutrient content of the remaining foodLETs that had been shipped to and stored in Cambodia during the trial was still in accordance with specifications. In addition, there was a 'PlbCON group.'

'Foodlet' is a generic term for a micronutrient supplement format that is like a 'petite food' (Gross, 2001; Briend and

**Table 1** Quantitative micronutrient formulation of the two iron-containing foodLETs administered on a semi-weekly basis as the intervention treatments

| |
|---|
| *FoodLET #1 (iron-folate (FeFOL), 2 micronutrients)* |
| Iron—30 mg as ferrous sulfate |
| Folic acid—300 $\mu$g |
| *FoodLET #2 (iron plus multi-micronutrients (FeMMN), 14 micronutrients)* |
| Iron—30 mg as ferrous sulfate |
| Folic acid—300 $\mu$g |
| Vitamin A[a]—375 retinal activity equivalents |
| Vitamin D—10 $\mu$g |
| Vitamin E—12 mg of $\alpha$-tocopherol equivalents |
| Vitamin B1—1 mg |
| Vitamin $B_6$—1 mg |
| Niacin—12 mg |
| Vitamin $B_{12}$—1.8 $\mu$g |
| Vitamin C—70 mg |
| Zinc—10 mg[a] |
| Copper—1.2 mg |
| Selenium—40 $\mu$g[a] |
| Iodine—100 $\mu$g |
| *FoodLET #3 (placebo control (plbCON) micronutrients)* |

Note: The amounts of nutrients were equally divided into two foodLET units, consumed twice weekly during the intervention.
Whereas the iron and folic acid dosage represented three RDAs for children of this age, the remaining micronutrients are at two RDAs included in the semi-weekly dosing.
[a]Only one RDA of vitamin A, Zn and selenium included in the semi-weekly dosing.

Solomons, 2003). The sweet, discoid, crushable and chewable version, which is analogous to placing food into a tableted form, is designated as the foodLET (uppercase 'LET') of the genre (Lock 2003). For technical reasons in the manufacturing process, it was necessary to utilize two foodLET units to contain the entire nutrient dosage desired.

Field distribution began on 1 Sep 2003 and ended on 25 Feb 2004. On two occasions each week, with a minimum separation of 3 days over 21 calendar weeks of the trial allowing for the 41 dosing days to have passed, field workers distributed and fully supervised the consumption of two foodLET units of either the iron-folate or the multiple micronutrient varieties, or the PlbCON foodLET. Hence, aside from other food sources, the multi-micronutrient intervention was to provide 85.7% of the weekly recommended intake of iron and folate, 28.6% of vitamin A, Zn and Se, and 57.1% of that for the remaining micronutrients of interest.

The national distribution protocols in Cambodia mandate administration of one dose of 100 000 IU of vitamin A to children at the age of 6 months and 200 000 IU of vitamin A to those aged 12 months or more on a semi-annual basis. Moreover, distribution of 250 mg of the anthelmintic drug, mebendazol, at 6-month intervals for children 12–23 months old, was part of a mandated public health program. Children continued to participate in these programs throughout the study.

*Anthropometry and global nutrition evaluation*

At the beginning and the end of the study, weight was measured to the nearest 100 g with Salter UNICEF scales imported from Denmark. Similarly, supine length was measured to the nearest 0.1 cm with a locally made wooden board, using tailor tape applied to the surface. The same tools were used by the same field workers on pre- and post-trial measurements. *z*-Scores were calculated by use of ANTHRO Pediatric Anthropometric Software program, version 1.02 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

*Laboratory analysis of hematological and iron status*

Hemoglobin, serum ferritin and C-reactive protein (CRP) were determined by means of the CELL-DYN 3200 automated laboratory analyser (Abbott, Abbott Park, IL, USA), and by use of respective commercial test kits (ferritin: AXSYM, Abbott; CRP: bioMérieux, Lyon, France) within 6 h of blood sampling after transport in 4 °C cool boxes. The following cut-off values were used: anemia: Hb concentration $<110\,g\,l^{-1}$; iron deficiency: serum ferritin $<12\,\mu g\,l^{-1}$; non-IDA: Hb concentrations $<110\,g\,l^{-1}$ and serum ferritin $12\,\mu g\,l^{-1}$ or above; inflammation: CRP $>8\,mg\,l^{-1}$.

*Hemoglobinopathies*. Hemoglobin E and F varieties were identified at baseline within 3 days of blood sampling, using electrophoresis on cellulose acetate membranes at alkaline pH with Sebia material and electrophoretic position markers (PerkinElmer Life Sciences, Shelton, CT, USA). This procedure permits separation and quantification of A1, F, S and A2 hemoglobins. C hemoglobins are very rare in Asia (Flint *et al*., 1993); therefore, all abnormal hemoglobin migrating at the range of hemoglobin A2 at a rate over 20% at alkaline pH were considered as hemoglobin E and no complementary tests were performed to differentiate A2, C and E. Patients were considered heterozygous when the rate of HbE was between 20 and 50%, and homozygous when the rate was over 50%.

*Morbidity indicators*

Field workers conducted weekly interviews with the mothers or caretakers to monitor occurrence of listlessness, vomiting, acute respiratory infection (ARI) and watery stools. Moreover, the health workers visited all children daily and monitored occurrence of severe symptoms, the number of symptoms and of total observations. The ratio between observed disease symptoms and total observations was summed over the entire period for all children in each of the intervention arms and expressed as percent. 'Watery stools' were defined as more than one 'watery stool' per day. ARI was marked when cough was observed along with fever ($>38\,^{\circ}C$). Vomiting was differentiated from regurgitation by a time interval of over 15 min following food intake. A child was defined as 'listless' if he or she were sleepier, showed less activity or consumed less food than usual.

*Pattern of consumption of food items*

A weekly questionnaire was used to assess different kinds of food consumed. Variables included rice soup, plain rice, fish (including crab, squid, snail and frog), beef, pork, chicken (including ducks and eggs), vegetables and fruits. Answers were recorded as 'yes' or 'no'. This system allowed us to analyze whether a child consumed any of these food items at least once during the previous week.

*Data handling and statistical analysis*

The *a priori*, main effective hypothesis of the study was that supplementation of iron with or without additional multi-micronutrients would lead to higher increments in hemoglobin and ferritin concentrations than PlbCON. The other results were exploratory in nature. The descriptive statistics of arithmetic means (or geometric means as indicated) and standard deviations or ranges (25th and 75th percentile) were calculated. Significant differences among means and increments across the three intervention groups were analyzed by analysis of variance (ANOVA), Kruskal–Wallis test or $\chi^2$ test ($\alpha = 0.05$, power 80%, two-sided testing). To analyze time-dependent intervention effects within the groups, the Student's *t*-test and the nonparametric Wilcoxon

test were used, as appropriate. Nevertheless, hedging against any undetected influences of environment or geography across the units of randomization, the analyzed probability from statistical tests was also compared to an $\alpha = 0.025$ instead of $\alpha = 0.05$. A number of linear models were calculated to control the effect of the intervention for potential confounders. For *post hoc* analysis, the intervention groups were split into an iron-deficient subgroup (ferritin $< 12\,\mu g\,l^{-1}$) and an iron-adequate subgroup (ferritin $> 12\,\mu g\,l^{-1}$). The same statistical procedures were repeated for *post hoc* subgroup analysis as for *a priori* analysis. A probability level of 0.05 was established as the level of significance for hypothesis testing. Calculations were made with statistical software programs (SAS for Windows, Version 6.12; SAS Institute Inc., Cary, NC, USA) and SPSS (Statistical Package for Social Sciences, Version 11.5; SPSS Inc., Chicago, IL, USA).

## Results

*Population characteristics*

*Demographic findings*. Overall, a total of 250 children, initially aged 6–24 months, were enrolled. Four children out of the 250 later were identified as 32 months old when age data were rechecked with the parents, but were retained in the analysis. The total baseline sample included 141 (56%) boys and 109 (44%) girls, with an average age of $13.5 \pm 5.5$ months on the day of enrolment. After a loss of 19 (7.6%) participants (13 boys and 6 girls), a total of 231 subjects were available for data analysis, including 128 (56%) boys and 103 (44%) girls with an average age of $20.1 \pm 5.2$ months at the end of the trial. Compliance and number of dropouts are given in Figure 2. Though the number is small ($n = 6$), withdrawal during the trial was highest in the FeFOL group.

*Anthropometry*. The mean weight of the enrollees at baseline was $8.4 \pm 1.2$ kg (min–max: 5.4–11.4 kg). Table 2 provides the *z*-scores for anthropometric indices across treatment groups (rows) and over the 20.5-week interval (columns). As indicated, the WHZ and HAZ were not different, whereas the WAZ values were different across groups at baseline. Post-intervention averages across groups remained significantly different for WAZ and became so for HAZ. However, applying the attenuation approximation for design effect, that is, using $\alpha = 0.025$ instead of $\alpha = 0.05$, would eliminate each of the aforementioned inter-group differences at the end of the study. In terms of time-dependent changes in absolute mean values, on average the children gained $1.1 \pm 0.4$ kg, corresponding to an increase of 13% between baseline and the end of the study. Weight increments amounted to $1.16 \pm 0.46$ kg and $1.10 \pm 0.43$ kg after intervention with FeFOL and FeMMN, respectively. Weight gain was significantly lower in the PlbCON group ($0.91 \pm 0.41$ kg; (one-way ANOVA, $P = 0.008$). The overall height of the enrollees at baseline was $72.4 \pm 5.7$ cm (min–max: 59.1–86.7 cm). The

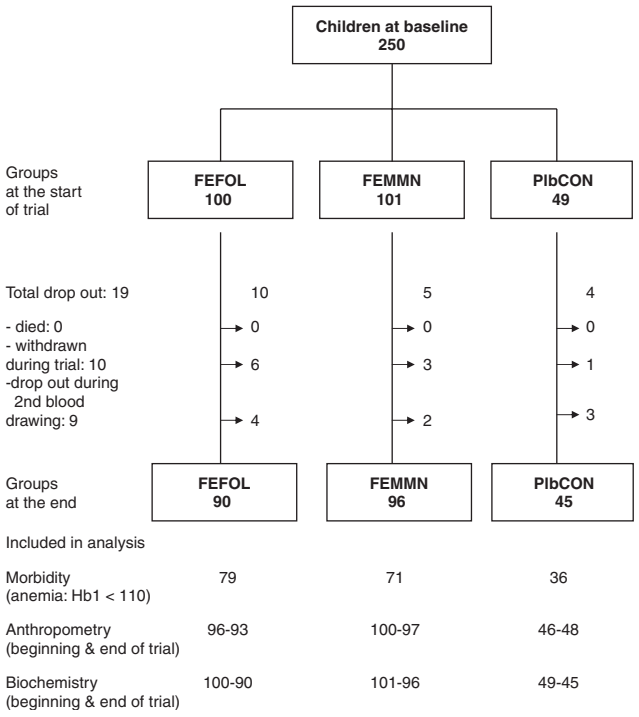


**Figure 2** Flow diagram of study participants.

children gained an average of $6.1 \pm 2.4$ cm in stature during the intervention period ($= 8.4\%$) without significant differences between groups (one-way ANOVA, $P = 0.404$). Linear modeling analysis revealed an impact of HbE on increments in weight, but not on height.

The mean *z*-scores WHZ (wasting) ($z < 0.0001$ in all groups) and WAZ (global malnutrition) ($z < 0.0001$–$0.002$) decreased significantly over time in all intervention groups, whereas no time-dependent change was observed in HAZ (Table 2). In the PlbCON group, the decreases in mean *z*-scores WAZ were more marked than in the iron-treated groups ($P = 0.032$), reflecting the aforementioned lower weight gain. WAZ were lower in the FeMMN group at baseline which may, in part, be responsible for less pronounced increments in this group. The HAZ (stunting) showed no significant changes over time in any group ($z = 0.125$–$0.480$; Wilcoxon test) (Table 2). However, these differential, time-dependent changes were not always robust enough to remain statistically significant after application of the design-effect attenuation (that is, using $\alpha = 0.025$ instead of $\alpha = 0.05$).

*Compliance with the intervention*

Over the 20.5 weeks of the intervention, the target number of supplement dosings (two foodLETs/intervention day) was 41. Ninety-eight percent of the subjects analyzed had actually received 40 or more dosings. The formats for administering the foodLET to the children tended to be constant within a given household on a week-to-week basis,

**Table 2** Comparison of mean *z*-scores for weight-for-height (wasting), weight-for-age (global malnutrition) and height-for-age (stunting) over time and between intervention groups (M ± s.d.)

| *Time (week)* | *PlbCON* | *FeFOL* | *FeMMN* | *Across-row P-value* |
|---|---|---|---|---|
| *WHZ (weight-for-height)* | | | | |
| 0 | −0.69 ± 0.83 ($n=46$) | −0.60 ± 0.97 ($n=96$) | −0.85 ± 1.09 ($n=100$) | $P=0.122$ |
| 20.5 | −1.19 ± 0.84 ($n=48$) | −1.01 ± 0.72 ($n=93$) | −1.19 ± 0.73 ($n=97$) | $P=0.139$ |
| Δ *WHZ* | −0.50 ± 0.76 ($n=46$) | −0.39 ± 0.71 ($n=93$) | −0.36 ± 0.85 ($n=97$) | $P=0.177$ |
| | $z<0.0001$ | $z<0.0001$ | $z<0.0001$ | |
| *WAZ (weight-for-age)* | | | | |
| 0 | −1.30 ± 1.05 ($n=46$) | −1.30 ± 0.93 ($n=96$) | −1.67 ± 0.96 ($n=100$) | $P=0.014$ |
| 20.5 | −1.73 ± 0.84 ($n=48$) | −1.53 ± 0.75 ($n=93$) | −1.81 ± 0.84 ($n=97$) | $P=0.044$ |
| Δ WAZ | −0.43 ± 0.54 ($n=46$) | −0.22 ± 0.56 ($n=93$) | −0.15 ± 0.54 ($n=97$) | $P=0.032$ |
| | $z<0.0001$ | $z=0.002$ | $z=0.007$ | |
| *HAZ (height-for-age)* | | | | |
| 0 | −1.23 ± 1.07 ($n=46$) | −1.27 ± 1.09 ($n=96$) | −1.53 ± 1.47 ($n=100$) | $P=0.13$ |
| 20.5 | −1.36 ± 0.94 ($n=48$) | −1.19 ± 0.96 ($n=93$) | −1.50 ± 1.12 ($n=97$) | $P=0.047$ |
| Δ HAZ | −0.12 ± 0.76 ($n=46$) | 0.06 ± 0.57 ($n=93$) | 0.04 ± 1.10 ($n=97$) | $P=0.172$ |
| | $z=0.480$ | $z=0.329$ | $z=0.125$ | |

Abbreviations: FeFOL, iron-folate; FeMMN, iron-multi-micronutrients; PlbCON, placebo control.
Across-row comparison was performed by the Kruskal–Wallis test; time-dependent changes within columns were analyzed by the Wilcoxon test for paired samples.

**Table 3** Variation in the manner in which the semi-weekly foodLET was consumed by infants and toddlers

| |
|---|
| 54.8% with *bobor* (rice soup) |
| 32.0% chewed/sucked as candy |
| 8.7% swallowed with water |
| 1.7% swallowed with coconut milk |
| 2.9% assorted miscellaneous formats |

but varied across families in terms of the format with which it was administered. Variation in the mode of foodLET consumption is summarized in Table 3. No systematic differences in the way of foodLET intake were observed between the three intervention groups.

*Dietary intake pattern*

As determined by use of a weekly questionnaire, all children ate the traditional rice soup (*borbor*), rice or both every week; on an average 6% had four servings per day, 57% had three servings per day, 27% had only two and 4% only one serving per day (6% did not return the questionnaire). Fish was regularly available at least once every week to 65–77% of the children and vegetable to 38–58%. Fruits were consumed at least once every week in only 18–32% of the families and chicken (10–12%), pork (4–7%) and beef (<1%) were available to very few participants on a weekly basis. No significant differences in food patterns were seen between the different intervention groups.

*Hematological and iron status variables*

*Hb values and changes in Hb.* Mean Hb concentration at baseline for all subjects averaged $101.5 \pm 10.6\,g\,l^{-1}$, with no differences among treatment groups (Table 4). Based on the cut-off criterion for anemia of Hb concentration $<110\,g\,l^{-1}$, 74.4% ($n=186$) of the enrolled population of 250 was classified as anemic and 44% ($n=110$) was classified as iron-deficient based on a ferritin concentration below $12\,\mu g\,l^{-1}$. Of the 186 anemic children, 91 (49%) had IDA. Correspondingly, the remaining 95 (51%) children had anemia without iron-deficiency (non-IDA). Hemoglobin phenotypes were determined for all 250 enrolled subjects. Of these, 164 (66%) had normal HbA, 79 (32%) had HbE hemoglobinopathy and 7 (3%) had HbF hemoglobinopathy.

The mean increment of Hb in the three intervention groups during the observation interval were significant for FeFOL ($+9.8 \pm 12.0\,g\,l^{-1}$) and FeMMN ($+6.4 \pm 10.6\,g\,l^{-1}$) ($z<0.0001$; Wilcoxon test), whereas no time-dependent increment was seen in the PlbCON group ($z=0.384$). The difference between Hb in the PlbCON group and that of both treatments containing iron was highly significant ($P<0.0001$) at the end of the study. The difference in Hb between FeFOL and FeMMN groups, however, was not significant (Wilcoxon test, $P=0.058$) (Table 4).

The prevalence of anemia among all 250 subjects enrolled at baseline was 74.4%. No difference in the number of anemic children, that is, with basal Hb concentrations $<110\,g\,l^{-1}$, was found across treatment assignments at time of enrollment: 79.0 ± 8.0% FeFOL; 70.3 ± 8.9% FeMMN; 73.5 ± 12.4 PlbCON; $P=0.36$, $\chi^2$ test). In terms of recovery from anemia across the treatment-groups over the 20.5 weeks of intervention, there was a 6.8 percentage point decline in prevalence in PlbCON from 73.5 to 66.7%, a 47.9 percentage point change in FeFOL, from 79.0 to 31.1%, and a 27.6 percentage point reduction in FeMMN, from 70.3 to 42.7%. The reduction in anemia prevalence was significantly

**Table 4** Analysis of hemoglobin, ferritin and C-reactive protein concentrations as well as changes in these parameters over 20.5 week of intervention in all children independent of Hb phenotype

| *Time (week)* | *PlbCON* | *FeFOL* | *FeMMN* | *Across-row* P-*value* |
|---|---|---|---|---|
| *Hb concentration ($g\,l^{-1}$)* | | | | |
| 0 | 103.1±8.5 ($n=49$) | 100.5±11.4 ($n=100$) | 101.8±10.8 ($n=101$) | $P=0.279$ |
| 20.5 | 101.8±9.6 ($n=45$) | 110.2±10.2 ($n=90$) | 108.4±8.4 ($n=96$) | $P<0.0001$ |
| *Δ Hb ($g\,l^{-1}$)* | −1.4±10.2 ($n=45$) | 9.8±12.0 ($n=90$) | 6.4±10.6 ($n=96$) | $P<0.0001$ |
| | $z=0.384$ | $z<0.0001$ | $z<0.0001$ | |
| *Ferritin concentration ($\mu g\,l^{-1}$)* | | | | |
| 0 | 12.0 (5.5,27.0) ($n=49$) | 12.5 (6.0,20.0) ($n=100$) | 15.0 (8.0,23.5) ($n=101$) | P = 0.421 |
| 20.5 | 15.0 (8.5,26.5) ($n=45$) | 30.0 (19.0,42.0) ($n=90$) | 36.0 (22.0,50.8) ($n=96$) | $P<0.0001$ |
| Δ Ferritin ($\mu g\,l^{-1}$) | 0.2±20.0 ($n=45$) | 16.4±29.1 ($n=90$) | 14.9±39.3 ($n=96$) | $P<0.0001$ |
| | $z=0.599$ | $z<0.0001$ | $z<0.0001$ | |
| *CRP concentration (mg per 100 ml)* | | | | |
| 0 | 1.1 (1.0, 2.0) ($n=49$) | 1.0 (1.0, 1.2) ($n=100$) | 1.0 (1.0, 1.4) ($n=101$) | P = 0.001 |
| 20.5 | 1.4 (1.0, 6.6) ($n=45$) | 1.6 (1.0, 7.3) ($n=90$) | 1.4 (1.0, 4.5) ($n=96$) | P = 0.605 |
| Δ CRP (mg per 100 ml) | 0.1±14.7 ($n=45$) | 4.2±9.8 ($n=90$) | 2.9±9.9 ($n=96$) | P = 0.051 |
| | $z=0.297$ | $z<0.0001$ | $z<0.0001$ | |

Abbreviations: CRP, C-reactive protein; FeFOL, iron-folate; FeMMN, iron-multi-micronutrients; PlbCON, placebo control.
Values are broken down by treatment groups: Hb concentrations: M±s.d.; ferritin and CRP values: geometric mean; range: 25th and 75th percentile; changes over time for ferritin and CRP are normally distributed and, therefore, are given as differences of arithmetic means ($n=250$ at t0).
Across-row comparison was performed by the Kruskal–Wallis test; time-dependent changes within columns were analysed by the Wilcoxon test for paired samples.

greater for both iron-containing groups as compared to PlbCON ($P=0.011$) and FeFOL produced a significantly greater reduction in the anemia prevalence than did FeMMN ($P=0.001$, $\chi^2$ test). The linear model showed no impact of high or low CRP values on Hb increments or Hb concentrations at the end of the study.

*HbE-hemoglobinopathy*

Subjects with normal HbA and abnormal HbE phenotypes were found almost equally distributed across the three treatment arms: HbE was 30.0% in FeFOL; 35.6% in FeMMN; 26.5% in PlbCON ($\chi^2$ test, $P=0.457$). A 6.2% increase in hemoglobin concentration from 101.5 to 107.8 $g^{-1}l$ was seen over the 20.5 weeks of observation when all children are included in the analysis. In those children with HbE, Hb concentrations increased 7.2% from 98 to 104 $g\,l^{-1}$ (Student's *t*-test, $P=0.0001$). This suggests that iron supplementation had the same relative capacity to improve IDA in both HbA and HbE children, but the hemoglobinopathic subgroup had more severe anemia at all stages (Tables 5a and b). Time-dependent increases in Hb were significant in HbE children on both iron-containing treatments. However, the number of children in this *post hoc* analysis was small and significant differences in the across-row (treatment-wise) comparisons were not seen (Table 5b). In contrast, ferritin values and increments were significantly higher after intake of iron-containing supplements in HbA and HbE children alike (Tables 5a and b). The linear model showed an impact of HbE on increases in weight, but not in height, no impact on Hb increments after iron-supplementation and no impact on the prevalence of 'watery stools' or ARI. There was no time-dependent change in Hb concentration in the small subgroup of children with HbF.

*Ferritin*. The prevalence of plasma ferritin concentrations $<12\,\mu g\,l^{-1}$ was 49.0% (PlbCON), 45.0% (FeFOL) and 40.6% (FeMMN; $P=0.604$), respectively, at baseline sampling. During intervention, the prevalence of low ferritin fell by nonsignificant 6.8% points to 42.2% ($P=0.467$) in the PlbCON group, by a significant 33.8% points to 12.2% in the FeFOL group ($P<0.0001$) and by a significant 30.2% points to 9.4% in the FeMMN group ($P<0.0001$, $\chi^2$ test). There was no significant difference between the final prevalence rates for low ferritin in the iron-containing treatment arms ($P=0.532$), but both prevalences were significantly lower than in the PlbCON group (Kruskal–Wallis test, $P<0.0001$).

*C-reactive protein values*. A total of 14 values of CRP (5.6%) were elevated above the 8 mg per 100 ml criterion at the baseline evaluation and 44 elevated CRP values (19.4%) were detected in the final assessment period (Tables 4 and 5a). The prevalence of post-trial CRP elevations showed no significant differences among treatment groups ($\chi^2$ test, $P=0.746$: 20.5% (PlbCON); 21.3% (FeFOL) and 17.0% (FeMMN)). Eliminating the Hb values of these individuals with evidence of inflammation from the analysis, moreover, did not alter the findings regarding the relative effects of the treatment arms on ferritin concentrations nor on final Hb concentrations or Hb increments over the 20.5-week intervention (data not shown).

The baseline CRP values were significantly higher in the 50 placebo-treated children than in the 200 iron-exposed

**Table 5a** Analysis in HbA children: hemoglobin, ferritin and C-reactive protein concentrations as well as changes in these parameters over 20.5 weeks of intervention

| *Time (week)* | *PlbCON* | *FeFOL* | *FeMMN* | *Across-row P-value* |
|---|---|---|---|---|
| *Hb concentration ($g\,l^{-1}$)* | | | | |
| 0 | 104.5±8.5 ($n=36$) | 101.7±11.7 ($n=66$) | 103.3±11.3 ($n=62$) | $P=0.304$ |
| 20.5 | 102.1±10.2 ($n=33$) | 113.0±9.8 ($n=58$) | 111.0±7.7 ($n=59$) | $P<0.0001$ |
| *Δ Hb ($g\,l^{-1}$)* | −2.5±10.4 ($n=33$) | 11.2±13.0 ($n=58$) | 7.1±10.7 ($n=59$) | $P<0.0001$ |
| | $z=0.175$ | $z<0.0001$ | $z<0.0001$ | |
| *Ferritin concentration ($\mu g\,l^{-1}$)* | | | | |
| 0 | 10.5 (5.0,26.0) ($n=36$) | 13.0 (5.0,23.3) ($n=66$) | 11.0 (7.0,20.3) ($n=62$) | $P=0.652$ |
| 20.5 | 18.0 (8.6,26.5) ($n=33$) | 27.0 (18.8,42.0) ($n=58$) | 34.0 (22.0,45.0) ($n=59$) | $P<0.0001$ |
| Δ Ferritin ($\mu g\,l^{-1}$) | 2.4±18.6 ($n=33$) | 13.8±33.4 ($n=58$) | 19.0±21.9 ($n=59$) | $P=0.005$ |
| | $z=0.264$ | $z=0.002$ | $z<0.0001$ | |
| *CRP concentration (mg per 100 ml)* | | | | |
| 0 | 1.2 (1.0, 3.2) ($n=36$) | 1.0 (1.0, 1.3) ($n=66$) | 1.0 (1.0, 1.2) ($n=62$) | $P<0.0001$ |
| 20.5 | 1.4 (1.0, 6.3) ($n=33$) | 1.8 (1.0, 7.3) ($n=58$) | 1.3 (1.0,4.4) ($n=59$) | $P=0.701$ |
| Δ CRP (mg per 100 ml) | −1.3±16.1 ($n=33$) | 4.4±11.2 ($n=58$) | 3.2±8.9 ($n=59$) | $P=0.109$ |
| | $z=0.540$ | $z<0.0001$ | $z=0.004$ | |

Abbreviations: CRP, C-reactive protein; FeFOL, iron-folate; FeMMN, iron-multi-micronutrients; PlbCON, placebo control.
Values are broken down by treatment groups: Hb concentrations: M±s.d.; ferritin and CRP values: geometric mean; range: 25th and 75th percentile; changes over time for ferritin and CRP are normally distributed and, therefore, are given as differences of arithmetic means ($n=164$).
Across-row comparison was performed by the Kruskal-Wallis test; time-dependent changes within columns were analysed by the Wilcoxon test for paired samples.

**Table 5b** Analysis in HbE children: hemoglobin, ferritin and CRP concentrations as well as changes in these parameters over 20.5 weeks of intervention

| *Time (week)* | *PlbCON* | *FeFOL* | *FeMMN* | *Across-row P-value* |
|---|---|---|---|---|
| *Hb concentration ($g\,l^{-1}$)* | | | | |
| 0 | 99.5±7.8 ($n=13$) | 97.0±10.5 ($n=30$) | 98.2±9.2 ($n=36$) | $P=0.854$ |
| 20.5 | 100.8±7.9 ($n=12$) | 104.5±9.0 ($n=29$) | 104.1±8.2 ($n=34$) | $P=0.482$ |
| *Δ Hb ($g\,l^{-1}$)* | 1.5±9.5 ($n=12$) | 7.7±9.8 ($n=29$) | 6.0±10.5 ($n=34$) | $P=0.308$ |
| | $z=0.505$ | $z<0.0001$ | $z=0.005$ | |
| *Ferritin concentration ($\mu g\,l^{-1}$)* | | | | |
| 0 | 21.0 (8.5,32.0) ($n=13$) | 11.5 (6.3,18.3) ($n=30$) | 16.0 (12.0,27.8) ($n=36$) | $P=0.029$ |
| 20.5 | 15.0 (7.8,28.5) ($n=12$) | 34.0 (24.0,50.5) ($n=29$) | 42.0 (29.0,57.3) ($n=34$) | $P=0.001$ |
| Δ Ferritin ($\mu g\,l^{-1}$) | −5.7±23.3 ($n=12$) | 22.4±19.3 ($n=29$) | 8.2±59.3 ($n=34$) | $P=0.006$ |
| | $z=0.328$ | $z<0.0001$ | $z=0.010$ | |
| *CRP concentration (mg per 100 ml)* | | | | |
| 0 | 1.0 (1.0, 1.5) ($n=13$) | 1.0 (1.0, 1.1) ($n=30$) | 1.0 (1.0, 1.5) ($n=36$) | $P=0.416$ |
| 20.5 | 1.1 (1.0, 8.7) ($n=12$) | 1.5 (1.0, 10.1) ($n=29$) | 1.7 (1.0, 5.1) ($n=34$) | $P=0.743$ |
| Δ CRP (mg per 100 ml) | 3.9±9.3 ($n=12$) | 4.2±6.8 ($n=29$) | 2.4±12.0 ($n=34$) | $P=0.516$ |
| | $z=0.237$ | $z<0.0001$ | $z=0.104$ | |

Abbreviations: CRP, C-reactive protein; FeFOL, iron-folate; FeMMN, iron-multi-micronutrients; PlbCON, placebo control.
Values are broken down by treatment groups: Hb concentrations: M±s.d.; ferritin and CRP values: geometric mean; range: 25th and 75th percentile; changes over time for ferritin and CRP are normally distributed and, therefore, are given as differences of arithmetic means ($n=79$).
Across-row comparison was performed by the Kruskal–Wallis test; time-dependent changes within columns were analysed by the Wilcoxon test for paired samples.

children (Table 4). There were significant increases in CRP concentration in the iron-exposed groups over time ($z<0.0001$), but not in the PlbCONs. Seasonal influences or infectious changes are unlikely to be responsible for these increments, as no such changes were observed in the PlbCON group. Thus, exposure to supplemental iron may possibly have produced some inflammatory responses, though due to difference in CRP values between groups at baseline, this is not provable.

*Frequency of infectious manifestations*

No clustering of disease symptoms was observed in any particular calendar week in any of the intervention groups.

Thus, no short-term epidemic diarrhea or ARI events seem to have been a confounding factor. Using the background prevalence in the PlbCON group as standard, the relative excess or reduction in symptom prevalence in the FeFOL and FeMMN treatment arms was assessed (Figure 3).

For listlessness, there was a 3.2% greater prevalence in the FeFOL subjects than in the PlbCON group ($P=0.340$) (Figure 3), whereas prevalence of listlessness was 6.9% lower than PlbCON in the FeMMN group ($P=0.001$). FeFOL and FeMMN administration had no significant impact on vomiting ($P=0.636$). Semi-weekly FeFOL led to a 4.5% higher rate over PlbCON ($P=0.105$), whereas FeMMN supplementation produced a 1.6% lower prevalence ($P=0.536$). 'Watery stools' were significantly more prevalent with FeFOL administration than with PlbCON (16.7%; $P=0.002$), whereas FeMMN treatment caused no significant increment ($+1.6\%$) of this symptom ($P=0.798$). Finally, for ARI the prevalence in FeFOL subjects was significantly higher (15.4%) than in the PlbCON group ($P=0.006$), whereas FeMMN supplementation reduced ARI prevalence by significant 13.3% ($P=0.014$) (Figure 3, Table 6). No impact of HbE on watery stools or ARI was evidenced by linear analysis.

Post hoc *interaction hypotheses*

The complex mixture of hemoglobin phenotypes, etiologies of anemias and differential responses in the treatment arms permits to address some additional hypotheses. This is done within the caveats that (1) the original study was not designed to examine them prospectively and (2) the sample size available for the analysis was not established *a priori* and may lack the power for a robust test of the null hypotheses.

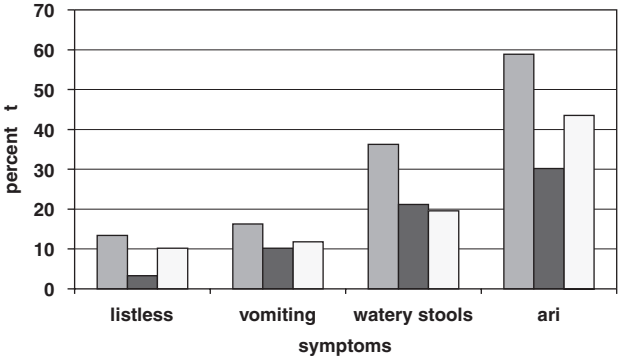


**Figure 3** Disease symptoms: listlessness, vomiting, diarrhea and acute respiratory infections (ARIs) were monitored by weekly questionnaires and during twice-weekly visits of trained village health workers. Symptom prevalence is given in percent of cases as related to total observations (weeks × participants). The frequency of all symptoms was close to evenly distributed over the observation period, so that no local epidemic events interfered with the prevalence of symptoms in any intervention groups (left bars = iron-folate (FeFOL); middle bars = iron-multi-micronutrients (FeMMN); right bars = placebo control (PlbCON)). Listlessness was significantly more frequent in FeFOL and PlbCON as compared to FeMMN; no significant difference was found between FeFOL and PlbCON. Vomiting showed no significant differences between any of the intervention groups. Watery stools were significantly more frequent in FeFOL than in FeMMN or PlbCON. ARI was significantly more frequent in FeFOL than in FeMMN or PlbCON and significantly more frequent in PlbCON than in FeMMN ($P<0.05$).

*Differential responsiveness of IDA and non-IDA after different treatments*. At baseline, the functional distribution between IDA and non-IDA cases showed no significant differences between the treatment arms. Figure 4 shows the impact of intervention on the fractional distribution between non-anemia, IDA and non-IDA. The iron-containing interventions significantly mitigated IDA. By contrast, non-IDA did not improve to a major extent, neither after FeFOL nor after FeMMN intervention.

*Different utilization of supplemental iron in iron-deficient and non-iron-deficient children*. It seemed appealing to examine whether the tenets of sequential changes in diagnostic indicators for hematological and iron status proposed by Cook and Finch (1979) were borne out by our data. For this, we isolated the two treatment arms, which received 30 mg Fe twice weekly, one with and one without added micronutrients. Eighty-six (42.8%) of the 201 children, receiving FeFOL or FeMMN, had serum ferritin concentrations below $12\,\mu g\,l^{-1}$ at baseline and 115 (57.2%) children did not. Subtracting the dropouts, 80 and 106 children were left in these two groups at the end of the study. Those with high ferritin at baseline showed ferritin increments of $22.7\pm18.0\,\mu g\,l^{-1}$; initial iron-deficiency (low ferritin at baseline) led to significantly lower ferritin increments ($10.3\pm42.5\,\mu g\,l^{-1}$; Wilcoxon test, $P=0.006$). Related to this observation is the question whether children who received iron, although they were initially iron-replete and had non-IDA, showed pathological symptoms more frequently than the initially iron-deficient fraction. No significant differences in symptom prevalence were observed between initially iron-adequate/iron-replete or iron-deficient children: listlessness 32 vs 24% ($n=189$); vomiting 50 vs 42% ($n=191$); watery stools 65 vs 69% ($n=191$) and ARI 84 vs 83% ($n=191$). Throughout the primary and *post hoc* analysis, with respect to the voluntary attenuation of statistical power assumption within our cluster-design, setting the threshold for the $\alpha$-value to 0.025 instead of 0.05 failed to change any conclusion about statistical significance, except as noted for anthropometric *z*-scores; this affirmed the robustness of the findings.

## Discussion

Southeast Asia is a region of the world with a major burden of IDA (Scrimshaw, 2004) and anemia is a major public health problem in Cambodia (Longfils *et al*., 2005), in particular during the first 2 years of life (HKI, 1999). Adequate nutrition is considered to be a basic human right

**Table 6** Prevalence of symptoms in the three supplementation arms given in percent of the total number of observations over the study period in all children on the respective treatment

| *Symptom* | *Listlessness* | | | *Vomiting* | | | *'Watery stools'* | | | *ARI* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Observed Child-month* | *Total* | *With symp* | *Without symp* | *Total* | *With symp* | *Without symp* | *Total* | *With symp* | *Without symp* | *Total* | *With symp* | *Without symp* |
| FeFOL | 464 | 62 | 402 | 465 | 76 | 389 | 465 | 169 | 296 | 465 | 274 | 191 |
| | 100% | 13.4% | 86.6% | 100% | 16.3% | 83.7% | 100% | 36.3% | 63.7% | 100% | 58.9% | 41.1% |
| FeMMN | 489 | 16 | 473 | 490 | 50 | 440 | 490 | 104 | 386 | 490 | 148 | 342 |
| | 100% | 3.3% | 96.7% | 100% | 10.2% | 89.8% | 100% | 21.2% | 78.8% | 100% | 30.2% | 69.8% |
| PlbCON | 225 | 23 | 202 | 228 | 27 | 201 | 230 | 45 | 185 | 230 | 100 | 130 |
| | 100% | 10.2% | 89.8% | 100% | 11.8% | 88.2% | 100% | 19.6% | 80.4% | 100% | 43.5% | 56.5% |
| sum | 1178 | 101 | 1077 | 1183 | 153 | 1030 | 1185 | 318 | 867 | 1185 | 522 | 663 |
| | 100% | 8.6% | 91.4% | 100% | 12.9% | 87.1% | 100% | 26.8% | 73.2% | 100% | 44.1% | 55.9% |

Abbreviations: ARI, acute respiratory infection; FeFOL, iron-folate; FeMMN, iron-multi-micronutrients; PlbCON, placebo control; symp, symptom.

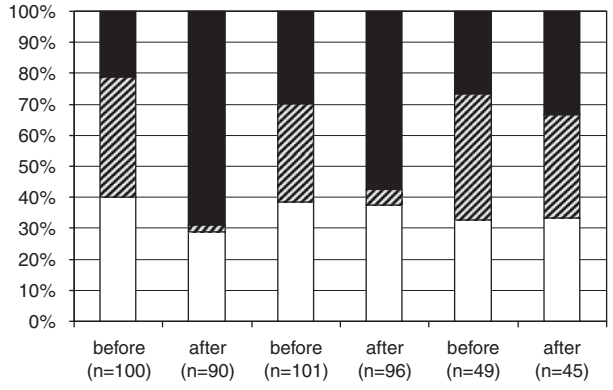


**Figure 4** Differences in prevalence between iron-deficiency anemia (IDA) and non-iron-deficiency anemia (non-IDA) between baseline and study end: Each column represents the percentage of non-anemic children (black), children with IDA (hatched) and with non-IDA (white). The two left columns stand for the prevalence before and after iron-folate (FeFOL) intervention, the two middle columns before and after iron-multi-micronutrients (FeMMN) intervention and the two right columns represent the placebo control (PlbCON) group. At baseline, prevalence of IDA and non-IDA is close to equally frequent among the anemic children in all three groups. No significant changes are observed in the PlbCON group while IDA prevalence decreases significantly and drastically after FeFOL and FeMMN intervention. Non-IDA prevalence, however, was not significantly affected by any of the interventions.

(Eide and Kracht, 1999; Rongguang and Kent, 2004). With the adverse consequences to early-life anemia on long-term cognitive impairment (Lozoff *et al*., 1991; Pollitt, 2001), any measure to improve performance and competence in later life will contribute positively to the economic emergence that seems crucial in the context of rapid social and economic development in this region of the world.

*Hematological and iron-status response*

The conventional iron-supplementation approach is daily administration, but weekly dosing appears to be associated with lower risks of adverse effects (Beard, 1998; Casanueva *et al*., 2005). As asserted by Hallberg (1998) and demonstrated in the IRIS study, the other field trial of a foodLET, with a weekly vs daily iron supplement format (Schultink *et al*., 2002; Smuts *et al*., 2003), daily iron administration has superior efficacy to reduce anemia. The respective cumulative iron doses over a week, in that study, however, were 20 mg (2 RDAs) and 70 mg (7 RDAs). This study offered 60 mg of iron, albeit at 3–4 day intervals, intermittent-day regimes.

As such, it is not unexpected that prorated supplementation of approximately 1 RDA of iron provided to infants and toddlers with IDA would produce a salutary response (Dallman *et al*., 1981; Yip *et al*., 1985). Reduction of anemia prevalence in our study was similar to that observed after daily supplementation of 12.5 mg Fe and 150 $\mu$g folic acid to infants in Kampong Chhnang, Cambodia (Giovannini *et al*., 2006). We may have been aided by a more efficient uptake of iron from the 30 mg presentation by the $>$48-h inter-dose interval, which seems to avoid the DMT-1 transport protein-mediated downregulation called 'mucosal block' (Oates *et al*., 2000; Frazer *et al*., 2003).

Iron-deficient children with HbE hemoglobinopathy can utilize supplemented iron effectively. Moreover, when controlling for any distortion of circulating ferritin behavior by inflammation, the interval increase in plasma ferritin was greater in the iron-containing treatment groups, again independent of the HbE or HbA phenotype status. This finding suggests that plasma ferritin values, indeed, represent the state of iron depletion and repletion, and that HbE hemoglobinopathy has no impact on iron storage under conditions of nutritional iron-deficiency.

Implications in this study come as a further reminder in a growing chain of evidence in young children around the world that much less than 100% of anemia is attributable to iron deficiency or responsive to iron administration (Allen *et al*., 2000; Asobayire *et al*., 2001; Verhoef *et al*., 2001; Zlotkin *et al*., 2001; Schümann *et al*., 2005). In accordance with earlier experiences (Allen *et al*., 2000; Zlotkin *et al*.,

2001; Schümann *et al*., 2005), non-IDA did not respond markedly to iron supplementation. The entire lack of any response of non-IDA prevalence to the four-weekly RDAs of vitamin B12 and riboflavin, and the two-weekly RDAs of vitamin A provided by the FeMMN seems to suggest that micronutrients deficiency may not be the cause of non-IDA.

A poor quality diet is another factor that can predispose one to deficiency of micronutrients. We recorded that plain boiled rice, the rice soup (*bobor*) or both were the food items most regularly consumed by our subjects. This is in accordance with the FAO balance sheet for Cambodia for 1995, rice accounted for 76% of the average 2000 kcal per capita food supply (UNICEF Report Rf. CSRC 2001/07/06, 2001). Recently, low zinc status was detected in 75% of stunted Cambodian preschoolers in Phnom Penh (Jack *et al*., 2004). Although no nutritional-status laboratory tests other than hematology were conducted, we can make inferences from the observed dietary intake patterns that other micronutrients were also in poor supply in this population. This feature of presumptive multiple micronutrient deficiency made the Kampot region of Cambodia an ideal setting for the exploration of the effects of exclusive iron supplementation as compared to iron plus multi-micronutrient supplementation.

*Morbidity*

Iron is a particular source of concern on the efficacy-safety continuum (Gross and Solomons, 2003), as it is the fulcrum of intracellular oxidative reactions (Schümann, 2001; Fang *et al*., 2002), as well as an essential nutrient for microbial pathogens (Weinberg, 1993). Gastrointestinal side effects are those most commonly associated with oral iron (Ganzoni *et al*., 1974; Brock *et al*., 1985). Epidemiological observations, summarized in a recent systematic review of the literature by Gera and Sachdev (2002) suggest an impact of iron supplementation on the prevalence of diarrhea, consistent with our observation of increased prevalence of watery stools with the FeFOL intervention.

*Post hoc* analysis revealed that initially adequate iron stores led to significantly higher increments in plasma ferritin at the same iron-intake levels than found in children with initially deficient iron stores, consistent with the proposition of Cook and Finch (1979). However, additional *post hoc* analysis showed no increased prevalence of adverse consequences of iron exposure in initially iron-replete children, as our hypothesis would project.

The weekly iron and folic acid, in the context of small multiples of the RDAs for other vitamins and minerals in the FeMMN preparation, had a net protective effect on part of the symptom panel. Not only was the pattern opposite to that of FeFOL, but the improvement compared to PlbCON suggests that, beyond mitigating the negative effects of iron, the broad assortment of micronutrients improves constitutional health of these infants and children. Iron is capable of inducing oxidative stress (Troost *et al*., 2003; Schümann *et al*., 2005), most likely through *in vivo* Fenton chemistry reactions (Schümann, 2001). The antioxidant micronutrients such as vitamins A, E and C, and selenium could have counteracted the oxidant stress induced by iron as well as other oxidant stresses induced by the background environment. Restoration of antioxidant defenses and repletion of deficiencies for nutrients with other essential structural and metabolic functions, that is, general micronutrient repletion, could explain the lower morbidity in the FeMMN group than in the other two groups. The multiple micronutrient formulation also contained zinc, which was shown to mitigate diarrhea with oral iron supplementation (Bhutta *et al*., 1999). If these speculations are accurate, the beneficial effects of multi-micronutrient supplementation on health may be less obvious in countries with more balanced diets.

Another trial in Cambodian infants (Giovannini *et al*., 2006) showed no impact of additional micronutrients on prevalence of symptoms. However, these studies are not directly comparable, due to differences in age at baseline, duration of the study, iron status, dosing schedule and choice of additional micronutrients.

*Growth*

Weight increments were higher in FeFOL and FeMMN groups as compared to PlbCON. Correspondingly, the *z*-score weight-for-age decreased significantly more in the PlbCON group as compared to those children with iron-containing interventions. Reversal of zinc (Brown *et al*., 2002) or vitamin A (Hadi *et al*., 2000) deficiencies stimulated compensatory catch-up growth in height in earlier studies. In this study, weight increments were observed in the FeFOL and the FeMMN groups. Iron deficiency seems to have impaired weight development, though most probably in conjunction with other deficiencies. Idjradinata *et al*. (1994) in Indonesia and Majumdar *et al*. (2003) in India showed that administration of iron to anemic children improved hematological recovery and ponderal growth. However, in those same studies, giving iron to non-anemic children produced no hematological response and had an adverse effect on weight gain. This study was not powered to detect small differences in weight increments between children with different iron status at baseline, but the group effect of experimental iron exposure, compared to PlbCON, reveals intriguing new aspects to the growth issues surrounding iron interventions.

*Operational and acceptance features of foodLETS*

The concept of the 'foodLET' used in this study was derived at a policy workshop in Rio de Janeiro, Brazil in 1999 (Gross, 2001). It is in the same genre as 'sprinkles' and high-nutrient density spreads (Nestel *et al*., 2003). In Kampot, as in the four international sites of IRIS (Smuts *et al*., 2003, 2005; Gross *et al*., 2005; Hop and Berger, 2005; IRIS Study Group, 2005; Lopez de Romaña *et al*., 2005; Untoro *et al*., 2005), acceptability of the intervention was good and cumulative

compliance was high. This is despite the fact that iron preparations produce darker stools and can discolor the serving dishes hours after the meals.

Moreover, in an analogous fashion to the South African site of IRIS (Smuts *et al*., 2003), in which the foodLETs were crushed and added to porridge, the majority of the Kampot caretakers chose to add the supplement to their child's complementary food. The efficacy to reduce anemia rates by the iron-containing interventions at two-weekly doses, despite its contact with potentially inhibitory constituents in the diet, attests to an adequate biological availability of the metal.

## Conclusion

This study in Kampot confirmed a >70% prevalence of anemia in Cambodian infants and toddlers, but places it in the context of highly prevalent hemoglobinopathy E and of a high background of non-IDA of approximately 50%. Over a 5-month period, twice-weekly iron dosing as FeFOL or FeMMN effectively eliminates almost all of the anemias attributable to iron-deficiency, including that in subjects with hemoglobinopathy E; however, they do not influence the prevalence of non-IDA. An additional effect of the iron-containing treatments was the support of increased weight gain. Our successful experience with the twice-weekly foodLET format for micronutrient delivery replicated the positive experience in the IRIS trial (Schultink *et al*., 2002; Smuts *et al*., 2005); it is a versatile and acceptable modality to deliver the nutrient packet to infants and toddlers.

The recent report of increased mortality from daily dosing of iron or iron–zinc supplements in malaria-endemic areas (Sazawal *et al*., 2006) strikes a prudent note of caution in the risk-benefit balance in anemic child populations. We show, however, that for a minimal additional cost of the micronutrients, a treatment that bolsters protection against the same symptoms can be placed into the public health arena. Even multiple micronutrient regimes are not without adverse consequences in some settings (Christian *et al*., 2003; Fawzi *et al*., 2004; Penny *et al*., 2004), such that close prospective health monitoring and evaluation should be an obligatory part of public health interventions of this nature.

## Acknowledgements

The authors acknowledge the inspiration of a vision on multiple nutrient interventions to resolve micronutrient malnutrition gained from our association with the late Dr Rainer Gross. The study was organized and funded by FSNPSP–GTZ. We thank Ung Kim Heang and Hay Soeng for assistance in the field. Our thanks go to Lomapharm for custom-tailored production and supply of the foodLETs and to Roche for donation of the vitamin mix. We are indebted to Jenny Bush-Hallen for her input in this study and to UNICEF Cambodia for additional financial support. The study was organized and funded by FSNPSP-GTZ with additional financial support from UNICEF Cambodia.

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