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Reprint

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Original Article

A comparative intervention trial on fish sauce fortified with NaFe-EDTA and FeSO4+citrate in iron deficiency anemic school children in Kampot, Cambodia

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Background: Inhabitants of agrarian villages of rural Cambodia suffer from high prevalences of iron deficiency and anemia in the context of a monotonous diet. **Objective**: To compare the efficacy and safety of placebo Khmer fish sauce to that of 10 mL of fish sauce containing 10 mg of iron, added to daily school meals either as NaFe-EDTA or as $FeSO_4$+ citrate. **Methods**: 140 students aged 6-21 years were enrolled in a double-blinded, placebo-controlled intervention trial. They were randomly allocated to one of three treatment groups, and followed for 21 weeks during which 114 school meals seasoned with 10 mL of fish sauce were consumed by each participant. Changes in the concentrations of hemoglobin (hb), serum ferritin (SF), and C-reactive protein (CRP) and in body weight and standing height were determined. Prevalences of vomiting, diarrhea, and acute respiratory infections were monitored weekly. **Results**: Both iron-fortified fish sauces increased hb and SF concentrations significantly as compared to placebo. No significant differences were observed between $FeSO_4$+citrate and NaFe-EDTA fortification, regarding mitigation of iron-deficiency anemia (IDA) or regarding CRP, growth, infections, or side-effects. **Conclusions**: Iron-fortified Khmer fish sauce added to Khmer food is a suitable vehicle for iron fortification in children and adolescents. $FeSO_4$+citrate and NaFe-EDTA show equivalent efficacy and safety.

Key Words: NaFe-EDTA, FeSO4+citrate, iron fortification, fish sauce, efficacy, safety

INTRODUCTION

Anemia is widely distributed in South-East Asia. Its prevalence in Vietnamese children and women of reproductive age in 2000 was ~34% and ~25%, respectively; the corresponding figures for Cambodia in 2000 were 57% and 62%, half of which was associated with reduced serum ferritin (SF) concentrations in infants.[1-4] Iron-deficiency anemia (IDA) in tropical countries is caused by poor iron bioavailability from predominantly vegetarian dietary fare and by intestinal blood losses due to hookworm infection.[3] Besides targeted iron supplementation programs with all of their recognized organizational problems, food fortified with iron is an alternative, cost-effective strategy for IDA-mitigation, capable of reaching all segments of the population.[5,6] In South-East Asia, fish sauce is an optimum candidate condiment, insofar as it is consumed e.g. by >80% and >90% of the Vietnamese and Cambodian population, respectively.[1,7]

NaFe-EDTA and $FeSO_4$+citrate are used in fish sauce fortification in Vietnam and Thailand, and both compounds produce similar shelf-lives.[8] NaFe-EDTA showed high bioavailability, efficacy, and efficiency in Vietnamese field trials.[1,9] Iron bioavailability from $FeSO_4$+citrate-fortified fish sauce was high under metabolic ward conditions[10], but no trial on its efficacy under field conditions has yet been reported. Khmer fish sauce of Cambodia goes through a different fermentation process, leading to a darker product than the light fish sauce found in Thailand and Vietnam. Therefore, this trial compares the repletion of hb- and SF-concentrations in school children with IDA after intake of Khmer fish sauce served with school meals in Kampot, Cambodia. The fish sauces contained either no additional iron (placebo), or iron added as NaFe-EDTA, or as $FeSO_4$+citrate at the same dose level. The impact of the intervention on weight gain and linear growth, and on the frequency of diarrhea, vomiting, and acute respiratory tract infections, as well as on CRP was monitored. Results are

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discussed with regards to efficacy, safety, and economic factors.

SUBJECTS AND METHODS

Population, Subjects, and Exclusion Criteria

Hun Sen High School in Chhuk district, Kampot province, Cambodia, with over 3,000 students was selected for the study. Based on an expected anemia prevalence of 57% anemia in women (Demographic Health Survey (DHS), quoted in the Cambodian Nutrition Investment Plan, 2002) over 200 cases of IDA in female student of reproductive age were expected. However, hb determination in 805 students identified only 57 IDA cases (~7%). Therefore, recruitment was extended to a nearby primary school where 89 IDA cases of both sexes were identified after analysis of 699 blood specimens (~13%). Demographic variables including sex and age of the subjects were recorded. Acute malnutrition below 80% weight for height score (W/H), severe anemia (hb < 70 g/L), chronic diseases, iron-supplementation from other sources, or lack of parental approval served as exclusion criteria. Six students with severe anemia (hb < 70 g/L) were excluded and received iron supplementation; 140 students with confirmed IDA joined the study at baseline.

Study Design and Treatment Assignments

The study was designed as a randomized double-blinded, placebo-controlled, longitudinal intervention trial. All subjects at both schools were shown to have IDA at baseline. The study began on January 21st and ended on June 15th, 2005. Students of each sex were assigned randomly to the different intervention arms. The protocol was approved by the National Ethical Committee of the Cambodian Ministry of Health (MoH). Informed written consent was obtained from adolescents over 18 years of age, parents or care-takers during the subscription procedure with the minors enthusiastically assenting to the extra school meal.

Sample size consideration

Sample size estimation was based on the results of an iron fortified fish sauce (IFFS) trial in Vietnam.[1] A sample size of 45 children per group was calculated to detect a difference in hb increase of 7.7 g/L between the 3 intervention groups on a significance level of 5% with a power of 80% (two-sided testing). The intra- and inter-individual variances for SF, CRP, morbidity and anthropometric variables did not enter sample size calculations.

Fish sauce fortification with $FeSO_4$+citrate and NaFe-EDTA

Non-fortified fish sauce was produced in the routine production process by Ngor Heng Fish Sauce Factory, Kampot, Cambodia, with an endogenous iron content of 86 ± 8 mg Fe/L. Iron was added as sodium iron(III)ethylene-diamine tetraacetic acid trihydrate (NaFe-EDTA; MW: 421; Akzo Nobel Chemicals Pte Ltd, Singapore) or as anhydrous ferrous sulfate ($FeSO_4$, MW: 152; Paul Lohmann, Emmerthal, Germany) plus 3.5 g/L citric acid (Otsaha Kamakor Manao Factory Ltd., Bangkok, Thailand) at a concentration of 1 mg Fe/mL. Production was supervised by researchers with experience in the process. Iron content was controlled in randomly sampled bottles of ready-to-use IFFS of all 3 varieties by use of Flame Atomic Absorption Spectrometry (AAS; Spectr AA-20, Varian Associates, Australia) at a wave length of 248.3 nm at Mahidol University after 6 and 12 weeks of storage.[12] In accordance to earlier results in Vietnam and Thailand, the concentration of added iron remained constant over the experimental period.[1,8] Ten mL of each variety of IFFS were mixed with each portion of daily food.

Nutrient Fortification Intervention

The primary health issue of interest in the study was iron deficiency anemia, and the nutrient to be fortified was fish sauce. The daily recommendation by the US Food and Nutrition Board (FNB) is 8 mg Fe/d for children 9 – 13 years, and 11 mg and 15 mg Fe/d for adolescent boys and girls 14 – 18 years of age, respectively.[11] Addition of 10 mg Fe/day to 10 mL of fish sauce added as $FeSO_4$+citrate or NaFe-EDTA approximates this recommendation for the age range 6 – 21 years; moreover, within 6 months of intake this dose should increase hb sufficiently to observe a measurable impact. IFFS was prepared monthly and was stored for six weeks to simulate the routine production and distribution processes. Fortified meals (~330 kcal/d) consisted of rice soup or noodle soup or rice plus vegetables, fish, chicken or meat. They were served for six days a week over 21 calendar weeks of the school semester, totaling to 114 dosing days. Field workers distributed and fully supervised the ingestion of the meals mixed with the 3 varieties of fish sauce. Food that was not consumed was re-weighed and recorded. Subjects received 500 mg Mebendazol at the beginning, in the middle, and at the end of the study to control intestinal helminths that might contribute to blood (iron) losses during the intervention.

Anthropometry and Global Nutrition Evaluation

At the beginning, middle, and end of the study, body weight and standing height were measured with SECA UNICEF scales to the nearest 100 g and with a professional stadiometer to the nearest 0.1 cm, respectively.

Diagnostic Assessment of Hematological and Iron Statuses

Anemia was defined by a hb concentration of <120 g hb/L. Iron deficiency was defined by a SF concentration ≤15 µg/L. The cut-off to define inflammation by CRP was >8 mg/L.

Analysis. Whole blood was collected in EDTA vials and hb concentration was analysed within 6 h of blood sampling (CELL-DYN® 3200 automated laboratory analyser; Abbott®; internal quality control material: CELL-DYN® 26, Abbott®). Serum ferritin was measured with a commercial enzyme-linked immunoassay (AXSYM® System, Abbott; quality control material: AXSYM®, Abbott). C-reactive protein was determined by immunoturbidimetry (Cobas mira plus®, Roche) using a commercial test kit (bioMérieux®; internal quality control kit, bioMérieux®).

Morbidity Indicators

Four teachers were trained to monitor occurrence of vomiting, acute respiratory infection (ARI), and diarrhea through weekly interviews and by daily recording of compliance with scheduled food intake and prevalence of symptoms. These activities were controlled by the study supervisor. The ratio between observed disease symptoms and total observations

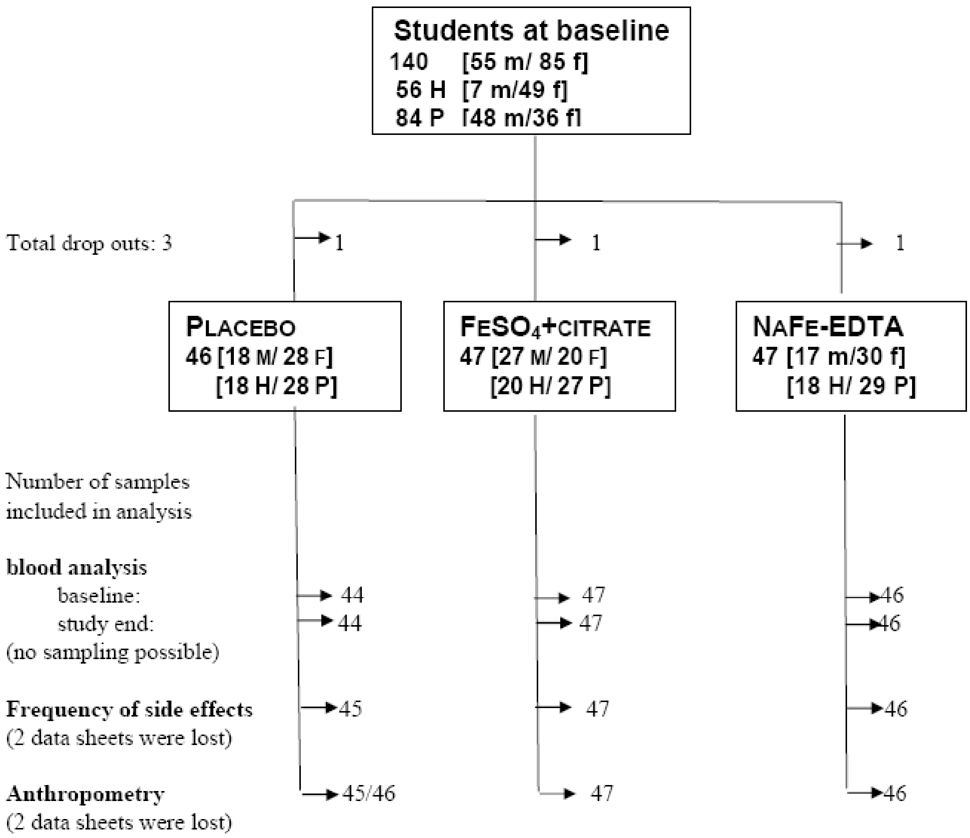

Figure 1. The groups showed normal distribution. No significant differences were found regarding age [Kruskal Wallis test], sex (m = male, f = female), or the ratio of children visiting high school (H) or primary school (P) [Chi-square test].

over the entire period was expressed as percent for each subject. "Diarrhea" was defined as three "watery stools" per day, and ARI as "cough" with fever. Vomiting differed from regurgitation by a time interval of over 15 min after food intake.

Food Intake at Home

The different diets consumed by students at home were monitored by use of a weekly food-group frequency questionnaire. Variables included: plain rice, fish, beef, pork, chicken, vegetables, and fruits. Answers were recorded as "yes" or "no".

Data Handling and Statistical Analysis

The *a priori*, main effective hypothesis of the study was that supplementation of iron would lead to significantly different increments in hb and SF concentrations between the $FeSO_4$+citrate group, the NaFe-EDTA group, and the placebo group. Data with a normal distribution are presented as mean ± standard deviation (M ± SD). If not normally distributed, data are given as geometric means and ranges (25^{th} and 75^{th} percentile). Comparison between the three intervention-groups was performed by the Kruskal-Wallis test. Changes in variables over time within each intervention group were analyzed by the Wilcoxon test for paired samples. Distribution of sex and "high school" vs. "primary school" were analyzed *post hoc* using Chi-square or Fisher's exact test. 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, USA) was used for statistical testing. A probability value of 5% was the criterion for statistical significance.

RESULTS

Demographic Findings

Of 146 anemic students, six were excluded and received iron supplements after their hb values were found to be below 70 g hb/L. Thus, a total of 140 students (55 males and 85 females), initially aged 6 to 21 years were enrolled at baseline (56 from high school: 7 males and 49 females; 84 from primary school: 48 males and 36 females). No significant difference in sex distribution was found among the three intervention groups (Fig 1). Three female subjects dropped out (= 2.2%); two of them abandoned and one was excluded when hb dropped to 59 g/L. Thus, data on 137 subjects were available for analysis [55 males (40%) and 82 females (60%)] with an average age of 163.4 months (13.6 years). No deaths were reported during over 665 child-months of the study.

Compliance with the Intervention

In Cambodia, pupils rotate among sessions. Schools are visited 6 days a week, either in the mornings or in the afternoons, changing morning and afternoon shifts monthly. School meals seasoned with fish sauce were provided during morning and afternoon courses alike. The 21 weeks of intervention included 12 days of public holiday so that 114 meals were part of each treatment. The meals were prepared for both schools at the same kitchen. All 137 students who completed the study received 114 dosings; a missing num-

Table 1. Changes in hb, SF, and CRP concentrations after intervention; (M ± SD, SF values: geometric mean, range: 25th and 75th percentile, n = 164)

Time (week)	Placebo	$FeSO_4$+citrate	NaFe-EDTA	Across-row p-value
hb concentration (g/L)				
Baseline	108.4 ± 10.9 (n=46)	106.9 ± 10.9 (n=47)	106.6 ± 12.2 (n=47)	*p*=0.76
end of study	104.9 ± 11.3 (n=44)	109.8 ± 8.8 (n=47)	109.7 ± 8.6 (n=46)	*p*=0.09
Δ hb (g/L)	-4.3 ± 7.6* (n=44) z<0.01	2.9 ± 11.5 (n=47) z=0.21	3.2 ± 9.9 (n=46) z=0.07	*p*<0.01 Comparison 1-2: *p*<0.01 (W) 1-3: *p*<.0.01 (W) 2-3: *p*=0.74 (W)
SF concentration (μg/L)				
Baseline	9.5 (5.0, 11.0) (n=46)	8.0 (5.0, 12.0) (n=47)	8.0 (4.0, 12.0) (n=47)	*p*=0.95
study end	12.2 (6.5, 19.8)* (n=44)	20.4 (12.0, 33.1) (n=47)	19.6 (13.8, 35.8) (n=46)	*p*<0.01 Comparison 1-2: *p*<0.01 (W) 1-3: *p*<0.01 (W) 2-3: *p*=0.48 (MW)
Δ SF (μg/L)	5.8 ± 9.9* (n=44) z<0.01	13.5 ± 10.9 (n=47) z<0.01	17.3 ± 14.7 (n=46) z<0.01	*p*<0.001 Comparison 1-2: *p*<0.01 (W) 1-3: *p*<0.01 (W) 2-3: *p*=0.26 (W)
CRP concentration (mg/100 mL)				
Baseline	1.0 (1.0, 1.2) (n=45)	1.0 (1.0, 1.2) (n=44)	1.0 (1.0, 1.0) (n=47)	*p*=0.32
study end	1.0 (1.0, 1.1) (n=44)	1.0 (1.0, 1.2) (n=47)	1.0 (1.0, 1.2) (n=46)	*p*=0.41
Δ CRP (mg/100 mL)	1.5 ± 8.7 (n=43) z=0.55	0.3 ± 2.2 (n=44) z=0.26	0.6 ± 2.8 (n=46) z=0.08	*p*=0.36

Across-row comparison was performed by the Kruskal-Wallis test (KW); changes over time within columns were analysed by the Wilcoxon test (= W) for paired samples. * signifies significant differences across rows. Δ hb (i.e. the change in hb over time) was sign. lower in group 1, while there was no sign. difference between groups 2 & 3. At the end of the study the SF concentration was sign. lower in group 1, while there was no sig. difference between group 2 & 3. No significant difference between groups was seen in CRP values. Changes in hb over time decreased significantly in group 1. Changes of SF concentration over time are significant in all groups while changes in CRP are not (see z-values).

ber of meals due to sick-leave were handed out at the beginning of holidays (maximum 6 days, no difference between intervention groups). All meals were fully consumed with no left-over.

Iron Status and Hematological Parameters

Haemoglobin concentration. Whereas no significant differences among the 3 intervention groups were observed at baseline, hb concentrations in the two iron-fortified groups were significantly higher than in the placebo group at the end of the study (p = 0.09) (Table 1). Correspondingly, the hb increments were significantly higher in the two IFFS-groups as compared to placebo (p < 0.01). However, there was no significant difference between the two varieties of IFFS. In the placebo group hb concentration decreased significantly over time (z < 0.01), whereas hb increments over time in the two iron groups were not significant (z = 0.21; z = 0.07).

Serum concentrations of SF and CRP. SF concentrations at the end of the study as well as SF increments were significantly higher in the two IFFS groups than in the placebo group (p <0.01) (Table 1). SF concentrations increased significantly over time in all 3 groups (z <0.01). No significant differences were found between the two varieties of IFFS. There was no significant change in CRP-values over time (z < 0.05) or among the 3 groups at baseline or at the end of the study (Table 1).

Food Intake at Home

At home, 16% of high school students had 3 meals/d, 29% had between 2 and 3 meals/d and 53% only two meals/d. The home meal pattern for primary school children consisted of 17% receiving 3 meals/d, 80% with 2 meals/d, and 3% with less than 2 meals/d. Fish was regularly available at least once a week to 80% of the whole group, pork and vegetable to 72%, chicken to 64%, fruits to 39%, and beef was available to <1% on a weekly basis.

Frequency of Infectious Manifestations

There was no clustering of vomiting, diarrhea, and ARI in any particular week in any of the intervention groups, which

Table 2. Frequency of side effects stratified according to the type of intervention.
Values are given as M ± SD or as frequency distribution (5th, 25th, 50th, 75th, and 95th percentile).

	placebo	$FeSO_4$+citrate	NaFe-EDTA	Across-row p-value
Vomiting				
	0.009 ± 0.28 0, 0, 0, 0, 0.10 (n=45)	0.011 ± 0.033 0, 0, 0, 0, 0.09 (n=47)	0.0158 ± 0.056 0, 0, 0, 0, 0.11 (n=46)	*p*=0.98 (KW)
Diarrhea				
	0.02 ± 0.37 0, 0, 0, 0, 0.12 (n=45)	0.01 ± 0.02* 0, 0, 0, 0, 0.06 (n=47)	0.03 ± 0.09 0, 0, 0, 0, 0.15 (n=46)	*p*=0.04 (KW) Comparison 1-2: *p*=0.08 (W) 1-3: *p*=0.42 (W) 2-3: *p*=0.01 (W)
ARI				
	0.01 ± 0.18 0, 0, 0, 0, 0 (n=45)	0.01 ± 0.01 0, 0, 0, 0, 0 (n=47)	0.01 ± 0.09 0, 0, 0, 0, 0 (n=46)	*p*=0.99 (KW)

KW = Kruskal Wallis test; W = Wilcoxon test; The table gives the "events" (e.g. observation of vomiting) as related to the number of observations (0.01 = 9.4 events of vomiting in 1000 observations); Diarrhoea was significantly less frequent in group 2 as compared to the other two groups. However, overall frequency was very low. So, one may doubt if the frequency of this event is really casually related to fish sauce intervention.

Table 3. Weight, height, and body mass, as well as changes in these parameters after intervention (e.g. ΔWeight and ΔHeight) broken down by treatment groups. Values are given as M ± SD or as frequency distribution (5th, 25th, 50th, 75th, and 95th percentile).

Time	Placebo	$FeSO_4$+citrate	NaFe-EDTA	Across-row p-value
Weight (kg)				
1(baseline)	33.7 ± 10.2 (n=46)	34.8 ± 11.1 (n=47)	32.7 ± 11.7 (n=47)	*p*=0.56
2 (mid-term)	34.0 ± 10.5 (n=45)	35.2 ± 11.2 (n=47)	33.4 ± 12.0 (n=45)	*p*=0.68
3 (study end)	34.7 ± 10.5 (n=45)	35.9 ± 11.4 (n=47)	33.7 ± 11.9 (n=46)	*p*=0.54
Δ Weight (kg) (2-1)				
	0.5 ± 1.0 (n=45) z=0.002	0.4 ± 0.7 (n=47) z=0.001	0.6 ± 0.9 (n=45) z<0.0001	*p*=0.62
Δ Weight (kg) (3-1)				
	0.9 ± 1.5 (n=45) z<0.01	1.2 ± 1.1 (n=47) z<0.01	1.1 ± 1.0 (n=46) z<0.01	*p*=0.35
Height (cm)				
1 (baseline)	139.3 ± 14.6 (n=46)	140.6 ± 13.3 (n=47)	137.0 ± 16.2 (n=47)	*p*=0.53
2 (midterm)	140.1 ± 14.1 (n=45)	141.7 ± 12.9 (n=47)	138.5 ± 15.9 (n=45)	*p*=0.60
3 (study end)	141.4 ± 14.3 (n=45)	142.7 ± 13.0 (n=47)	139.0 ± 15.8 (n=46)	*p*=0.52
Δ Height (cm) (2-1)	1.1 ± 1.2 (n=45) z<0.01	1.1 ± 1.1 (n=47) z<0.01	1.3 ± 0.9 (n=45) z<0.01	*p*=0.46
Δ Height (cm) (3-1)	2.0 ± 1.4 (n=45) z<0.01	2.1 ± 1.5 (n=47) z<0.01	2.2 ± 1.4 (n=46) z<0.01	*p*=0.68
BMI (kg/m^2)				
1 (baseline)	16.8 (14.1, 15.0, 18.2, 22.2) 17.0 ± 2.3 (n=44)	16.7 (13.1, 14.4, 19.3, 22.2) 17.1 ± 2.8 (n=47)	16.3 (13.4, 14.4, 18.6, 21.2) 16.7 ± 2.5 (n=46)	*p*=0.80
3 (study end)	16.6 (13.9, 14.8, 18.6, 22.1) 16.9 ± 2.4 (n=44)	16.8 (13.3, 14.6, 19.8, 22.7) 17.1 ± 3.1 (n=47)	16.0 (13.4, 14.6, 18.7, 21.5) 16.8 ± 2.6 (n=46)	*p*=0.95
Δ BMI (kg/m^2)	-0.04 ± 0.55 (n=44) z=0.75	0.03 ± 0.60 (n=47) z=0.57	0.07 ± 0.48 (n=46) z=0.45	*p*=0.75

Across-row comparison was performed by the Kruskal-Wallis test; time dependent changes within columns were analysed by the Wilcoxon test for paired samples.

excluded confusion by short-term epidemic events. The frequency of events was between 0.13% for ARI and 2.6% for diarrhea and was independent of treatment groups, except for diarrhea; diarrhea was significantly less frequent in the $FeSO_4$+citrate-group (p = 0.04) **(Tab. 2)**.

Anthropometry

There were no significant differences in anthropometric parameters between the intervention groups at any point in time (Table 3). As expected, increments in weight and height over time were significant for all intervals, showing that the children grew to the same extent in all 3 intervention groups.

Post hoc Analysis

No differences were found for hb at baseline, as only anemic children were included. Primary school students differ from high school students with respect to age and related anthropometric parameters, such as weight, height, BMI, as well as sex distribution. At high school, adolescent girls were preferentially included because of the higher prevalence of anemia. The increments in SF were lower in menstruating adolescent girls than in pupils at primary school (data not shown).

DISCUSSION

Epidemiological Observations

All enrolled students showed IDA at baseline which may lead to low birth weight, impaired psychomotoric development and reduced physical work performance. [13,14,15,16] However, only 7% and 13% of screened high school girls and primary school children were affected by IDA. The reduction as compared to earlier results (62% and 93% in primary school children 5 – 11 and 12 – 14 years of age in 2000[3,4], presumably ~50% being IDA) seems related to a selection bias. Only wealthy Khmer families (~6%) can afford to send their daughters to high school and the selected primary school children participated in a school feeding program run by the World Food Program before our trial. This may explain the low IDA prevalence observed during the screening process.

Impact of Iron-Fortified Fish Sauce on Growth, Intestinal Bleeding, Inflammation, and on hb and SF Concentrations

The lack of IFFS effects on weight and height is in accordance with expectation, as IFFS added no additional energy calories to the meal. Moreover, growth effects as a consequence of iron intake have only been observed in iron-adequate children and not in iron deficiency.[17,18] During the study period the children gained ~2 cm in standing height and ~1 kg in body weight without significant differences between the 3 intervention groups (Table 3). The growth-related increments in blood volume significantly reduced hb concentration in the placebo group, while hb concentrations increased in both IFFS groups. This observation suggests that iron was the rate-limiting factor for hb synthesis. In the present study, Mebendazol was given twice in addition to the doses provided by the National Health Program. Therefore, substantial interference of hookworm-induced intestinal bleeding with the impact of iron intervention can be ruled out.[3] Serum CRP concentration did not increase over time and showed no differences between treatment groups (Table 1), excluding interfering influences of infections or inflammation on changes in SF or hb concentrations.

Impact of NaFe-EDTA- and $FeSO_4$+citrate fortified Fish Sauce on hb and SF Concentrations

In cereal flour and phytate-rich diets iron absorption from NaFe-EDTA is 2-3 times higher than from other iron sources.[19-21] It is a recommended food additive (JECFA[22]) with an upper limit of 0.8 mg Fe/kg body weight/d which supplies enough iron to even cover the demand of infants with fortified food.[6] Accordingly, NaFe-EDTA in fish sauce showed convincing efficacy and efficiency in an earlier hb repletion trial in Vietnam which is in line with our Cambodian results (Table 1).[1,9] In consequence, NaFe-EDTA-fortified fish sauce has been approved for large scale production in Vietnam, the process being well established. This is helpful to apply for funding and approval in neighboring countries.

The repletion of hb- and SF-concentrations was equal after intake of $FeSO_4$+citrate and NaFe-EDTA (Table 1). This favorable finding was not surprising, as an earlier stabile isotope study with $FeSO_4$+citrate in fish sauce showed high iron bioavailability under metabolic ward conditions.[10] Moreover, sensory characteristics and stability tests in Thai fish sauce showed no differences 3 months after addition of $FeSO_4$+citrate and NaFe-EDTA.[8] In our study, iron-fortified Khmer fish sauce was routinely produced in a commercial plant. It was 6 - 21 weeks old when consumed and showed no precipitates in any of the 3 varieties. The taste of the school meals, corresponding to an average Cambodian fare remained unaffected after addition of either IFFS variety. Thus, $FeSO_4$+citrate and NaFe-EDTA performed equally well and the statement that "NaFe-EDTA is the only soluble iron compound that does not precipitate peptides in fish sauce"[6] is no longer valid.

Differential Food Technology

Ease of production, cost- and safety-aspects show little difference between both fortificants. $FeSO_4$+citrate dissolve faster than NaFe-EDTA which does not require separate mixing tanks. Moreover, reaching the same biological impact $FeSO_4$+citrate is approx. 5 times less expensive than NaFe-EDTA (Omar Dary, personal communication 2007).[23] Today in Cambodia fish sauce is produced in hundreds of small plants[7] that help to maintain local occupation and self-sufficiency in rural areas. For these small producers, fish sauce fortification with $FeSO_4$+citrate offers technical and cost advantages. The expenses for the fortificants itself contribute only ~6% of total costs[24] and the price advantage of $FeSO_4$+citrate accounts for only a few US cents per bottle. However, this corresponds to the total profit margin of small-scale manufacturers for the cheaper local Cambodian fish sauce varieties (Ngov Heng Fish Sauce Factory, personal inquiries) which may influence the sustainability of IFFS programs after the end of a funded launching period. Though advantages and disadvantages of NaFe-EDTA and $FeSO_4$+citrate are subtle and balanced, it seems good to have the choice between two alternatives of equivalent efficacy for the production of IFFS.

Safety Aspects of Iron Fortification

No acute side effects of IFFS are to be expected at a dose level of 1 mg Fe/mL (Table 2) and the wider margin of safety for "citrate" as compared to "EDTA"[25,26] is negligible at dosages used for fish-sauce fortification. Regarding long-term use of IFFS, however, some care seems warranted. In Vietnam, IFFS long-term consumption increased SF concentration to values as high as 67% of those observed for the 50th percentile for women in the USA at a fortification level of 0.5 mg Fe/mL.[9] This is half the concentration used in the corresponding short-term efficacy trial in Vietnam and in the present Cambodian trial.[1] Van Thuy et al. prudently proposed to lower the fortification level to 0.4 mg Fe/mL IFFS in a national program.[9] WHO guidelines recommend to add no more than 22% of the daily requirement with market-driven foods.[28] Even at this reduced level 20 mL of IFFS would supply 8 mg Fe/d which is 80% of the RDA of 10 mg Fe/d for adult men and 30% of the RDA for pregnant women of 27 mg Fe/d.[11] This data exemplifies the high potential of IFFS in the fight against IDA. Preliminary studies suggest that the average fish sauce consumption in Cambodia may be below 10 ml/d. Still, the data also suggest to monitor changes in the iron status of the population on a regular basis in national IFFS programs using highly available iron compounds. The iron concentration in IFFS needs to be reduced when the average iron status of the population has increased to adequate levels, as observed earlier, in order to avoid potential harm in iron-adequate children.[4,9,29]

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AUTHOR DISCLOSURES

Philippe Longfils, Didier Monchy, Heike Weinheimer, Visith Chavasit, Yukiko Nakanishi and Klaus Schümann, no conflicts of interest.

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Original Article

A comparative intervention trial on fish sauce fortified with NaFe-EDTA and FeSO4+citrate in iron deficiency anemic school children in Kampot, Cambodia

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魚露以 Na-Fe-EDTA 或 $FeSO_4^+$檸檬酸鹽強化在柬埔寨鐵缺乏貧血學童的比較介入性研究

背景：柬埔寨的鄉村，以農業為主的村落居民，在單調的飲食背景下，有高盛行率的鐵缺乏及貧血。目的：添加 NaFe-EDTA 或 $FeSO_4^+$檸檬酸鹽於日常學校餐點中使用的魚露，以比較安慰劑魚露與每 10 mL 含 10 mg 鐵的魚露之功效與安全性。方法：Kampot 省 140 名學生，年齡 6-21 歲，參與這個雙盲、安慰劑控制介入研究。他們被隨機分配至 3 介入組中的 1 組，追蹤 21 週，每名學生計攝取加入 10 mL 魚露調味的餐點 114 餐。隨後測量每名學生的血紅素(hb)、血清運鐵蛋白(SF)、C 反應蛋白(CRP)及體重和站著的身高之改變。每週監測嘔吐、腹瀉及急性呼吸道感染的盛行率。結果：鐵強化魚露均顯示 hb 及 SF 濃度顯著地較安慰劑組高。以 $FeSO_4^+$檸檬酸鹽或 NaFe-EDTA 強化，在減輕鐵缺乏貧血(IDA)，或是 CRP 濃度、生長、感染率及副作用方面，兩者均無顯著差異。結論：鐵強化之魚露添加至柬埔寨人的飲食，對兒童及青少年的鐵強化是適合的媒介物。以 $FeSO_4^+$檸檬酸鹽或 NaFe-EDTA 添加，顯示有相同的效力及安全性。

關鍵字：Na-Fe-EDTA、$FeSO_4^+$檸檬酸鹽、鐵強化、魚露、效力、安全性