Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women[1–3]

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ABSTRACT

Background: Fish sauce is consumed daily by a large proportion of the Vietnamese population and could therefore be a potentially useful food vehicle for iron-fortification programs.

Objective: We evaluated the efficacy of iron-fortified fish sauce in improving the iron status of anemic women.

Design: In a randomized, double-masked study of 152 anemic (hemoglobin concentration of 81–119 g/L) women, a meal based on noodles or rice was served 6 d/wk with 10 mL fish sauce containing either 10 mg Fe as NaFeEDTA (iron-fortified group) or no added iron (control group). Concentrations of hemoglobin, serum ferritin (SF), and serum transferrin receptor (TfR) were measured at baseline and after 3 and 6 mo.

Results: After 6 mo, hemoglobin and SF concentrations were higher and TfR concentrations were lower in the iron-fortified group than in the control group [hemoglobin: 116.3 ± 8.7 ($\bar{x}$ ± SD) compared with 107.6 ± 11.0 g/L ($P < 0.0001$); SF: 30.9 (95% CI: 23.4, 40.6) compared with 14.6 (11.3, 19.0) μg/L ($P = 0.0002$); TfR: 7.2 (6.4, 7.9) compared with 9.0 (8.1, 9.9) mg/L ($P = 0.002$)]. The prevalence of iron deficiency (SF < 12 μg/L or TfR > 8.5 mg/L) and iron deficiency anemia (iron deficiency with hemoglobin < 120 g/L) was lower in the iron-fortified group than in the control group [32.8% compared with 62.5% ($P = 0.0005$) and 20.3% compared with 58.3% ($P < 0.0001$), respectively].

Conclusions: Regular consumption of iron-fortified fish sauce significantly reduced the prevalence of iron deficiency anemia in Vietnamese women during the 6-mo intervention. Fortifying fish sauce with iron by using a water-soluble, highly bioavailable compound (NaFeEDTA) is a promising strategy for combating iron deficiency anemia in Vietnam. *Am J Clin Nutr* 2003;78:284–90.

KEY WORDS Iron, food fortification, anemia, iron deficiency, transferrin receptor, ferritin

INTRODUCTION

Micronutrient malnutrition—"the hidden hunger"—includes deficiency disorders of iron, vitamin A, and iodine and represents widespread nutritional problems in resource-poor areas. The consequences of these deficiency disorders on health and economic development are major public health concerns in many countries.

Iron deficiency (ID) in its most severe form results in anemia, and a recent review (1) concluded that iron deficiency anemia (IDA) is associated with impaired child development and decreased work productivity. Although estimates of the magnitude of this public health problem vary widely (1) and accurate prevalence data are often missing, it can be assumed that a significant proportion of children and women of childbearing age in many developing countries are anemic and that ID is a major factor in the etiology of anemia. For example, recent data from Cote d'Ivoire showed that 42–46% of schoolchildren and adult women were anemic and that IDA accounted for ≈50% of the anemia cases (2). In preschool children, the prevalence of anemia was 50% and IDA represented 80% of all anemia cases.

In Vietnam, detailed information about the etiology of anemia is not available. The 1995 National Anemia and Nutrition Risk Factor Survey reported a high prevalence (≈40–50%) of anemia in young children and adult women, including both pregnant and nonpregnant women. Insufficient iron intake and low iron bioavailability were identified as important factors in the etiology of anemia although other important contributing factors such as hookworm infection, which increases iron losses, were also highlighted (3). A more recent Nutrition Risk Factor Survey, based on data collected in 2000, showed that the prevalence of anemia had decreased to ≈34% in young children and to ≈25% in women. However, anemia remains a significant public health problem in Vietnam (HH Khoi, NC Khan, and LB Mai, unpublished observations, 2001), and strategies for combating anemia, ID, and IDA are needed in large segments of vulnerable population groups.

Food fortification is often suggested as one of the most cost-effective and sustainable strategies for increasing iron intake in the general population (4, 5). The food vehicle that is the most commonly fortified with iron is cereal. However, condiments have been proposed as alternative vehicles for iron-fortification programs, particularly in countries where rice is the staple food,

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because rice grains are difficult to fortify. Fish sauce was suggested as a potentially useful vehicle for iron fortification in Vietnam because > 80% of the population consumes fish sauce regularly (Khoi et al, unpublished observations, 2001). The aim of the present study was to evaluate the efficacy of iron-fortified fish sauce in improving iron status and reducing the prevalence of anemia in adult, nonpregnant anemic Vietnamese women.

SUBJECTS AND METHODS

The study, which had a randomized, double-masked, placebo-controlled design, was implemented from April to October 2000 with factory workers in Hai Duong and Hung Yen provinces, which are located in the Red River delta of northern Vietnam. Selection criteria for the study site included lack of interventions to control IDA in nonpregnant women and approval of the study by local health authorities.

The women recruited into the study were 17–49 y old, were employed in 1 of the 6 selected factories in the study area, and had a hemoglobin concentration > 80 but < 120 g/L in an initial hemoglobin-screening study. A brief medical examination was included in the screening study to exclude women with gastrointestinal or metabolic disorders. Pregnant women were excluded on the basis of self-reported amenorrhea during the screening study and were referred to the local health center for antenatal care. The anemic women who met the inclusion criteria were randomly divided into 2 groups: the iron-fortified group, who received daily 10 mL of fish sauce fortified with 10 mg Fe, and the control group, who received daily 10 mL of nonfortified fish sauce. Meals with added fish sauce were served 6 d/wk for 6 mo.

The Scientific Committees of the National Institute of Nutrition (Hanoi, Vietnam) and of the Ministry of Health (Hanoi, Vietnam) reviewed and approved the study protocol. All women were informed orally and in writing about the aims and procedures of the study, and written informed consent was obtained from all women before enrollment in the study. Women who had a hemoglobin concentration < 80 g/L at any blood sampling during the study were provided with medicinal iron.

The sample size was estimated on the basis of the expectation that the iron-fortified group would have a higher hemoglobin concentration (7 g/L) at the end of the study period than would the control group, with a confidence interval of 95% and a power of 90%. The SD was estimated to be 13 g/L. The sample size was estimated to be 61 per group; with the assumption of a 20% dropout rate, a total of 152 women were recruited for the study.

Nonfortified fish sauce (Type I, 15 g N/L) was produced via natural fermentation by the Cat Hai Fish Sauce Company (Hai Phong, Vietnam). The salt content was ≈280 g/L, and the native iron content was 11 mg/L. Fish sauce was fortified with a water-soluble, highly bioavailable iron compound (NaFeEDTA; Akzo Nobel Chemicals Pte Ltd, Singapore). A concentration of 1 mg Fe as NaFeEDTA/mL of fish sauce was achieved in the factory by mixing 1104 g NaFeEDTA with 160 L fish sauce for 2 h before bottling. Because of limitations in the factory facilities, batches of fortified and nonfortified fish sauce were prepared every 3 mo. The fish sauce was put into brown glass bottles, coded, and stored in the dark to prevent losses of NaFeEDTA (M Fidler et al, unpublished observations, 2001). Before the fish sauce was used in the study, the iron content in random bottles of both the NaFeEDTA-fortified and the nonfortified batches were measured with the use of atomic absorption spectrophotometry at Showa Women's University, Tokyo, Japan. The stability of NaFeEDTA in the fish sauce was evaluated in a separate study by exposing fortified fish sauce in clear and amber bottles to different light conditions (artificial and natural sunlight, fluorescent light or indirect sunlight, and darkness) during different periods of time ranging from 14 to 364 d (Fidler et al, unpublished observations, 2001).

Ten milliliters of fish sauce, which was either fortified with iron (iron-fortified group) or not fortified (control group), was added to a midmorning snack that was based on noodles or rice and served in the factories. The study was implemented in the 6 different factories for 6 d/wk during a 26–28-wk period; each factory had an iron-fortified group and a control group. Field assistants served the meals and monitored meal intake. Compliance was monitored daily.

Blood samples were collected in the morning (0800–1100) at baseline (t_0) and after 15 (t_3) and 26–28 wk (t_6). At each sampling, 4 mL venous blood was drawn into EDTA-coated tubes. The tubes were kept cool and were transported to the laboratory at the National Institute of Nutrition in Hanoi, Vietnam, within 8 h. Concentrations of hemoglobin, serum ferritin (SF), serum transferrin receptor (TfR), and C-reactive protein (CRP) were measured at baseline, t_3, and t_6. Screening for hemoglobinopathies was made at baseline by electrophoresis at the Children's Hospital, Hanoi, Vietnam. Whole blood was screened within 1 wk of blood sampling by using electrophoresis on cellulose membranes. Hemoglobin was measured in whole blood within 12 h of sampling by using the cyanomethemoglobin method with Sigma diagnostic kits (Sigma, St Louis). A 3-level quality-control material (Dia-HT-1,2,3; Diamed AG, Cressier sur Morat, Switzerland) was analyzed in parallel. Samples were analyzed in duplicate, and the analysis was repeated if the results differed by > 5%. An aliquot of whole blood was stored at −20 °C for later analysis if needed. Serum was separated from cells by centrifugation at 5000 × g for 10 min at 4 °C. Aliquots of serum were stored at −20 °C until analysis of SF, TfR, and CRP at the end of the study. SF was measured by using a 2-site enzyme-linked immunosorbent assay with monoclonal reagents for both the capture and indicator antibodies as described previously (6). The assay was standardized with purified human liver ferritin, which was calibrated against a World Health Organization reference standard obtained from the National Bureau of Standards (7). TfR was measured by using a similar assay with double monoclonal antibodies against intact TfR purified from human placenta (8). SF and TfR were measured in duplicate at the Division of Hematology, Kansas University Medical Center, Kansas City, and measurements were repeated when the duplicates differed by > 10%. CRP was measured in duplicate with the use of nephelometry (Turbox; Orion Diagnostica, Espoo, Finland) at the Laboratory for Human Nutrition, Swiss Federal Institute of Technology, Zurich, Switzerland.

Anemia was defined as a hemoglobin concentration < 120 g/L, low iron stores were defined as an SF concentration < 12 μg/L (9), and tissue ID was defined as a TfR concentration > 8.5 mg/L (10). TfR and SF concentrations were used to calculate TfR-SF ratios. Body iron content was calculated according to Skikne et al (10) by using the following formula:

$$\text{Body iron (mg/kg)} = [\ln(\text{TfR/SF}) - 6.5]/-0.278 \qquad (1)$$

ID was defined as a low SF concentration or an elevated TfR concentration, and IDA was defined as the simultaneous presence of ID and anemia.

Body height was measured at baseline by using a stadiometer (precision = 0.1 cm). Body weight was measured at baseline, t_3, and t_6 by using an electronic scale (precision = 0.1 kg). To screen for gastrointestinal parasites at baseline and t_6, fecal samples were

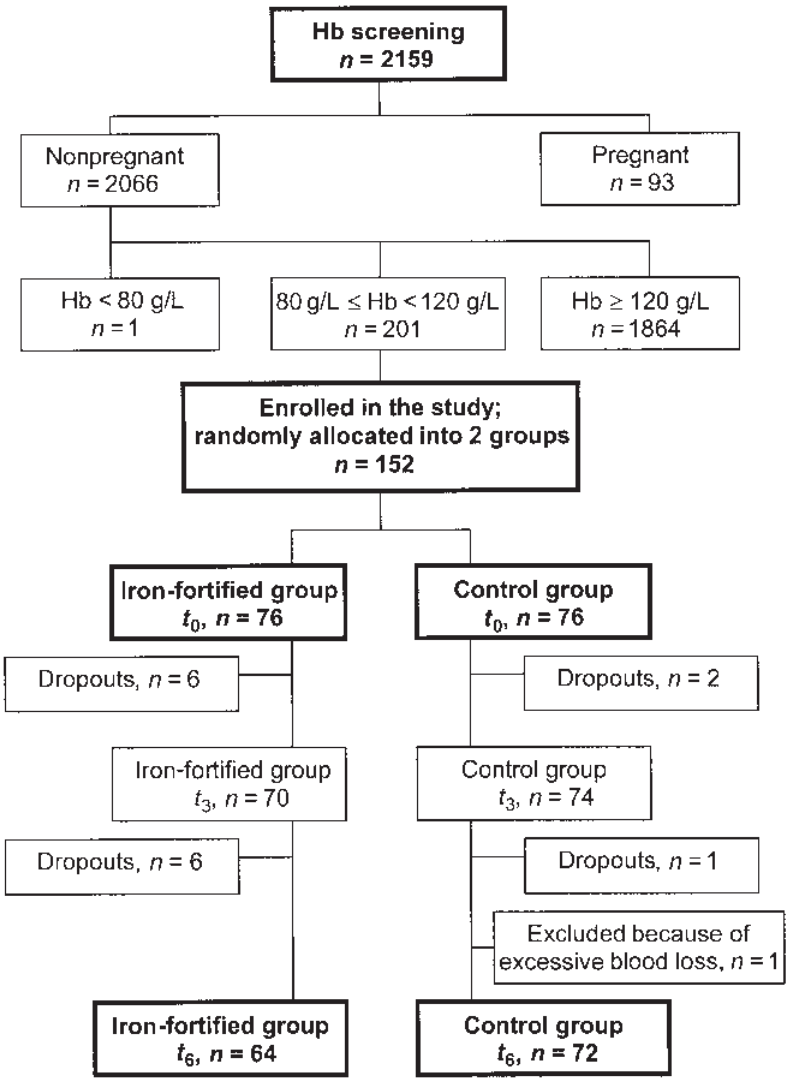

FIGURE 1. Study profile: initial screening to enroll anemic women in the study, followed by a 6-mo intervention. Hb, hemoglobin; t_0, baseline; t_3, 3 mo; t_6, 6 mo.

collected and prepared for microscopic analysis by using Kato-Katz methodology. Dietary intakes of energy and nutrients in the 2 study groups were estimated from 24-h recalls at baseline and t_6 by using *Nutritive Composition Table of Vietnamese Foods* (Hanoi, Vietnam: Hanoi Medical Publishing House, 2000).

The principal hypothesis was that the women in the iron-fortified group would have higher hemoglobin concentrations at the end of the study period than would the women in the control group. In addition, we evaluated the influence of iron-fortified fish sauce on laboratory measures of iron status (SF and TfR) and on the prevalence of ID and IDA during the intervention study.

Statistical analysis was performed by using repeated-measures models, ie, analysis of variance for quantitative response variables and logistic regression for binary response variables. The effect of treatment on the response variables was assessed by using a group × time interaction term (null hypothesis of no difference between the 2 groups). Differences between groups at each time period were tested when the group × time interaction term was significant ($P < 0.05$). The models were fitted with PROC GLM and PROC GENMOD in SAS release 8.2 for WINDOWS (SAS Institute Inc, Cary, NC). All P values for differences between groups at each time period were Bonferroni corrected.

When data were not normally distributed, statistical analysis was carried out after log transformation. For continuous response variables (hemoglobin, SF, TfR, and TfR-SF ratio), results are presented as means ± SDs or as geometric means for log-transformed data. Prevalence data are given for binary indicators (anemia, ID indicators, and elevated CRP).

RESULTS

The study profile is presented in **Figure 1**. The screening study was implemented in 2159 women. During the screening study, one woman with a low hemoglobin concentration (< 80 g/L), 93 pregnant women, and 1864 nonpregnant women with a hemoglobin concentration ≥ 120 g/L were excluded from the study; thus, 201

TABLE 1
Physical characteristics, dietary intakes estimated from 24-h recalls, and prevalence of intestinal parasites in the study subjects at baseline[1]

	Iron-fortified group (n = 64)	Control group (n = 72)
Physical characteristic		
Age (y)	33.3 ± 8.3[2]	35.2 ± 8.8
Height (cm)	154.2 ± 3.9	154.2 ± 5.1
Weight (kg)	47.9 ± 5.5	47.5 ± 5.2
BMI (kg/m^2)	20.2 ± 2.1	20.0 ± 2.0
CRP (mg/L)	5.6 ± 9.8	8.0 ± 18.5
Prevalence of elevated CRP (%)	7.8	18.1
Dietary intake		
Energy (kJ)	7178 ± 2133	7336 ± 2115
Iron (mg)	8.9 ± 4.0	8.6 ± 2.9
Vitamin C (mg)	68 ± 48	59 ± 47
Rice and other cereals (g)	374 ± 126	370 ± 147
Vegetables (g)	160 ± 116	151 ± 101
Meat (g)	84± 76	78 ± 70
Fish (g)	26 ± 54	25 ± 59
Seafood (g)	17 ± 68	16 ± 56
Eggs and milk (g)	20 ± 33	27 ± 44
Fruit (g)	51 ± 78	43 ± 73
Prevalence of intestinal parasites (%)[3]		
Ascaris lumbricoides	20.0	28.3
Hookworm	11.1	7.7
Trichuris trichuria	28.9	34.0
≥1 parasite	42.2	49.1

[1] CRP, C-reactive protein. There were no significant differences between the groups.
[2] $\bar{x}$ ± SD.
[3] n = 45 and 55 in the iron-fortified group and the control group, respectively.

(9%) women met the inclusion criteria. Of those 201 women, 152 were enrolled in the study (76 in the iron-fortified group and 76 in the control group). Slightly elevated hemoglobin A_2 (>3.5%) was observed in 13 women (6 in the iron-fortified group and 7 in the control group). One woman was homozygous for hemoglobin E, and 2 women were heterozygous for hemoglobin E. None of these women was excluded from the data analysis. Fifteen women did not complete the study (data at t_3 were also missing for 8 of these women) and were not included in the data analysis. Of the 137 women who completed the study, one woman in the control group was excluded from the data analysis because of excessive blood loss (based on estimated body iron). None of the women became pregnant during the study. The data analysis was performed with 136 women (64 in the iron-fortified group and 72 in the control group). Initial nutritional status and iron status were not significantly different between the women who dropped out of the study and those who were included in the data analysis.

At baseline, the 2 groups were not significantly different in age, body weight and height, body mass index, estimated dietary intakes (energy, the selected nutrients iron and vitamin C, and selected foods such as cereals, vegetables, meat, and fish), CRP concentration, and prevalence of elevated CRP concentration (>10 mg/L) (**Table 1**). The 2 groups also did not differ significantly at baseline in hemoglobin, SF, and TfR concentrations or in TfR-SF ratio (**Table 2**).

At baseline, 69.9% of all the women had IDA (anemia plus either a low SF concentration or an elevated TfR concentration); 41.9% of these women had both a low SF concentration and an elevated TfR concentration. There were no significant differences between the 2 groups in the prevalence of anemia, low SF concentration, elevated TfR concentration, and elevated TfR-SF ratio (**Table 3**) or in the prevalence of ID and IDA [70.3% (95% CI: 59.1%, 81.5%) and 69.4% (58.8%, 80.1%) for the iron-fortified and control groups, respectively (P = 0.94)] (**Figure 2**).

TABLE 2
Iron-status indicators in the 2 study groups at baseline (t_0) and after 3 (t_3) and 6 (t_6) mo of intervention[1]

Indicator	Iron-fortified group (n = 64)	Control group (n = 72)
Hemoglobin (g/L)		
t_0	110.7 ± 8.0[2]	110.4 ± 8.7
t_3	111.9 ± 8.7[3]	106.5 ± 10.3
t_6	116.3 ± 8.7[4]	107.6 ± 11.0
SF (μg/L)		
t_0	13.6 (10.1, 18.2)[5]	14.6 (11.0, 19.4)
t_3	22.0 (16.5, 29.5)	13.6 (10.3, 18.1)
t_6	30.9 (23.4, 40.6)[6]	14.6 (11.3, 19.0)
TfR (mg/L)		
t_0	10.1 (8.9, 11.3)	9.8 (8.8, 10.9)
t_3	7.8 (7.0, 8.8)[3]	9.7 (8.6, 10.7)
t_6	7.2 (6.4, 7.9)[7]	9.0 (8.1, 9.9)
TfR:SF		
t_0	742 (510, 1074)	645 (451, 923)
t_3	354 (248, 509)[3]	692 (488, 979)
t_6	233 (164, 327)[4]	614 (443, 850)

[1] SF, serum ferritin; TfR, serum transferrin receptor. The group × time interaction term was significant for each variable, P < 0.0001.
[2] $\bar{x}$ ± SD.
[3,4,6,7] Significantly different from the control group (ANOVA with Bonferroni correction): [3] P < 0.05, [4] P < 0.0001, [6] P = 0.0006, [7] P = 0.006.
[5] Geometric $\bar{x}$; 95% CI in parentheses.

TABLE 3
Prevalence and 95% CIs of anemia and iron deficiency in the 2 study groups at baseline (t_0) and after 3 (t_3) and 6 (t_6) mo of intervention[1]

Condition	Iron-fortified group (n = 64)	Control group (n = 72)
	%	
Anemia (Hb < 120 g/L)		
t_0	100	100
t_3	87.5 (79.4, 95.6)	94.2 (88.8, 99.6)
t_6	66.2 (55.7, 78.7)	88.9 (81.6, 96.1)
Low SF (<12 μg/L)		
t_0	53.1 (40.9, 65.4)	48.6 (37.1, 60.2)
t_3	31.3 (19.9, 42.6)	50.7 (39.2, 62.3)
t_6	15.6 (6.7, 24.5)[2]	50.0 (38.5, 61.5)
Elevated TfR (>8.5 mg/L)		
t_0	57.8 (45.7, 69.9)	63.9 (52.8, 75.0)
t_3	45.3 (31.1, 57.5)	60.9 (49.6, 72.1)
t_6	28.1 (17.1, 39.1)	51.4 (39.9, 62.9)
Elevated TfR:SF (>500)		
t_0	57.8 (45.7, 69.9)	56.9 (45.5, 68.4)
t_3	39.1 (27.1, 51.0)	56.5 (45.1, 68.0)
t_6	20.3 (10.5, 32.0)[2]	56.9 (45.5, 68.4)

[1] Hb, hemoglobin; SF, serum ferritin; TfR, serum transferrin receptor. The group × time interaction term was significant for low SF (P < 0.0001) and elevated TfR:SF (P < 0.0001).
[2] Significantly different from the control group, P < 0.0001 (logistic regression analysis with Bonferroni correction).

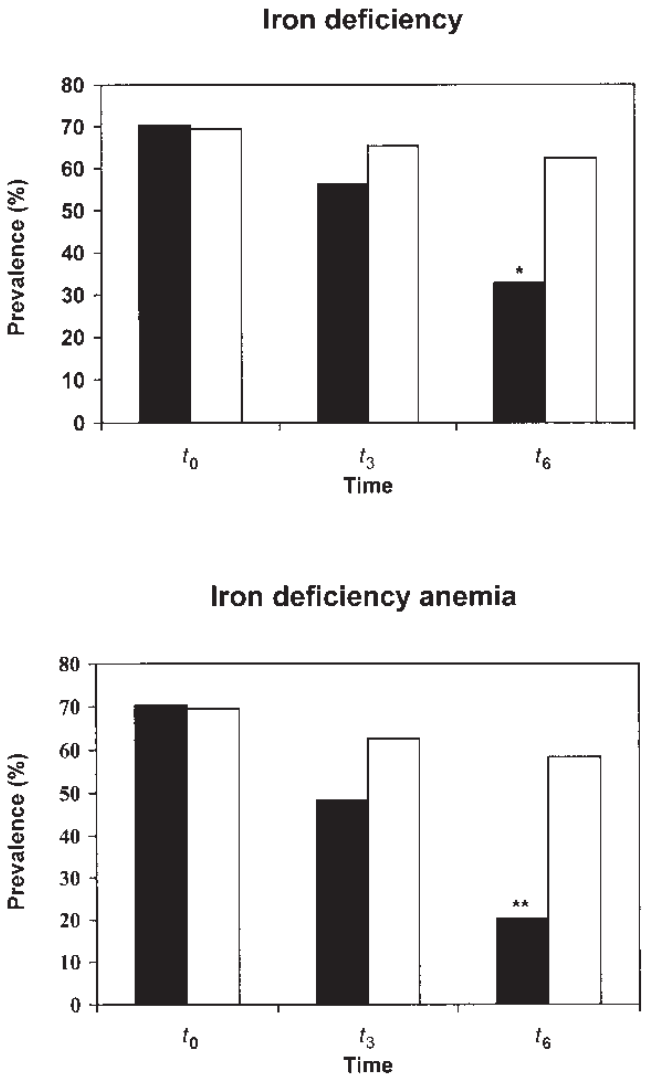

FIGURE 2. Prevalence of iron deficiency (ID) and of iron deficiency anemia (IDA) at baseline (t_0) and after 3 (t_3) and 6 (t_6) mo of intervention in the iron-fortified group (■; $n = 64$) and the control group (□; $n = 72$). The group × time interaction was significant for ID ($P = 0.003$) and for IDA ($P < 0.0001$). *,**Significantly different from the control group (repeated-measures logistic regression with Bonferroni correction): $^{*}P = 0.0006$, $^{**}P < 0.0001$.

Of the 100 women from whom fecal samples were obtained at baseline, 46.0% were infected with ≥1 intestinal parasite, 24.5% were infected with *Ascaris lumbricoides*, 31.6% were infected with *Trichuris trichuria*, and 9.3% were infected with hookworm (434 ± 32.3 eggs/g feces). There was no significant difference between the 2 groups (Table 1).

The group × time interaction terms were significant for hemoglobin, Sf, TfR, TfR:SF, and calculated body iron ($P < 0.0001$ for each variable). At t_3 and t_6, hemoglobin and SF were significantly higher in the iron-fortified group than in the control group, whereas TfR and TfR:SF were significantly lower in the iron-fortified group than in the control group (Table 2). Total body iron was not significantly different between the 2 groups at t_0 (−15 ± 252 compared with 13 ± 260 mg in the iron-fortified group and the control group, respectively; $P = 0.67$). At t_3 and t_6, body iron was significantly higher in the iron-fortified group than in the control group [t_3: 108 ± 218 compared with 15 ± 267 mg ($P = 0.04$); t_6: 186 ± 192 compared with 39 ± 260 mg ($P < 0.0001$)].

The repeated-measures logistic regression analysis indicated highly significant group × time interactions for prevalence of low SF ($P < 0.0001$), elevated TfR:SF ($P < 0.0001$), ID ($P = 0.003$), and IDA ($P < 0.0001$). At t_6, the prevalence of low SF and elevated TfR:SF was significantly lower in the iron-fortified group than in the control group (Table 3). Similar results were found for the prevalence of ID [32.8% (95% CI: 21.3%, 44.3%) compared with 62.5% (95% CI: 51.3%, 73.7%) in the iron-fortified group and the control group, respectively; $P = 0.0006$] and IDA [20.3% (95% CI: 10.5%, 30.2%) compared with 58.3% (95% CI: 46.9%, 69.7%) in the iron-fortified group and the control group; $P < 0.0001$] (Figure 2).

Changes in hemoglobin, SF, and TfR between t_0 and t_6 were significantly greater in the iron-fortified group than in the control group [hemoglobin: 5.7 ± 10.3 compared with −2.8 ± 8.7 g/L ($P < 0.0001$); SF: 18.7 ± 14.9 compared with 2.8 ± 14.7 μg/L ($P < 0.0001$); and TfR: −3.3 ± 3.4 compared with 1.2 ± 3.5 mg/L ($P = 0.0005$)]. Changes in TfR:SF between t_0 and t_6 were also significantly greater in the iron-fortified group than in the control group (−1260 ± 1568 compared with −324 ± 1909; $P = 0.003$). Body iron increased significantly more from t_0 to t_6 in the iron-fortified group than in the control group (201 ± 144 compared with 26.0 ± 110 mg; $P < 0.0001$). By the end of the study, the prevalence of anemia had decreased significantly (33.8%) in the iron-fortified group, whereas the decrease in the prevalence of anemia in the control group was not significant (11.1%). The prevalence of ID decreased 37.5% and 6.9% in the iron-fortified group and the control group, respectively, and the prevalence of IDA decreased 50.0% and 11.1% in the iron-fortified group and the control group, respectively. No significant differences between the 2 groups were observed for changes in other variables such as body weight or body mass index.

The mean iron content of the nonfortified fish sauce was 28.2 ± 0.03 mg/L, and the mean iron content of the fortified fish sauce was 1250 ± 11 mg/L. There were little or no losses of NaFeEDTA when clear bottles of fish sauce were stored in indirect sunlight or in the dark or when amber bottles of fish sauce were stored in sunlight (Fidler et al, unpublished observations, 2001). The median numbers of servings of fish sauce consumed by the iron-fortified group and the control group were 149 (range: 109–162) and 152 (range: 98–163), respectively ($P = 0.25$). Only 5 women consumed <120 servings (2 in the iron-fortified group and 3 in the control group). At t_6, fecal samples were obtained from 135 women: 71.1% were infected with ≥1 intestinal parasite, 37.8% were infected with *A. lumbricoides*, 47.4% were infected with *T. trichuria*, and 13.5% were infected with hookworm. The prevalence of infection with all parasites and with *A. lumbricoides* did not differ significantly between the 2 groups, but the prevalence of infection with hookworm and with *T. trichuria* was significantly higher and significantly lower, respectively, in the iron-fortified group than in the control group [hookworm: 20.6% compared with 7.1% ($P = 0.02$); *T. trichuria*: 36.5% compared with 56.9% ($P = 0.02$)]. At t_3, 15 women had an elevated CRP concentration (3 in the iron-fortified group and 12 in the control group; $P = 0.02$). At t_6, 15 women had an elevated CRP concentration (7 in the iron-fortified group and 8 in the control group; $P = 0.95$).

DISCUSSION

The results of this study clearly show that fish sauce fortified with NaFeEDTA is efficacious in improving iron status and

reducing the prevalence of IDA in anemic Vietnamese women. These results add to the body of evidence that food fortification with iron compounds having high bioavailability is a useful approach to combat ID and IDA. Although food-fortification programs have been implemented in several countries, information about the efficacy of this strategy in improving iron status is lacking, except for condiments fortified with NaFeEDTA (11). Previous studies reported encouraging results with NaFeEDTA fortification of fish sauce in Thailand (12), of sugar in Central America (13), and of curry powder in South Africa (14). All these earlier studies reported a positive effect on iron status after 12–24 mo of intervention. The results from the present study, which featured a double-masked study design and strict monitoring of compliance, showed that regular consumption of fortified fish sauce, which provided an additional 10 mg Fe as NaFeEDTA/d for 6 d/wk, also had a major effect on iron status after a shorter intervention period, ie, 6 mo.

NaFeEDTA is a water-soluble, highly bioavailable form of iron (5) that has been suggested for fortification of fish sauce and soy sauce because NaFeEDTA does not provoke unacceptable organoleptic changes in the fortified condiment (4, 15, 16). Although NaFeEDTA is not currently used in large-scale food-fortification programs, the evaluation by the Joint Expert Committee on Food Additives of the World Health Organization/Food and Agriculture Organization concluded that NaFeEDTA can be considered to be safe when used in supervised food-fortification programs that provide ≈0.2 mg Fe · kg body $wt^{-1} \cdot d^{-1}$ (17).

Preliminary evaluations of the feasibility of fortifying fish sauce with iron in Vietnam have been encouraging because fish sauce can be fortified without major modifications to the production process. Moreover, there were little or no losses of NaFeEDTA when fish sauce was stored in clear bottles under artificial light or in the dark or when the fish sauce was stored in amber bottles in sunlight. In addition, the existing network of fish-sauce factories, which are supervised by the Ministry of Fishery, would facilitate the implementation of the fortification program, and the additional cost has been estimated to be $0.02/L (18).

All the women included in the present study were anemic at baseline, and 69.9% of them were iron deficient. ID was thus the major factor in the etiology of anemia. Information about dietary intake, which was based on 24-h recalls, indicated low dietary iron from a largely plant-based diet in the 2 study groups. The role of other nutritional deficiencies, such as folate, vitamin B-12, and vitamin A deficiency, in the etiology of anemia in the study population is unknown. Helminth infections were prevalent although the intensity of hookworm infection was low; therefore, gastrointestinal blood loss was not assumed to be a significant contributing factor to ID in this study population (19). Malaria is not a public health problem in the Red River delta and was therefore not screened for in the present study.

During the present efficacy study, we monitored changes in hemoglobin and in 2 variables of iron status, SF and TfR. The prevalence of low SF (< 12 μg/L) decreased significantly from 53.1% to 31.3% and 15.6% after 3 and 6 mo of consumption of fortified fish sauce, respectively. Changes in SF concentration were positively correlated with the number of iron-fortified servings of fish sauce consumed during the study. SF was thus a useful indicator of iron status in this study. However, ferritin is an acute-phase reactant and thus has limited usefulness in settings where malaria and other infections are prevalent (2). SF is also influenced by mild infection (20). In the population in the present study, the prevalence of infection, which was evaluated by measurements of CRP concentration, was low.

In addition, a novel indicator of iron status, TfR, was monitored during the present study. TfR has been shown to be a sensitive indicator of tissue ID: elevated plasma TfR concentrations occur during ID and when erythropoiesis increases. The usefulness of this indicator was recently shown in population groups having a high prevalence of malaria and other infections (2). Until now, TfR has not been used to monitor the effect of food fortification although TfR has been shown to respond to iron supplementation in iron-depleted nonanemic women (21). In the present study, the continuous decrease in TfR concentration in the women who consumed iron-fortified fish sauce indicates the usefulness of this iron-status variable when evaluating the effect of iron-fortified foods on iron status.

Furthermore, the TfR-SF ratio has been suggested as a tool for estimating body iron (10), and its usefulness was further evaluated in the present study. At baseline, almost 60% of the women had an elevated TfR-SF ratio (> 500), indicating body iron depletion. This was confirmed by the low mean value for body iron in the control group and by the negative mean value in the iron-fortified group (22). By the end of the 6-mo intervention, TfR-SF ratios had decreased significantly in the women who consumed iron-fortified fish sauce, corresponding to a mean increment of 201 mg body iron. During the same period, the mean increment was 26 mg body iron in the control group. On the basis of the mean increment in the iron-fortified group, it can be estimated that ≈1.2 mg additional iron from the fortified fish sauce was absorbed on average per day during the intervention study, representing ≈12% fractional absorption from the NaFeEDTA added to the fish sauce. This estimate seems realistic because it is comparable to estimates in anemic subjects of 8–10% fractional iron absorption from NaFeEDTA added to fish sauce (12), sugar (13), and curry powder (14). A recent study in nonanemic women reported geometric mean iron absorption values ranging from 3.3% to 6.7% when a fish sauce similar to the one used in the present study was added to a test meal of rice and vegetables or to a meal based on rice alone (23).

No significant changes in iron status or in the prevalence of anemia were observed in the control group, except for a decrease in TfR concentration at the end of the study. Thus, these results indicate that the women in the control group increased their intake or absorption of iron during the study. Although the change in TfR concentration was modest, these observations highlight the importance of including a control group in efficacy studies.

In conclusion, regular consumption of fish sauce fortified with NaFeEDTA (10 mg Fe/d for 6 d/wk) for 6 mo significantly improved iron status and reduced the prevalence of IDA in anemic women. Iron fortification of fish sauce is a promising approach for controlling IDA in Vietnam. However, note that because of the short duration of the present study, a relatively high level of iron fortification (1 mg/mL) was used.

Before implementation of a large-scale iron-fortification program at the national level, the effectiveness of iron-fortified fish sauce should be evaluated under realistic conditions. Such an evaluation is currently being undertaken in 2 districts in the Red River delta of northern Vietnam, with fish sauce fortified with NaFeEDTA at 0.5 mg Fe/mL, ie, at a fortification level 50% lower than that used in the present study. Effectiveness will be monitored by changes in iron status over 18 mo in representative segments of the population.

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PVT, JB, and LD were the principal investigators of the study. They were responsible for the design and the implementation of the study. JB was responsible for data management and analysis. JB and LD were responsible for the preparation of the manuscript. PVT was responsible for day-to-day supervision of the field staff. NCK, HHK, and NTL contributed to the development of the study protocol and supervised the fieldwork. JDC was responsible for the analysis of the SF and TfR iron-status measurements. RFH participated in the development of the study protocol. JDC and RFH participated in the evaluation of the results and contributed to the preparation of the manuscript. RFH is a member of the Technical Advisory Board of Project IDEA (Iron Deficiency Elimination Action) of the International Life Sciences Institute. At the time of the study, LD was a member of the Scientific Advisory Committee of Project IDEA of the International Life Sciences Institute. None of the other coauthors reported any conflict of interest.

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