Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women[1–3]

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ABSTRACT

Background: Although elemental iron powders are widely used to fortify cereal products, little data exist on their efficacy in humans.

Objective: We compared the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced iron in Thai women with low iron stores.

Design: A double-blind intervention was conducted in 18–50-y-old women ($n = 330$) randomly assigned into 4 groups to receive either no fortification iron or 12 mg Fe/d for 6 d/wk for 35 wk as ferrous sulfate, electrolytic iron, or hydrogen-reduced iron in a baked, wheat-flour–based snack. Snacks were not consumed with meals, and consumption was monitored. At baseline, 20 wk, and 35 wk, hemoglobin status and iron were measured and the groups were compared.

Results: Between baseline and 35 wk, geometric mean serum ferritin (SF) increased significantly in all 3 groups receiving iron ($P <$ 0.01), and geometric mean serum transferrin receptor (TfR) decreased significantly in the groups receiving ferrous sulfate and electrolytic iron ($P < 0.05$). Calculated mean ($\pm$SD) body iron stores increased from 1.5 ± 2.8 to 5.4 ± 2.9 mg/kg in the ferrous sulfate group, from 1.5 ± 3.5 to 4.4 ± 3.6 mg/kg in the electrolytic iron group, and from 1.3 ± 3.2 to 3.2 ± 4.3 mg/kg in the hydrogen-reduced iron group ($P < 0.01$ for all 3 groups) but did not change significantly in the control group.

Conclusions: Ferrous sulfate, electrolytic iron, and hydrogen-reduced iron, fortified into wheat-based snacks, significantly improved iron status. On the basis of the change in body iron stores during the 35-wk study, the relative efficacy of the electrolytic and hydrogen-reduced iron compared with ferrous sulfate was 77% and 49%, respectively. *Am J Clin Nutr* 2005;82:1276–82.

KEY WORDS Elemental iron, electrolytic iron, hydrogen-reduced iron, iron fortification, Thailand

INTRODUCTION

Iron deficiency is an important global public health problem that mainly affects infants, children, and women of childbearing age in poor populations in developing countries (1, 2). Food fortification may be an effective, long-term approach to combat iron deficiency (3, 4). Worldwide, cereals are the foods most often fortified with iron. The United States, Canada, the United Kingdom, and several Latin American and African countries require iron be added to white wheat flour (5). Fortification of cereals with ferrous sulfate or other soluble iron compounds may cause adverse sensory changes (5, 6). Less-soluble iron compounds, such as the elemental iron powders, cause fewer organoleptic problems, are relatively inexpensive, and are commonly used in cereal fortification. However, most elemental iron powders are poorly absorbed relative to ferrous sulfate (3, 4), and, despite their widespread use, their efficacy as cereal fortificants has never been properly tested in humans.

The elemental iron powders most often used in cereal fortification are electrolytic iron and hydrogen-reduced iron. Electrolytic iron is the only elemental powder currently recommended for fortification of cereals (7) and is mainly used in infant cereals. It is made by electrolytic migration of iron from an iron anode through a ferrous sulfate solution. The resulting elemental iron powder is >99% iron and can be finely ground. Food Chemicals Codex (FCC) specifications require that electrolytic iron powders pass through a 325-mesh sieve (particle size < 44 μm) (5). The relative bioavailability (RBV) of electrolytic iron compared with ferrous sulfate is estimated to be 16–70% in rats and 75% in humans (3–5). Hydrogen-reduced iron is the most widely used elemental iron powder for cereal fortification (5). It is made by reduction of ground iron oxide to its elemental state with hydrogen at an elevated temperature and has the lowest purity of the food-grade iron powders (>96% iron). Most hydrogen-reduced powders used commercially in Europe and North America have a particle size of 300 or 325 mesh, although powders with larger particle sizes are often used in developing countries (5). The RBV of reduced iron powders is estimated at 13–54% in rats and 13–148% in humans (3–5).

Iron deficiency anemia (IDA) in young women increases the risk of complications during pregnancy and has an adverse effect on work performance and productivity (2, 8). Women of child-bearing age are therefore one of the main target groups of iron fortification. In Thailand, young women often have low iron body stores (9), and the prevalence of IDA is 25–30% in pregnant women and 15–20% in women of reproductive age (10). The frequency of the thalassemias and related hemoglobinopathies is also high in the Thai population (11). Our study aim was to compare the efficacy of a fortified wheat-based snack that contained electrolytic iron, hydrogen-reduced iron, or ferrous sulfate in improving iron status in Thai women with low iron stores.

SUBJECTS AND METHODS

Screening studies

To determine whether Thai women of reproductive age were a suitable population for the feeding trial, a preliminary study was done to estimate the prevalence of IDA and thalassemia in this group. In a convenience sample of 18–50-y-old women employed in 4 factories near Bangkok ($n = 201$), age was recorded, weight and height were measured, and 5 mL whole blood was collected by venipuncture into EDTA-containing tubes. Samples were analyzed for concentrations of hemoglobin, serum ferritin (SF), and transferrin receptor (TfR), and hemoglobin typing was done. From the encouraging results of this preliminary study, women from 8 factories near Bangkok were screened for participation in the efficacy study. The inclusion criteria for the screening were 1) age 18–50 y; 2) female sex; 3) nonpregnant state; 4) no chronic medical illnesses; and 5) no use of vitamin and mineral supplements. The women were registered, weight and height were measured, and 5 mL whole blood was collected by venipuncture into EDTA-containing tubes. Samples were analyzed for concentrations of hemoglobin, SF, and TfR, and hemoglobin typing was done. Women from the screening were invited to join the intervention trial if they had a SF < 25 μg/L, a hemoglobin > 90 g/L, and one of the following hemoglobin types: 1) normal hemoglobin A (HbA); 2) α-1 thalassemia heterozygote; 3) α-2 thalassemia heterozygote; 4) HbE heterozygote; or 5) hemoglobin Constant Spring. Women with HbH, EA Bart's disease, or β-thalassemia were excluded because these traits were expected to confound measures of iron status and response to iron repletion (12, 13). Women with hemoglobin < 90 g/L and iron deficiency (<1% of the screening population) were excluded and received oral iron supplementation. Ethical review committees at the Swiss Federal Institute of Technology and Mahidol University approved the study. Informed written consent was obtained from the participants.

Women who met all inclusion criteria were individually randomly assigned into 4 groups by factory to receive a baked snack providing 1) no fortification iron, 2) 12 mg Fe as ferrous sulfate monohydrate (Ferrous Sulfate Monohydrate USP/FCC; Crown Technology Inc, Indianapolis, IN), 3) 12 mg Fe as electrolytic iron (Electrolytic A131; North America Högänäs High Alloys LLC, Johnstown, PA), or 4) 12 mg Fe as hydrogen-reduced iron (H-Reduced AC 325; North American Högänäs Alloys LLC). The ferrous sulfate, electrolytic iron, and hydrogen-reduced iron used to fortify the snacks were drawn from the pool of iron powders supplied by SUSTAIN (Washington, DC).

TABLE 1
Ingredients of the iron-fortified baked snacks fed during the efficacy trial

Ingredients	Cookies	Bread
		g
Low-extraction wheat flour	1990	2400
Baking soda	30	—
Yeast	—	24
Milk		
Condensed	160	—
Powdered	—	72
Water	110	1350
Salt	20	32
Butter	355	132
Margarine	855	—
Dough developer[1]	—	9.6
Butter flavor	10	—
Calcium proprionate[2]	—	9.6
Egg	355	—
Sugar	1065	150
Sodium citrate	29.7	11
Topping		
Raisins, chocolate chips, cashews, or plain	0.3–0.5	—
Apricot or strawberry jam	—	0.5–0.8
Iron compounds[3]		
Ferrous sulfate	8.59	3.16
Hydrogen-reduced iron	2.83	1.04
Electrolytic iron	2.83	1.04
No iron	—	—

[1] Dough developer consisted of 39% flour, 25% dextrose, 23% emulsifier, and 12% vegetable oil.

[2] Used as an anticaking agent.

[3] Only one compound was used for each intervention. No iron was used as control.

Preparation of the iron-fortified snacks

The iron compounds provided by the manufacturers were homogenized before drawing samples by turning the bulk containers top to bottom at least 5 times to correct any settling or segregation that may have occurred during shipping. Samples were drawn by using a sampling probe (GP-4 Sampling Probe; Gilson, Lewis Center, OH) and packed as 2.27-kg batches. They were shipped and stored in plastic bags with a twist top, inside well-sealed metal cans with a desiccant. At Mahidol University, the containers were stored at 4 °C in the kitchen of the Institute of Nutrition. They were tightly resealed after each use, and the iron powders were checked for rust formation at the midpoint of the efficacy trial by sieving through an 80-mesh screen.

Several bakery products were tested for their suitability as the fortification vehicle (14). Cookies and bread were chosen because their recipes are simple and they are widely consumed in Thailand. Sweetened butter cookies (weight after cooking: 5 g) were produced with a topping of raisins, chocolate chips, cashew nuts, or plain. A daily serving consisted of 4 cookies. Sweetened white bread, with apricot or strawberry jam topping on the loaves, was sliced after baking into 30-g daily portions. The ingredients of the baked snacks and their iron content are shown in **Table 1**. The snacks provided 10–12 mg Fe as ferrous sulfate monohydrate, electrolytic iron, or hydrogen-reduced iron per serving; control snacks provided no added iron (14). Sodium citrate was added at a molar ratio of 2:1 with iron to reduce the metallic flavor and to increase the shelf life of snacks containing

the ferrous sulfate; this amount of sodium citrate was also added to the snacks for the other 3 groups. Before beginning the efficacy study, the iron concentration and its homogeneity in the baked snacks was tested (14). To check shelf life and stability, snacks were placed in sealed polypropylene bags and stored in metal boxes at 28 °C. The color, taste, and texture of the snacks containing the 3 iron compounds were compared after 2 wk of storage by using ranking tests and 9-point hedonic scales by an adult panel (*n* = 23) in the Sensory Science Laboratory at the Institute of Nutrition at Mahidol University.

During the feeding trial, the snacks were produced daily in color-coded batches under close supervision in the kitchen of the Institute of Nutrition. The amount of iron compound needed for each baking was measured by using a digital scale accurate to 0.01 g (Balance AG204; Mettler-Toledo, Columbus, OH). The iron for that day's dough was first dry-mixed into sugar by shaking them together by hand for several minutes in a small plastic bag. The sugar and iron premix was then added to the remaining ingredients and mixed for ≈10 min by using a noncutting, blade dough mixer (UM-20; Heng Wei, Guangzhou, China). The dough was formed into cookies of uniform size and weight by using a baker's syringe (Ampia Biscuits cookie press; Marcato, Padova, Italy) and baked at 180 °C for 30 min. The bread was baked at 180 °C for 40–45 min. For quality control, with the use of a prespecified sampling pattern from the baking trays and loaves, snacks were set aside after baking for determination of iron content. The snacks were packed into individual, color-coded plastic sachets containing a daily serving. They were stored in opaque plastic containers at room temperature in the Institute of Nutrition at Mahidol University kitchen for 1–2 wk and distributed weekly to participating factories.

Efficacy trial

At the factories, the snacks were consumed 6 d/wk at the midmorning break or at the end of a shift, with consumption directly monitored and recorded by either the factory nurse, the personnel officer, or a member of the study team. If a subject was absent from work, the missed snacks were compensated by provision of 2 snacks on the following days, consumed at midmorning and midafternoon. Snacks were consumed away from meals, and only water was provided with the snack. On scheduled work holidays, the subjects were sent home with enough snacks, packaged into individual daily portions, to cover the holiday and instructed to consume a snack each day. After 20 and 35 wk, measurements for concentrations of hemoglobin, SF, and TfR were repeated, and the relative efficacy of the intervention was judged by comparing iron status among the 4 groups. The primary outcome variable was change in body iron stores (15). The study design was double blind, and the intervention period was from July 2004 to April 2005.

Laboratory analyses

Whole blood was transported on ice to the laboratory at Mahidol University. Hemoglobin and red cell indexes were measured on the day of collection by using a hematology analyser (Advia 120; Bayer Diagnostics, Leverkusen, Germany) with 3-level control material provided by the manufacturer. Hemoglobin typing was done with the use of automated HPLC (Variant; Bio-Rad, Hercules, CA). DNA was isolated from the buffy coat by using the QIAamp DNA Blood Kit (QIAGEN, Valencia,

CA). Genotyping for α-thalassemia was performed by the Gap polymerase chain reaction technique as previously described (16). Blood was centrifuged on the day of collection, and the serum was divided into aliquots and frozen at −20 °C. Serum was transported frozen on dry ice, and SF and TfR concentrations were measured by using immunoassays (Ramco, Houston, TX) (17) in Zürich. C-reactive protein was not measured. Normal reference values are 15–300 μg/L for SF and 2.9–8.5 mg/L for TfR (16, 17). Anemia was defined as hemoglobin < 120 g/L (1). Iron deficiency was defined as either SF < 15 μg/L or TfR > 8.5 mg/L (1, 17). IDA was defined as anemia and iron deficiency by the above-mentioned criteria. Phytic acid content of the baked snacks was measured by the modified Makower method (18). Iron content of the baked snacks was measured by atomic absorption spectroscopy (Spectra AA-50; Varian, Palo Alto, CA). Body iron was estimated from the TfR-to-SF ratio (15) by using the following formula:

Body iron (mg/kg) =

$$- [\log(TfR/SF) - 2.8229]/0.1207 \quad (1)$$

Statistical analysis

Data processing and statistics were done by using SPLUS-2000 (Insightful Corporation, Seattle, WA), Prism (version 3; GraphPad, San Diego, CA), and EXCEL (XP 2002; Microsoft, Seattle, WA). Normally distributed data were expressed as means ± SDs; nonnormally distributed data were expressed as geometric means or medians (range). One SF data point at both 20 and 35 wk, from the same individual in the ferrous sulfate group (210 and 234 μg/L, respectively) was excluded as outliers. Data not normally distributed (SF, TfR, body iron) were log transformed for comparisons. A two-factor repeated-measures analysis of variance was done to compare the effects of time × group and time × group interaction for hemoglobin, SF, TfR, and body iron. If the interaction effect was significant, *t* tests between groups and paired *t* tests within groups were done and adjusted for multiple comparisons (Bonferroni correction). Proportions were compared by using the chi-square tests. Logistic regression was done to compare the effects of time × group and time × group interaction for the binary variables of iron deficiency and anemia. Regression models were calculated with body iron stores at 35 wk and change in body iron stores by using baseline and 35 wk as the dependent variables and by using group, age, baseline body iron stores, and hemoglobin type (normal, HbA; α-thalassemia 2 heterozygote; HbE heterozygote) as independent variables. Women who dropped out of the study were not included in the regressions. *P* values < 0.05 were considered significant.

RESULTS

Screening studies

In the prescreening study (*n* = 201), the mean ± SD of hemoglobin was 126 ± 10 g/L, median SF and TfR were 66 μg/L (range: 7–280 μg/L) and 4.7 mg/L (range: 0.1–15.8 mg/L), respectively. On hemoglobin typing, 76% of subjects had normal hemoglobin (α-thalassemia 1 or 2 trait was not ruled out), and 19% were HbE heterozygotes. Prevalence of anemia, iron deficiency, and IDA were 25%, 10%, and 7%, respectively. From these data and good logistics and access at the factories, the

TABLE 2
Characteristics of the women in 8 Bangkok factories in the baseline screening to select subjects for the efficacy study[1]

Characteristic	Value
Age (y)	29.8 ± 7.7[2]
Weight (kg)	54.0 ± 9.2
Height (cm)	154.8 ± 5.4
BMI (kg/m^2)	22.5 ± 3.7
Hemoglobin (g/L)	126 ± 11
Serum ferritin (μg/L)[3]	46 (2–584)[4]
Serum transferrin receptor (mg/L)[5]	4.3 (2–35)
Hemoglobin typing[6]	
Normal	779 [52][7]
Normal, α-thalassemia trait not ruled out	245 [16]
HbE heterozygote	360 [24]
HbE homozygote	81 [5]
β-Thalassemia heterozygote	31 [2]
EA Bart's disease	7 [0.5]
Hb Constant Spring	6 [0.4]
HbH disease	5 [0.3]
Prevalence of anemia[8]	477 [24]
Prevalence of iron deficiency[9]	98 [5]
Prevalence of iron deficiency anemia[10]	98 [5]

[1] $n = 2010$. Hb, hemoglobin.

[2] $\bar{x} \pm$ SD (all such values).

[3] $n = 1956$.

[4] Median; range in parentheses (all such values).

[5] $n = 266$.

[6] $n = 1514$.

[7] n; percentage in brackets (all such values).

[8] Anemia was defined as a hemoglobin concentration < 12 g/L.

[9] Iron deficiency was defined as either a serum ferritin concentration $< 15 \mu$g/L or a serum transferrin receptor concentration > 8.5 mg/L.

[10] Iron deficiency anemia was defined as anemia and iron deficiency according to the abovementioned criteria.

population was judged suitable for the efficacy trial. The results of the subsequent baseline screening for the efficacy trial are shown in **Table 2**. Our original intention was to enroll only women with clear iron deficiency (SF $< 15 \mu$g/L) with or without anemia in the efficacy trial. However, the prevalence was lower than expected (10%), and the study protocol was changed to include women with low iron stores, defined as a SF $< 25 \mu$g/L.

Evaluation of the iron-fortified snacks

In the sensory studies to evaluate the baked snacks, all 3 iron-fortified snacks were judged acceptable after 2 wk of storage (14). The taste of the snacks containing the elemental iron powders was generally judged superior to those with ferrous sulfate (14). The mean phytic acid content of the baked cookies and bread were 77 ± 1 and 96 ± 2 mg/100 g, respectively.

In the quality control measurements during the efficacy trial, the weight per serving of 4 cookies ($n = 40$) was 20.9 ± 0.7 g, and the mean iron content was 13.1 ± 0.9 mg, with no significant difference in iron content among cookies containing the 3 different iron compounds (data not shown). The weight per serving of the bread ($n = 24$) was 33.3 ± 1.8 g, and the mean iron content was 9.2 ± 0.9 mg, with no significant difference in iron content among breads containing the 3 different iron compounds (data not shown). Because the snacks were administered in a rotating pattern of 4 d of cookies, then 1 d of bread, the mean daily iron dose was ≈ 12 mg. In the sieving tests for rust formation at the midpoint of the efficacy trial, no rust was found in the electrolytic iron. The hydrogen-reduced iron had > 5 rust balls/3 g powder and was replaced by a new batch of hydrogen-reduced powder for the second half of the trial.

Efficacy trial

Sixteen percent of the women in the screening met the inclusion criteria and were enrolled. The baseline characteristics of the subjects ($n = 330$) after random assignment for the efficacy trial are shown in **Table 3**. No significant differences were observed in baseline anthropometric characteristics, hemoglobin type, or iron status indicators among the 4 groups (Table 3 and **Table 4**). Of the 330 women who began the study, 216 completed it; 52 women in both the electrolytic and hydrogen-reduced iron groups completed the study, and 56 women in the control and ferrous sulfate groups completed the study. The high dropout rate was due to 1) subjects having employment contracts that ended during the study (89% of dropouts); 2) pregnancy (10%), and 3) venipuncture refusal (1%). When comparing the women, by group, who completed the study with those who dropped out, no significant differences were observed in baseline anthropometric or hematologic characteristics (data not shown).

The changes in hemoglobin and iron status, as well as the prevalence of anemia and iron deficiency at 20 and 35 wk are

TABLE 3
Baseline characteristics of the Thai women by group after random assignment for the efficacy trial of iron-fortified wheat-based snacks[1]

Group	Ferrous sulfate ($n = 83$)	Electrolytic iron ($n = 83$)	Hydrogen-reduced iron ($n = 82$)	No iron control ($n = 82$)
Age (y)	31.4 ± 8.0[2]	29.6 ± 7.6	29.7 ± 8.3	29.3 ± 8.0
Weight (kg)	53.3 ± 8.3	52.2 ± 9.5	53.5 ± 9.0	53.7 ± 9.7
Height (cm)	155 ± 5	155 ± 6	155 ± 5	155 ± 6
BMI (kg/m^2)	22.4 ± 3.4	22.0 ± 4.0	22.2 ± 3.5	22.2 ± 3.6
Number of women by Hb type				
Normal, HbA	50	58	57	52
α-Thalassemia 1 heterozygote	0	1	2	0
α-Thalassemia 2 heterozygote	12	12	9	6
HbE heterozygote	19	12	13	23
Hb Constant Spring	2	0	1	1

[1] No significant differences were observed between groups (ANOVA). Hb, hemoglobin.

[2] $\bar{x} \pm$ SD (all such values).

TABLE 4
Changes in hemoglobin and in iron-status indicators in Thai women with low baseline iron stores who consumed iron-fortified wheat-based snacks for 35 wk[1]

	Ferrous sulfate	Electrolytic iron	Hydrogen-reduced iron	No iron control
Hemoglobin (g/L)[2]				
Baseline	124 ± 10	122 ± 13	123 ± 13	124 ± 10
20 wk	128 ± 9	125 ± 10	127 ± 16	123 ± 11
35 wk	125 ± 9	124 ± 12	122 ± 12	121 ± 12
Serum ferritin (μg/L)[3,4]				
Baseline	13 (3–25)	12 (3–25)	12 (2–25)	10 (2–27)
20 wk	24 (3–59)[a,5]	20 (2–81)[b,6]	16 (5–63)[b,6]	11 (3–38)[c]
35 wk	29 (4–109)[a,5]	26 (3–98)[a,5]	22 (2–169)[b,5]	13 (3–69)[c]
Transferrin receptor (mg/L)[4]				
Baseline	5.8 (2.9–14.7)	5.5 (2.1–13.2)	5.7 (2.0–14.2)	5.3 (1.8–12.4)
20 wk	4.5 (2.3–9.5)[a,6]	4.6 (1.8–12.0)[a,6]	5.0 (1.7–12.7)[b,6]	5.3 (1.7–13.0)[b]
35 wk	4.5 (1.7–10.3)[a,6]	5.1 (2.3–19.3)[b,6]	6.0 (2.9–14.6)[c]	5.8 (2.1–17.8)[c]
Body iron stores (mg/kg body weight)[2,4]				
Baseline	1.5 ± 2.8	1.5 ± 3.5	1.3 ± 3.2	1.0 ± 3.4
20 wk	4.6 ± 2.8[a,5]	3.9 ± 3.5[b,5]	2.8 ± 4.1[c,6]	1.1 ± 3.4[d]
35 wk	5.3 ± 2.9[a,5]	4.4 ± 3.6[b,5]	3.2 ± 4.3[c,5]	1.4 ± 4.5[d]
Change from baseline	3.8 ± 3.1[a]	3.0 ± 3.4[b]	1.9 ± 3.2[c]	0.3 ± 3.5[d]
Number with anemia[7,8]				
Baseline	18	26	20	19
20 wk	12	18	15	25
35 wk	12[a]	21[b]	18[b]	20[b]
Number with iron deficiency[8,9]				
Baseline	34	28	30	39
20 wk	11	17	25	46
35 wk	7[a]	11[a]	21[b]	30[b]

[1] Number of women in the ferrous sulfate, electrolytic iron, hydrogen-reduced iron, and control groups at 20 and 35 wk are as follows: 69 and 56, 62 and 52, 63 and 52, and 64 and 56, respectively. Values in a row with different superscript letters are significantly different, $P < 0.05$ (t test).

[2] All values are $\bar{x} \pm$ SD.

[3] All values are geometric $\bar{x}$; range in parentheses.

[4] Significant group × time interaction, $P < 0.001$ (ANOVA).

[5,6] Significantly different from baseline (t test): [5] $P < 0.01$, [6] $P < 0.05$.

[7] Anemia was defined as a hemoglobin concentration < 12 g/L.

[8] Significant group × time interaction $P < 0.05$ (logistic regression).

[9] Iron deficiency defined as either a serum ferritin concentration < 15 μg/L or a serum transferrin receptor concentration > 8.5 mg/L.

shown in Table 4. Between baseline and 35 wk, a significant increase was observed in geometric mean SF in all 3 groups receiving iron ($P < 0.01$), and a significant decrease was observed in the geometric mean serum TfR in the groups receiving the ferrous sulfate and electrolytic iron ($P < 0.05$). Calculated mean ± SD body iron stores increased from 1.5 ± 2.8 to 5.3 ± 2.9 mg/kg in the ferrous sulfate group, from 1.5 ± 3.5 to 4.4 ± 3.6 mg/kg in the electrolytic iron group, from 1.3 ± 3.2 to 3.2 ± 4.3 mg/kg in the hydrogen-reduced iron group ($P < 0.01$ for all 3 groups), and did not change in the control group. In the regressions, addition of the other independent variables did not improve the predictions once the group variable had been included; baseline body iron stores and hemoglobin type were not significant predictors of response to iron.

DISCUSSION

The amount of fortification iron absorbed depends on meal or diet composition, the iron status of the individual, and the bioavailability of the iron fortificant (3–5). In this study, the iron compounds were fed in snacks made from low-extraction wheat flour that provided only modest amounts of phytic acid (<0.1 g/100 g). All of the subjects had low iron stores, but <50% were

iron deficient, and only ≈1 in 5 was anemic. The total dose of iron delivered during the trial was ≈2.5 g, based on a study period of 35 wk with snacks fed 6 d/wk and a fortification level of 12 mg Fe/snack. By comparing the total iron dose to the mean increase in total body iron in the 3 groups, ≈8% ferrous sulfate, 6% electrolytic iron, and 4% hydrogen-reduced iron were absorbed during the 35-wk trial in the ferrous sulfate, electrolytic iron, and hydrogen-reduced iron groups, respectively. Thus, in this population with low iron stores consuming snacks of moderate-to-high iron bioavailability, the relative efficacy of the electrolytic and hydrogen-reduced iron powders was 77% and 49%, compared with ferrous sulfate.

Although various screening tests (solubility in dilute acid, in vitro dialyzability, Caco-2 cell uptake, rat hemoglobin repletion studies, serum iron profiles) were proposed to evaluate the potential of iron compounds for food fortification, their predictive value is uncertain (5). Swain et al (19) studied elemental iron powders by using rat hemoglobin repletion tests and solubility in hydrochloric acid. From the hemoglobin repletion tests, the RBV of electrolytic and hydrogen-reduced iron powders (from the same SUSTAIN pool of iron powders used in the present study) compared with ferrous sulfate was 54% and 42%, respectively.

Solubility at pH 1.0 and surface area predicted RBV from the rat hemoglobin repletion studies (19). Hoppe et al (20) also studied elemental iron (from the same SUSTAIN pool of iron powders) but used serum iron profiles in iron-replete men and reported mean RBVs of 65% for electrolytic iron and 56% for hydrogen-reduced iron. By using radioisotopes in humans, Forbes et al (21) reported the absorption of electrolytic iron from a meal of wheat farina and milk was 75% of ferrous sulfate. Taken together, those studies suggest electrolytic and hydrogen-reduced iron powders are ≈40–70% as well absorbed as ferrous sulfate, with absorption of electrolytic iron superior to hydrogen-reduced iron. Ultimately, the true test of iron compounds for fortification is their ability to reduce iron deficiency in at-risk population groups. Extending the results from the surrogate tests (19–21), the superior efficacy of electrolytic iron compared with hydrogen-reduced iron is clearly evident in the present study. These data suggest potential iron fortificants can and should be evaluated by measuring the effect on iron status in rigorous field efficacy trials (22–25).

Thalassemia mutations are extremely common in Southeast Asia. The frequency of α-thalassemia reaches 25% in parts of Thailand, and the frequency of HbE approaches 60% in northern Thailand, Laos, and Cambodia (11, 26, 27). In homozygous thalassemia, plasma iron turnover is increased because of ineffective erythropoiesis and increased hemolysis, and this may increase iron absorption (28–30). If heterozygotes partially express the homozygote phenotype (ineffective erythropoiesis and increased plasma iron turnover), they may absorb more iron from fortified foods. In our study population, 12% of women were α-thalassemia 2 heterozygotes and 20% were HbE heterozygotes. However, in the regressions, hemoglobin type was not a significant predictor of an increase in body iron stores in response to the fortification iron. Although this finding suggests that the heterozygotes did not show increased iron absorption compared with individuals with normal hemoglobin, the number of affected individuals was small and the trial period was only 35 wk. This finding should be confirmed by studies with greater power to detect potential small differences in iron absorption in these heterozygotes.

Because of its superior bioavailability in this study and its low cost, ferrous sulfate may be the preferred choice for fortification of bakery flour (typically used within a month of milling) and low-moisture wheat products such as noodles and pasta (5). However, its wider use is limited by its tendency to cause rancidity in stored flours and color changes in several cereal products (5, 6). In this study, both the electrolytic and hydrogen-reduced iron powders were sufficiently well-absorbed to be nutritionally useful and could be alternatives to ferrous sulfate in stored flours and cereal products in which ferrous sulfate cannot be used. Compared with hydrogen-reduced iron, the greater efficacy of electrolytic iron may be due to its better solubility in gastric juice, as a result of its high surface area, its highly irregular, dendritic particle shape, and its high purity (5, 19).

Although often combined under the generic term "reduced iron," the large difference in efficacy of electrolytic and hydrogen-reduced iron in this study emphasizes that it is not feasible to give a single recommendation concerning the fortification of cereal with elemental iron powders. The efficacy of other elemental iron powders, such as carbonyl, atomized, and carbon monoxide–reduced iron, should be tested in efficacy studies before they can be recommended for flour fortification.

Overall, our findings support recent fortification guidelines by the World Health Organization, which recommend electrolytic iron as the elemental iron powder of choice for cereal fortification (7). In addition, the guidelines suggest that hydrogen-reduced iron, used at twice the concentration of ferrous sulfate, could be an effective fortificant.

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