Salt Dual-Fortified with Iodine and Micronized Ground Ferric Pyrophosphate Affects Iron Status but Not Hemoglobin in Children in Côte d’Ivoire

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ABSTRACT Deficiencies of iron and iodine are common in West Africa, and salt is one of very few food vehicles available for fortification. Salt dual-fortified with iodine and micronized ground ferric pyrophosphate (FePP) was tested for its efficacy in rural, tropical Côte d’Ivoire. First, salt and iron intakes, and iron bioavailability were estimated using 3-d weighed food records in 24 households. Local iodized salt was then fortified with 3 mg Fe/g salt as ground FePP (mean particle size = 2.5 μm), and stability, sensory and acceptability trials were done. The dual fortified salt (DFS) was distributed to households and its efficacy compared with that of iodized salt (IS) in a 6-mo, double-blind trial in 5- to 15-y-old iron-deficient children (n = 123). All children were dewormed at baseline. After 6 mo, serum ferritin (SF) and transferrin receptor (TfR) concentrations as well as body iron stores improved significantly in the DFS group but not in the IS group (P < 0.05). Body iron increased from 4.6 ± 2.7 to 5.9 ± 2.7 mg/kg (mean ± SD) in the DFS group; concentrations before and after treatment in the IS group were 5.5 ± 2.9 and 5.6 ± 3.1 mg/kg, respectively. The hemoglobin concentration and the prevalence of anemia did not change in either group. The prevalences of malaria, soil-transmitted helminths, and riboflavin deficiency were 55, 14, and 66%, respectively. In tropical West Africa, low-grade salt fortified with micronized ground FePP increased body iron stores but not hemoglobin in children. Iron utilization may have been impaired by the high prevalence of malaria and concurrent nutrient deficiencies. J. Nutr. 136: 1814–1820, 2006.

KEY WORDS: • iron • ferric pyrophosphate • salt • iodine • Africa

In regions of West Africa, 20–38% of school children may suffer from both iron (Fe) and iodine deficiencies (1). In countries in which existing food supplies and/or limited access fail to provide adequate levels of these nutrients in the diet, food fortification is a promising approach. However, there have been no published successful trials of food fortification with Fe in tropical Africa possibly due to other concurrent micronutrient deficiencies and the high prevalence of infection, which can reduce Fe absorption and decrease Fe mobilization from stores (2,3). Salt is one of very few food items consumed daily by rural African populations even in poor remote areas of subsistence farming (4). Salt has been iodized effectively in many African countries, and it would be advantageous to use the existing infrastructure for iodization to also fortify salt with Fe. An additional benefit of adding Fe to iodized salt, beyond the positive effect of Fe on cognitive development, school performance, immune function and work capacity (5), is that iron deficiency anemia (IDA) 3 impairs thyroid metabolism and reduces the efficacy of iodine prophylaxis in areas of endemic goiter (1,6).

However, when ferrous Fe is added to low-grade iodized salt in developing countries, it causes unacceptable color changes and iodine losses (7,8). One approach is to place a barrier around ferrous Fe by encapsulation. In Morocco, a dual-fortified salt (DFS) containing ferrous sulfate encapsulated with hydrogenated oil showed good efficacy, but the salt discolored when the moisture content was high (9). Micronized ground ferric pyrophosphate (FePP) is a promising fortificant for salt (8). It has a white color and although insoluble, FePP with a mean particle size (MPS) of 2.5 μm has a relative bioavailability value (RBV) of 69% that of ferrous sulfate in rats (10). In Morocco, iodized

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3 Abbreviations used: CRP, C-reactive protein; DFS, dual-fortified salt; EAR, Estimated Average Requirement; EGRAC, erythrocyte glutathione reductase activation coefficient; FePP, ferric pyrophosphate; Hb, hemoglobin; IDA, iron deficiency anemia; IS, iodized salt; MFP, meat, fish, and poultry; MPS, mean particle size; RAE, retinol activity equivalents; RBV, relative bioavailability value; SF, serum ferritin; TfR, transferrin receptor.
salt (IS) fortified with micronized ground FePP (MPS of 2.5 μm) showed high efficacy in reducing IDA in children (11). However, conditions are more challenging in tropical Africa, where the ambient temperature and humidity are high, salt is typically low grade, and parasitic infections and nutrient deficiencies are common and may blunt the response to Fe interventions (12).

We fortified local, low-grade iodized salt in Côte d’Ivoire with micronized FePP and tested its stability, organoleptic qualities in traditional meals, and acceptability. We measured the efficacy of the DFS to improve iron status in a 6-mo intervention trial in iron-deficient school children in rural Côte d’Ivoire. IS was used as a control.

MATERIALS AND METHODS

Study site. The study site was a rural village in the Dabou district of Côte d’Ivoire, 10 km from the southern coast. The climate is tropical, with temperatures ≥ 27°C and a relative humidity ≥ 80% much of the year. During the ~6-mo rainy season, the village experiences daily drenching rains. Most of the foods consumed are produced locally, and the staple food is cassava. Plantain, rice, yams and dried, smoked fish are eaten regularly.

Measurements of food and nutrient intake and calculation of dietary iron bioavailability. To establish the optimal fortification level for Fe in the salt, 3-d weighed food records were conducted in 24 randomly selected village households according to Hess et al. (13). In the village, family meals are traditionally eaten from shared bowls. To estimate individual food intake with greater accuracy, families ate from individual bowls during the 3-d of records.

Nutrient intakes (Fe, vitamin C, phytic acid, polyphenols, vitamin A, riboflavin) were calculated using food composition tables (4,14–17) and food analysis software EBI-Sprü (University of Hohenheim/Stuttgart). Vitamin A intakes were calculated as retinol activity equivalents (RAEs) using a conversion factor of 12 μg β-carotene to 1 μg retinol for a mixed diet (18,19). For β-carotene from palm oil, the standard equivalent of 6 μg for 1 μg of RAE was used based on the presence of fat and the absence of a plant matrix (18). Dietary Fe bioavailability was estimated using the algorithms of Tseng et al. (20) and Reddy et al. (21) and adjusted for body iron stores (22). The percentage heme Fe in cooked animal products was estimated to be 60% for beef (23–26), 40% for pork (25,26), 30% for chicken (24–26), 25% for fish (25) and 10% for shrimp (25). Estimated iron bioavailability was calculated for a mean meal size consumed by children 6–15 y containing 28% sauce and 72% staple.

Salt fortification. Iodized salt from Namibia imported by the major Abidian salt producer (Sagid Salt) was used for the study. This salt has a slight beige color, a range of a particle size from 0.2 to 2 mm, and a NaCl content of ~97%. To produce the DFS, 300 g food-grade micronized FePP (art. no. 3046449, ≥25% Fe, mean particle size ~2.5 μm, Dr. Paul Lohmann, GmbH KG, Germany) produced from routine FePP by grinding, was added to 25 kg of salt (3 mg Fe/g salt) and premixed by hand with a plastic spoon. The premix was then fed in 1 pass through a 2-m long, screw-ribbon blender set up by UNICEF at Sagid Salt for iodization. Homogeneity tests showed a homogenous mixing of the Fe into the salt after 1 pass with no further improvement with additional passes; the Fe concentration was 3.2 ± 0.2 mg Fe/g salt (n = 30) after 1 pass through the mixer. From each mixing for the DFS and from each prepackaged 25 kg bag for the IS, a sample of ~30 g was collected and stored until analyzed for color, iodine, and Fe (DFS only) according to Wegmuller et al. (8).

The bioavailability level of 3 mg Fe/g salt was chosen on the basis of a mean per capita salt intake of ~3.5 g/d in school children, an estimated iron bioavailability of ~14% from the diet (see analysis of the 3-d records in the Results section), a RBV of 70% of the micronized ground FePP compared with Fe sulfate in rats (10), and the recommended level of daily Fe absorption (27).

Stability testing. DFS and IS were stored as 10- and 5-kg portions in loosely woven, high-density polyethylene bags typically used to package salt at the production site and as two 300-g portions in transparent low-density polyethylene bags typically used at the retail level and in markets. Storage conditions and the sampling procedure at mixing and after storage for 1, 2, 3, 4, 5, and 6 mo were according to a earlier publication (8). Samples were frozen at −25°C until color, iodine, and Fe (DFS only) were measured. The mean temperature and relative humidity, measured daily during the 6-mo storage period, were 27.5 ± 2.1°C and 79.3 ± 9.9%.

Organoleptic testing. For the evaluation of sensory changes of local foods, IS and DFS were added to meals prepared by local women using traditional recipes. Two equal amounts of each of the common staples (rice, yam, cassava, and plantain) were prepared in 2 pots and the same quantity of IS or DFS added. Four common sauces (tomato, eggplant, okra, and palm nut) were prepared without salt, divided into 2 equal parts, and equal amounts of IS or DFS added. The color, odor, and flavor of these foods were compared by an untrained panel of 18–21 (per session) local adults (mean age 38 y; 20% female) using triangle tests (28). In each session, 1 staple and 1 sauce were evaluated.

Efficacy study. The subjects were 5- to 15-y-old children from 4 primary schools. Informed written consent was obtained from the chief medical officer and informed oral consent from the school directors and the parents of the children. The Swiss Federal Institute of Technology in Zurich, Switzerland, and the Ministry of Health of Côte d’Ivoire gave ethical approval for the study. Oral assent was obtained from participating children. All children at the 4 schools (n = 605) were screened. Height and weight were measured, a spot urine sample was collected for measurement of urinary iodine, and blood was collected by venipuncture for the determination of hemoglobin (Hb) and serum ferritin (SF), transferrin receptor (TfR), and C-reactive protein (CRP) concentrations. Children with iron deficiency with or without anemia were invited to join the intervention trial. Anemia was defined as Hb <120 g/L in children aged ≥12 y, and Hb <115 g/L in children aged 5–12 y (29). Iron deficiency was indicated by serum TfR >8.5 mg/L (30), or SF <30 μg/L (31). Two children with Hb <80 g/L were excluded and treated with oral Fe. The remaining children were divided into 2 groups: all children from 2 schools at one end of the village received the IS (n = 63); the children from 2 schools at the other end of the village received the DFS (n = 60). Both the investigators and schools were unaware of the group assignment. Each participating child was given a monthly 2.5-kg salt portion (based on a mean per capita salt intake of ~4 g/d and an mean household size of 12 persons) distributed at school to be used in their household. In a village meeting at the beginning of the study and at each of the monthly salt distribution, it was emphasized that the distributed salt should be used for all cooking and food preparation, as well as at the table. At the baseline screening and again at 4 mo, all children received an oral dose of 400 mg albendazole (BELTAPHARM SPA and SmithKline Beecham Pharmaceuticals). At 6 mo, all baseline measures were repeated. To determine the prevalence of parasitic infections and micronutrient deficiencies that may influence response to the iron fortification, blood, spot urine, and stool samples were taken from sub-samples of randomly selected children from all of the screened children of the 4 schools. In a first subsample, parasitic infections (malaria (n = 144), soil transmitted helminths (n = 107)) were measured 12 mo after the intervention, and in a second separate random subsamples (n = 152 and 182), vitamin A (measured only in children with normal CRP) and riboflavin status were determined at the 6-mo measures.

Acceptability testing and compliance. To judge DFS and IS acceptability, household interviews were conducted after 1 and 6 mo of salt use. The head of the household answered questions on patterns of salt use, acceptability, and health benefits. Households were selected randomly including 68 IS and 75 DFS households corresponding to a total of 35% of participating households. To estimate compliance, salt remaining in the household at the end of the month was weighed and the amount of salt consumed per day during the period since the last distribution calculated. This amount was divided by the number of people in the household and compared with the mean per capita salt intake from the 3-d weighed food records.

Laboratory analysis. Salt samples were stored at −25°C until analysis. Color was determined by colorimetry (Chroma-Meter CR-310, Minolta AG) as described in an earlier publication (8) using the Hunter Scale. The color of the DFS and IS at each time point was
Men, Children, and Population compared with IS at baseline and color lightness (L-value) and color difference (ΔE) were calculated. Salt iodine concentration was measured with a validated modified Sandell-Kolthoff method (8,32,33). The fortification level of Fe in the salt was verified by the standard addition method using flame atomic absorption spectroscopy (SpectraAA-400, Varian) according to (8).

Aliquots of serum and urine samples were frozen at −25°C until analysis. Hemoglobin was measured using an AcT8 Counter (Beckman Coulter). SF was measured using an automated chemiluminescent immunoassay system (IMMULITE, Diagnostic Products Corporation). Serum TfR was measured using an ELISA (RAMCO). Serum CRP was measured using nephelometry (TURBOX, Orion Diagnostics). CRP concentrations <10 mg/L were defined as normal (34). Body iron stores were calculated from the Tf:SF ratio (35). Only children with normal CRP concentrations at both time points, baseline, and 6 mo, were included in the calculation of body iron stores. Serum retinol (SR) was measured by HPLC (Merck-Hitachi) according to Tanumihardjo et al. (36) in children with normal CRP values; vitamin A deficiency was defined as a SR <0.70 μmol/L (37). Riboflavin was measured by the erythrocyte glutathione reductase activation coefficient assay using a modification of the method of Dror et al. (38). Riboflavin deficiency was determined as activation coefficients >1.2 (39). Urinary iodine was measured using the Sandell-Kolthoff reaction as modified by Pino et al. (32). Whole blood was used to prepare a thick smear and a thin smear for malaria parasites by the Giemsa coloration technique from which parasites/μL of blood were determined (40). The presence of blood in urine, an indication for schistosomas, was analyzed by Dip Sticks (Roche Diagnostics). Parasites/parasites/μL of blood were determined (40). The presence of blood in urine, an indication for schistosomas, was analyzed by Dip Sticks (Roche Diagnostics). Schistosoma haematobium infection was measured by counting eggs under the microscope in filtered spot urine samples (41). From the stool samples, a thick smear was prepared using the Kato-Katz method (42). The slides were examined using light microscopy for the presence of eggs of soil-transmitted helminths (Ascaris lumbricoides, hookworm, and Trichuris trichiura).

Statistical analysis. Data processing and statistical analysis were done using SPSS 12.0 and Excel (XP 2002; Microsoft). Normally distributed data were expressed as means ± SD and were compared by Student’s t test. Values in the text are means ± SD unless stated otherwise. Parameters not normally distributed were expressed as medians and ranges, and were compared by Mann-Whitney and Wilcoxon tests or log transformed and compared by t test. A 2-factor ANOVA was done to compare effects of time and group and time-by-group interaction for Hb, indices of Fe status, salt iodine, and salt color (lightness).

RESULTS

Salt and micronutrient intake. The 3-d weighed food records were collected in 24 families including 207 subjects (median age: 16 y, range: 2–81 y). Daily intakes of salt, iron, vitamins C and A, riboflavin, phytic acid, and animal tissue (MFP: meat, fish, and poultry) were compared with the Estimated Average Requirement (EAR) (Table 1). Per capita salt intake was lower than previously reported for rural Côte d’Ivoire (13): in 2- to 5-y-old children, 6- to 15-y-old children, and women, important target groups of iron and iodine fortification, median daily salt intakes were 1.3, 2.8, and 3.8 g, respectively. Median vitamin A intake was at the EAR for 2- to 5-y-old children and above the EAR for 6- to 15-y-old children, women, and men, due to high consumption of red palm oil and refined palm oil fortified with retinyl palmitate. Vitamin C intake was low (34–61% of the EAR) because consumption of fresh fruit was rare, and vegetables are consumed in sauces that are simmered for several hours, resulting in extensive losses of vitamin C. Riboflavin intake was low (47–59% of the EAR) due to infrequent consumption of animal foods with the exception of fish, and the negligible riboflavin content of cassava, the dietary staple.

Iron intake and bioavailability. Median iron intake was well above the EAR for all age groups (Table 1); 94% of MFP intake was from fish, and 10% of dietary iron was heme iron, assuming fish to contain 25% heme Fe (25). Phytic acid intakes were relatively low, in the range of 107–230 mg/d, and came mainly from cassava, plantain, and sauces with peanuts and okra. The molar ratio of phytic acid to iron in the diet was 1.4–1.7. Mean polyphenol intake was low in all age groups (55–157 mg/d). Total iron bioavailability in school age children assuming a SF concentration of 30 (67,41) and 20 μmol/L (44.94 pmol/L) was 8.0 ± 2.2 and 11.4 ± 3.2% for the Tseng model, and 11.9 ± 2.4 and 17.3 ± 3.4% for the Reddy model.

TABLE 1

<table>
<thead>
<tr>
<th>Population group</th>
<th>Salt</th>
<th>Iron</th>
<th>RAE</th>
<th>Riboflavin</th>
<th>Ascorbic acid</th>
<th>Phytic acid</th>
<th>MFP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/d</td>
<td>% &lt; EAR²</td>
<td>µg/d</td>
<td>% &lt; EAR³</td>
<td>mg/d</td>
<td>% &lt; EAR⁴</td>
<td>mg/d</td>
</tr>
<tr>
<td>Children, 2–5 y</td>
<td></td>
<td>1.3 (0–7.9)</td>
<td>5.5 (1.8–12.7)</td>
<td>25</td>
<td>247 (16–1733)</td>
<td>50</td>
<td>0.24 (0.09–0.53)</td>
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<tr>
<td>(n = 32)</td>
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<tr>
<td>Children, 6–15 y</td>
<td></td>
<td>2.8 (0–10.9)</td>
<td>9.8 (3.6–19.8)</td>
<td>21</td>
<td>598 (68–2650)</td>
<td>37</td>
<td>0.42 (0.17–1.16)</td>
</tr>
<tr>
<td>(n = 71)</td>
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<tr>
<td>Women, 6–15 y</td>
<td></td>
<td>3.8 (0–17.7)</td>
<td>13.4 (4.3–26.6)</td>
<td>14</td>
<td>822 (122–4363)</td>
<td>34</td>
<td>0.53 (0.20–1.13)</td>
</tr>
<tr>
<td>(n = 59)</td>
<td></td>
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<tr>
<td>Men, 6–15 y</td>
<td></td>
<td>3.8 (0–11.2)</td>
<td>11.7 (2.0–23.2)</td>
<td>7</td>
<td>760 (17–5582)</td>
<td>40</td>
<td>0.54 (0.10–1.19)</td>
</tr>
<tr>
<td>(n = 45)</td>
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</tbody>
</table>

1 Values are medians (range), n = 207 total.
2 % of the studied population with an intake below the EAR. EAR for iron (18) is 3.6 mg for children (2–5 y), 6.3 mg for children (6–15 y), 8.1 mg for women, and 6.0 mg for men.
3 EAR for vitamin A (RAE) (18) is 21.0 μg for children (2–5 y), 30.0 μg for children (6–15 y), 50.0 μg for women, and 625.0 μg for men.
4 EAR for riboflavin (57) is 0.5 mg for children (2–5 y), 0.9 mg for children (6–15 y), 0.9 mg for women, and 1.1 mg for men.
5 EAR for vitamin C (58) is 18 mg for children (2–5 y), 49 mg for children (6–15 y), 60 mg for women, and 75 mg for men.
respectively (20,21). In school age children with a SF concentration of 20 µg/L (44.94 pmol/L), mean total daily iron absorption was estimated to be 1.16 and 1.76 mg for the Tseng and Reddy models, respectively, indicating adequate iron intake and bioavailability and that low Fe intake is not a major reason for iron deficiency.

**Color and iodine stability.** There was no difference in color lightness between the two salts. The color difference ∆E of ~11 at 6 mo was due to the slight difference in color between the DFS (light beige) and the IS (white). Iodine loss at 6 mo was ~50% for the IS and ~70% for the DFS when stored in low-density polyethylene bags and close to 100% for both salts when stored in high-density polyethylene bags. The iron concentrations of the DFS at mixing and after 6 mo of storage was 3.28 ± 0.17 and 2.99 ± 0.24 mg Fe/g salt, respectively.

**Organoleptic testing.** In the triangle testing comparing DFS and IS, there was no detectable difference in color, odor, or taste in either the traditional staples (rice, cassava, yam, plantain) or the sauces (tomato, eggplant, okra, palm nut).

**Efficacy trial.** The iodine concentrations of the monthly salt aliquots (14/mo and type of salt) taken during mixing of the salt were 71.5 ± 18.6 and 53.8 ± 18.2 µg/g salt in the IS and the DFS, respectively. The iron concentration in the DFS at the monthly mixings (3–7 aliquots/mo) was 3.21 ± 0.21 mg/g salt.

The results of the screening showed that although nearly half of the children screened were anemic, the prevalence of IDA was only 12%, with another 11% of children iron deficient without anemia (Table 2). The median urinary iodine concentration was 358 µg/L (2.8 µmol/L) with a range from 19 to 1027 µg/L (0.2–8.1 µmol/L), indicating excessive iodine intake (43). The serum retinol concentration was 1.22 ± 0.38 µmol/L (measured only in children with normal CRP), and 7% of children had an SR <0.7 µmol/L, indicating very little vitamin A deficiency. The erythrocyte glutathione reductase activation coefficient (EGRAC) value was 1.26 ± 0.12, and 66% of children had a value >1.2, indicating extensive riboflavin deficiency. Overall malaria prevalence was 55%: 50 and 5% of the children were infected with *Plasmodium falciparum* and *P. malariae*, respectively. However, the parasite load was low (<10,000/µL blood) in 98% of the infected children. The prevalence of soil-transmitted helminths was only 14%, probably due to deworming 8 mo before measurement. Only 11% of the screened children had a CRP >10 mg/L.

A total number of 123 iron-deficient children agreed to take part in the study: 32 children with low SF, of these 17 also had a low Hb; 84 children with high TfR, of these 41 also had a low Hb; 7 children with both low SF and high TfR, of these 6 also had a low Hb. These children were administered either IS or DFS. At baseline, the mean age was 9.4 ± 0.16 and 1.34 ± 0.14 m, and weight was 28.5 ± 9.5 and 29.2 ± 8.9 kg in the IS and DFS groups, respectively, with no differences between groups. The groups did not differ in SF, serum TfR, or body iron concentration at baseline, but Hb was higher in the DFS group than in the IS group (P < 0.05) due to the randomization at school level. The Hb concentration did not change in either group over the 6-mo intervention (Table 3). Both SF (calculated for children with normal CRP at both time points) and serum TfR concentrations were greater than at baseline in the DFS group at 6 mo (P < 0.05); these concentrations did not change in the IS group. There was a significant increase in total body iron in the DFS group (P < 0.001), with no change in the IS group (Figure 1). Body iron concentration (calculated for children with normal CRP at both time points) increased in the DFS group from 4.6 ± 2.7 to 5.9 ± 2.7 mg/kg (P < 0.001), whereas it did not change in the IS group (5.5 ± 2.9 vs. 5.6 ± 3.1). At 6 mo, serum TfR differed between the groups (P < 0.05), but SF and body iron did not. The serum CRP concentration and the prevalence of elevated CRP (11–15%) did not differ between the groups throughout the study (data not shown). The prevalence of anemia did not change significantly, with incidences of 62 and 62% in the IS group and 42 and 47% in the DFS group at baseline and 6 mo, respectively. In the DFS group, the prevalence of iron deficiency anemia and iron deficiency without anemia decreased from 42 to 23% (P < 0.01) and from 58 to 28% (P < 0.001), respectively. In the IS group, the prevalence of iron deficiency anemia decreased from 62 to 38% (P < 0.02), but the decrease from 38 to 25% in the prevalence of iron deficiency without anemia was not significant (P = 0.08).

**Household acceptability and compliance.** Most of the 143 interviewed households reported that the 2.5-kg monthly distribution was adequate to cover all household needs. Salt was used to prepare sauces and the main staples in 92–100% of the households. All surveyed households rated both IS and DFS as acceptable. Although 52 and 36% of DFS households stated that addition of the DFS darkened food during the first and the

### Table 2

Results of the screening of all children in the 4 primary schools in Orbaff

<table>
<thead>
<tr>
<th>Age, 1y</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>Hemoglobin, g/L</th>
<th>Serum ferritin, 2 µg/L</th>
<th>Serum transferrin receptor, mg/L</th>
<th>Prevalence of anemia, n (%)</th>
<th>Prevalence of iron deficiency anemia, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2 ± 2.7</td>
<td>1.32 ± 0.15</td>
<td>27.8 ± 9.3</td>
<td>116 ± 12</td>
<td>66 (3–472)</td>
<td>6.5 (2.3–29.7)</td>
<td>303 (50)</td>
<td>72 (12)</td>
</tr>
<tr>
<td>6-11 y</td>
<td>&lt; Hb &lt; cut-off, 4 n</td>
<td>IDA due to TfR &gt;8.5 mg/L and Hb &lt; cut-off, n</td>
<td>Prevalence of iron deficiency (ID) without anemia, n (%)</td>
<td>ID due to SF &lt;30 µg/L and TIR &gt;8.5 mg/L, n</td>
<td>ID due to SF &lt;30 µg/L and TIR &gt;8.5 mg/L, n</td>
<td>Urinary iodine, 5 µg/L</td>
<td>Serum transferrin, 6 µmol/L</td>
</tr>
<tr>
<td>18</td>
<td>46</td>
<td>8</td>
<td>64 (11)</td>
<td>16</td>
<td>47</td>
<td>1</td>
<td>10 (7)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 605.
2 Values are medians (range).
3 Conventional conversion factor: 1 µg/L = 2.247 pmol/L.
4 Cut-off: Hb <120 g/L in children aged ≥12 y; Hb <115 g/L in children aged 5–12 y.
5 Conventional conversion factor: 100 µg/L = 788 mmol/L.
6 Measured in a subsample of 152 children with CRP <10 mg/L.
7 Measured in a subsample of 182 children.
8 Measured in a subsample of 142 children.
9 Measured in a subsample of 144 children.
10 Measured in a subsample of 107 children, 8 mo after second deworming with 400 mg albendazole.
TABLE 3

Hemoglobin, serum ferritin, and transferrin receptor concentration, and prevalence of iron deficiency with or without anemia of children in the IS and DFS groups at baseline and 6 mo†

<table>
<thead>
<tr>
<th>Time</th>
<th>Hemoglobin (g/L)</th>
<th>Serum ferritin (μg/L)</th>
<th>Transferrin receptor (mg/L)</th>
<th>Iron deficiency with or without anemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
<td>DFS</td>
<td>IS</td>
<td>DFS</td>
</tr>
<tr>
<td>mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>112 ± 13</td>
<td>117 ± 13d</td>
<td>58 (10–472)</td>
<td>48 (9–397)</td>
</tr>
<tr>
<td>6</td>
<td>113 ± 11</td>
<td>117 ± 12</td>
<td>61 (11–500)</td>
<td>63 (6–200)c</td>
</tr>
</tbody>
</table>

† Values are means ± SD or medians (range), n = 63 in IS group, n = 60 in DFS group. There was a significant treatment × time interaction for all variables, P < 0.001 (ANOVA). dDifferent from baseline: P < 0.01, cP < 0.05; different from the IS group: bP < 0.05.

For serum ferritin, only children with normal CRP values at both time points were included (IS, n = 49; DFS, n = 46).

second survey, respectively, no perceptible darkening of sauces or tubers was detected in the systematic triangle testing of the DFS. To estimate compliance, salt remaining in the household at the end of the month was weighed, and the amount of salt used was compared to the expected per capita salt consumption from the weighed food records. Per capita salt intake from the weighed food records was 3.7 ± 3.1 g/d. Calculated per capita salt consumption in the households using DFS was 4.0 ± 2.5 and 6.1 ± 4.0 g/d at mo 1 and 6, respectively, suggesting that compliance with the DFS was high.

DISCUSSION

Dual fortification of salt with iron and iodine is technically challenging. Its success depends on the chemical form of the iron and iodine compound, on salt quality, on storage conditions and packaging material (8,44). There have been several previous attempts to use ferrous iron in a DFS (8,9,45). However, unwanted color change is a common problem even if encapsulated iron compounds are used.

FePP is a white colored, insoluble, organoleptically inert iron compound. It can be fortified into even low-grade salt without causing color changes (8). Moreover, ferric iron is more stable than ferrous iron when used in a DFS together with iodine in the form of KIO3, the form of iodine recommended for salt fortification in developing countries (43). However, the lower relative bioavailability of FePP compared with ferrous sulfate has presumably limited its use in food fortification (10,46). However, Zimmermann et al. (11) recently showed that increasing the fortification level of an iron compound can compensate for its lower bioavailability. Regular FePP (MPS ≥ 10 μm) was reported to be 20–70% the RBV of ferrous sulfate (10,46). Reducing the particle size to 0.5 μm was reported to increase RBV of FePP in milk products (47). However, we have no evidence that the micronized ground FePP (MPS of ~2.5 μm) has a higher bioavailability than the regular FePP. In the present study, based on the change in body iron after 6 mo of DFS, considering the mean salt intake in school children of 3.4 g/d and the fortification level of 3 mg Fe/g salt, ~3–3.5% of the fortification iron was absorbed during the study period.

An advantage to the approach to salt fortification used in this study was the achievement of a homogenous mixture of the iron into the salt with 1 pass through the local ribbon screw mixer installed by UNICEF and in place for salt iodization in many countries. Consequently, the only additional costs would be the iron compound and installation of a dry-mixing device to dose the iron into the upper basket of the screw mixer.

This study was similar to a previous study conducted in Morocco in which a DFS-containing micronized FePP decreased the prevalence of IDA in children from 31 to 3% after 10 mo (11). There are several possible explanations for the blunted response seen in Côte d’Ivoire. The present study length was 6 mo compared with 10 mo in Morocco, and the daily iron dose given here was ~10 mg/d, compared with ~20 mg/d in Morocco. Another difference between the 2 study sites was the food vehicle. Although in Morocco, salt was baked mainly into bread, it was primarily added to sauces in Côte d’Ivoire; thus, some FePP may have been lost in the cooking pot because it is insoluble. Along with iron deficiency, anemia in children in developing countries may be due to concurrent micronutrient deficiencies, malaria, hookworm, and hemoglobinopathies (2,48). Malaria is endemic in Côte d’Ivoire (49,50), whereas there is no malaria in northern Morocco. Although the malaria prevalence was determined in the dry season, during which malaria is less common, 55% of children had low-level parasitemia. Studies of DFS efficacy in India also reported no effect on Hb, possibly due to a high prevalence of malaria in the study population (51). Because of parasites in Côte d’Ivoire, we treated with albendazole at baseline and 4 mo. This could explain the impressive decrease in IDA in the control IS group. Hookworm and amoebae infection are common in rural Côte d’Ivoire; with studies reporting infection rates of 35–45% and 42% of hookworm and Entamoeba histolytica/E. dispar (4,49). Blood loss due to hookworm infection can contribute to iron deficiency and anemia (52) and several studies have shown a strong association between intensity of infection and anemia (53,54).

Another comorbidity that may have blunted the response to iron fortification was the poor riboflavin status in Ivorian children. Riboflavin deficiency may produce alterations in iron
metabolism and Hb synthesis (2), and studies have shown the positive effect of riboflavin on iron utilization (55,56). The high prevalence of riboflavin deficiency in children in Côte d’Ivoire may have contributed to the lack of a Hb response despite an increase in body iron.

A similar pattern of response was reported in previous Fe repletion trials in tropical countries. In preschool children in Zanzibar, iron supplementation had no effect on Hb concentration or mild or moderate anemia but improved SF and erythrocyte protoporphyrin, likely due to endemic infections and concurrent nutrient deficiencies (3). Our findings indicate that a DFS-containing micronized ground FePP can increase body iron stores in children in Côte d’Ivoire, but does not increase hemoglobin. These data suggest that iron fortification programs may not be successful in reducing anemia in tropical West Africa without concurrent control of endemic parasitoses.

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