

# Dual fortification of salt with iodine and microencapsulated iron: a randomized, double-blind, controlled trial in Moroccan schoolchildren<sup>1-3</sup>

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## ABSTRACT

**Background:** In many developing countries, children are at high risk of both goiter and iron deficiency anemia.

**Objective:** In a series of studies in northern Morocco, we developed and tested a dual-fortified salt (DFS) containing iodine and microencapsulated iron.

**Design:** To establish the DFS fortification concentration, we measured salt intake by 3-d weighed food records and estimated iron bioavailability from the local diet by using published algorithms. We then formulated a DFS containing 25 µg iodine/g salt (as potassium iodide) and 1 mg iron/g salt (as ferrous sulfate hydrate encapsulated with partially hydrogenated vegetable oil). After storage and acceptability trials, we compared the efficacy of the DFS to that of iodized salt in a 9-mo, randomized, double-blind trial in iodine-deficient, 6–15-y-old children ( $n = 377$ ).

**Results:** Mean salt intake in school-age children was 7–12 g/d, and estimated iron bioavailability from the local diet was 0.4–4.3%. After storage for 20 wk, the DFS and iodized salt were not significantly different in iodine content, and color stability was acceptable when the compounds were added to local meals. During the efficacy trial, urinary iodine concentrations and thyroid volumes improved significantly ( $P < 0.001$  and  $< 0.05$ , respectively) from baseline in both groups. At 40 wk, mean hemoglobin concentrations in the DFS group had increased by 14 g/L ( $P < 0.01$ ), and serum ferritin, transferrin receptor, and zinc protoporphyrin concentrations were significantly better ( $P < 0.05$ ) in the DFS group than in the iodized salt group. The prevalence of iron deficiency anemia in the DFS group decreased from 35% at baseline to 8% at 40 wk ( $P < 0.001$ ).

**Conclusion:** A DFS containing iodine and encapsulated iron can be an effective fortification strategy. *Am J Clin Nutr* 2003;77:425–32.

**KEY WORDS** Iodine, iron, deficiency, dual, fortification, salt, anemia, goiter, hypothyroidism, children, Morocco

## INTRODUCTION

Iron deficiency anemia (IDA) and the iodine deficiency disorders (IDD) affect more than one-third of the world's population (1, 2). These deficiencies often coexist: in regions of West and North Africa, 20–30% of schoolchildren have both goiter and IDA (3, 4). IDA impairs the thyroid's metabolism of iodine and may reduce the efficacy of iodine prophylaxis in areas where goiter is

endemic (3, 5, 6). Although universal salt iodization in many countries has proven highly effective against IDD (2), there is no comparable, proven method for controlling IDA in populations on a national scale (7). In regions with a high prevalence of IDD and IDA, dual fortification of salt with iodine and iron could be an effective fortification strategy.

Ensuring iodine stability and iron bioavailability in dual-fortified salt (DFS) is difficult (8–11). Water-soluble iron compounds, which are the most bioavailable, react with moisture and impurities in salt and cause unacceptable changes in color (11). Moreover, in the presence of ferrous ions and oxygen, the iodine in DFS is unstable because of the catalytic oxidation of iodate or iodide to  $I_2$ . Insoluble iron compounds, such as elemental iron powders or iron phosphate compounds, cause fewer sensory changes but may be so poorly absorbed as to be of little nutritional value (7). The ideal iron compound for a DFS would be one with high bioavailability that causes no sensory changes or losses of iodine when the iron compound is added to salt. Ferrous sulfate has a high relative bioavailability (100%) and has been successfully used to fortify infant formula, bread, and pasta (12). In animals, bioavailability of ferrous sulfate encapsulated with partially hydrogenated vegetable oil is comparable to that of nonencapsulated ferrous sulfate (13). Encapsulated iron is used in infant formulas and infant cereals, and it has excellent potential for overcoming unwanted sensory changes and iodine losses in salt, while maintaining high bioavailability (7).

In rural areas of northern Morocco, the prevalence of goiter among schoolchildren is 53–64%, and that of IDA is 25–35% (4, 14, 15). In this region, salt is widely consumed at a level of 5–15 g/d, and DFS could be an effective vehicle for fortification against both iodine and iron deficiencies. Therefore, we

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developed a new DFS, with iodine added as potassium iodide and iron added as ferrous sulfate encapsulated with hydrogenated vegetable oil. We tested the stability of the DFS under local conditions and evaluated its organoleptic effects when added to traditional northern Moroccan meals. To set the fortification concentration of the DFS, we measured salt and iron intake and estimated iron bioavailability from the local diet. Finally, we compared the efficacy of the DFS to iodized salt (IS) in a randomized, double-blind trial in iodine-deficient Moroccan schoolchildren with a high prevalence of anemia.

## SUBJECTS AND METHODS

The study was done in villages in the Brikcha Rural Commune, an area in the Rif Mountains of northern Morocco where goiter is endemic (14). The region is 400–800 m above sea level, and the climate is temperate, with an 8-mo dry season (22–34 °C, mean rainfall 23 cm/mo) and a 4-mo damp season (10–22 °C, mean rainfall 77 cm/mo). The population is of mixed Berber and Arab descent. This region is isolated from commercial routes, and most foods consumed are produced locally (15). More than 95% of the population is rural, and they work on small farms.

### Measurement of salt intake and iron bioavailability

To establish the optimal fortification concentration for the DFS, we needed to know the daily salt intake and iron bioavailability from the local diet. Three-day weighed food records were kept in 50 households randomly selected from local census rolls. Households were asked to continue in their usual food choices and habits and their traditional ways of cooking and serving foods. To account for seasonal variations, 24 households were studied in the damp season and 26 in the dry season. Over 3 consecutive days, edible portions of all food and beverages consumed were weighed on a Kern 440–53 scale (Kern & Sohn GmbH, Albstadt, Germany) that is accurate to  $\pm 1$  g. In this region, meals consist of 1 or 2 communal dishes placed in the center of the table from which all family members eat with their hands. We therefore estimated individual food consumption by using the unit of consumption (UC) formula of the Department of Agriculture of Morocco (15): for each male  $\geq 14$  y old, UC = 1.0; for each female  $\geq 10$  y old, UC = 0.8; and for each male  $< 14$  y old and each female  $< 10$  y old, UC =  $0.3 + [0.05 \times \text{age (y)}]$ . Nutrient intakes were calculated by using the Moroccan food composition table (16) and the Food and Agriculture Organization food composition table for Africa (17). In addition, local legumes, cereals, and vegetables were directly analyzed for iron and phytic acid content. The algorithms of Tseng et al (18) and Reddy et al (19) were used to estimate iron bioavailability. To estimate iron absorption for a range of body iron, results of the algorithms were adjusted (20) for high, medium, and low body iron stores.

### Salt preparation

Nearly all of the salt used in this region is supplied by a local cooperative; the salt is produced in drying ponds of water from a salty spring. Morocco legislated mandatory salt iodization in 1997, but because of financial constraints, this cooperative has not yet begun iodization. The native salt has an iodine content  $< 2$  ppm (4). The IS and DFS were prepared by using unground, unwashed salt from the local cooperative. The moisture content of the stored

salt is  $< 1\%$  during the dry season but  $\approx 3\%$  during the damp season. To prepare the IS and DFS, iodine was added as reagent-grade potassium iodide (Sigma & Aldrich, Buchs, Switzerland) at a concentration of 25  $\mu\text{g I/g}$  salt. The DFS was fortified at a concentration of 1 mg Fe/g salt with microencapsulated ferrous sulfate. To prepare this compound, ferrous sulfate hydrate was encapsulated with partially hydrogenated vegetable oil by the use of fluidized bed coating (Cap-Shure FS-165E-50; Balchem, Slate Hill, NY); the final product contains 50% ferrous sulfate. First, concentrated mixes were made by adding 650 mg KI alone or 650 mg KI and 120 g encapsulated ferrous sulfate to 2-kg batches of salt by using a small electric rotating-drum mixer (MINI 80; Engelsmann, Ludwigshafen, Germany) for mixing at 26 rpm for 10 min. The 2-kg mixes were then added to 18-kg batches of salt in a large electric rotating-drum mixer (ELTE 650; Engelsmann) at 30 rpm for 10 min.

### Stability testing

The IS and DFS were stored as 2-kg batches in closed low-density transparent polyethylene sacks under local ambient conditions during both the dry and damp seasons. After storage for 1, 4, 8, 12, and 20 wk during both seasons, 50-g aliquots of salt ( $n = 6$  at each time point) were taken for measurement of iodine concentration. Colorimetry was used to measure color stability; in addition, a panel of 12 local adults (aged 24–52 y) visually inspected unmarked samples that were placed side-by-side on white backgrounds.

### Acceptability testing

We measured local acceptability of the DFS and IS twice. First, during the summer season, in preparation for the efficacy study, 400 village households were randomly selected from census rolls. At each household, a local interviewer showed unmarked 100-g samples of IS and DFS in identical clear polyethylene bags placed side-by-side on white backgrounds and asked 2 forced-choice questions: 1) If you were at the market and could choose between these 2 salts, which would you prefer? and 2) If your first choice was not available at the market, would you purchase and use the other one? Second, to judge acceptability after 8 mo of household salt use in the efficacy study, during which the salts were mixed fresh and distributed monthly, an interview was done in  $\approx 50\%$  of the participating households ( $n = 212$ ). Fifty-two households were randomly selected from each of the 2 study groups. Both the interviewer and the head of household were blind to group assignment. The head of household answered 8 forced-choice questions on patterns of salt use, acceptability of color and taste, and overall satisfaction with the salt.

### Organoleptic testing

To evaluate potential color, odor, and taste changes when the IS and DFS were added to local foods, sensory testing was done in both the dry and damp seasons. IS and DFS, after storage in closed low-density transparent polyethylene sacks for 2–3 wk, were added to northern Moroccan meals prepared by local women in their kitchens according to traditional recipes. Each type of salt was added in identical amounts in parallel to separate portions of 4 common foods: bread (with locally ground wheat flour), bisarra (fava bean and olive oil purée), harirra (chickpea and wheat flour soup), and couscous (semolina). Their flavor, odor, and color were then compared by the local panel with the use of triangle tests (21). During the triangle test, 3 coded samples of each of the 4

foods were given in random order in a private setting. The panelists were asked to determine which sample of each food differed from the other 2 samples and to describe how the samples differed (21).

### Efficacy study

The subjects were 6–15-y-old children from 2 neighboring primary schools. Informed written consent was obtained from the chief medical officer and the school director, and informed oral consent was obtained from the parents of the children. The University Children's Hospital in Zürich and the Ministry of Health in Rabat gave ethical approval for the study. All children in the 2 schools were invited to participate in the 9-mo study; all ( $n = 377$ ) accepted and were enrolled. At baseline, weight and height were measured and a spot urine sample was collected for measurement of urinary iodine (UI). Thyroid gland volume ( $T_{vol}$ ) was measured with the use of a portable SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5-MHz linear transducer (22). Ultrasound examination was performed on subjects sitting upright with the neck extended. Five milliliters of whole blood was collected by venipuncture for determination of hemoglobin, serum ferritin (SF), whole-blood zinc protoporphyrin (ZnPP), and serum transferrin receptor (sTfR). Whole blood was spotted onto filter paper for measurement of serum thyroxine ( $T_4$ ).

Because each participating family shared a monthly salt portion (see below), children were randomly divided by household into 2 groups. One group ( $n = 188$ ) was given IS, ie, salt fortified with 25  $\mu\text{g}$  I/g salt, and the second group ( $n = 189$ ) was given DFS, ie, salt dual-fortified with 25  $\mu\text{g}$  I and 1 mg Fe/g salt. The IS and DFS were prepared as described above. For monitoring, 50-g aliquots ( $n = 6$ ) of the salts were taken and measured for iodine content at each monthly mixing and for iron content at 1, 3, and 9 mo. Both investigators and households were blind to group assignment. On the basis of a per capita salt intake of 7–12 g/d and local census data indicating an average of 7.5 members/household, each household was provided with 2 kg salt at the beginning of each month for 9 mo to supply all household needs. The salt was dispensed directly to the head of the household from a central supply at the local health center. At baseline, the study was carefully explained to the participating families, and it was emphasized that the new salt should be used for all cooking and food preparation, as well as at the table. This message was reinforced at each of the monthly salt distributions.

At 10, 20, and 40 wk, weight and height were measured in the children and spot urine samples were collected for the measurement of UI. Whole blood was spotted onto filter paper for the measurement of  $T_4$ .  $T_{vol}$  was measured with ultrasound. At 20 and 40 wk, whole blood was collected by venipuncture for measurement of hemoglobin, SF, ZnPP, and sTfR. After completion of the study, all children with IDA were treated with oral iron [60 mg Fe (as ferrous sulfate) for 4 d/wk for 12 wk].

### Laboratory analyses

Serum and urine samples were aliquoted and frozen at  $-20^\circ\text{C}$  until they were analyzed. UI was measured at the Human Nutrition Laboratory in Zürich by the use of a modification of the Sandell-Kolthoff reaction (23). Dried blood spots on filter paper were analyzed for serum  $T_4$  at the Children's Hospital in Zürich by the use of an immunoassay (24), with normal reference val-

ues of 65–165 nmol/L. Hb was measured with an AcT8 Counter (Beckman Coulter, Krefeld, Germany), and ZnPP was measured on washed red blood cells with a hematofluorometer (Aviv Biomedical, Lakewood, NJ), at a laboratory of the Chefchaouen Provincial Hospital in Morocco. SF and sTfR were measured at the University of Kansas Medical Center (Lawrence, KS) with the use of separate enzyme-linked immunosorbent assays (25, 26). Normal reference values are as follows: ZnPP,  $<40 \mu\text{mol/mol}$  heme; SF, 12–300  $\mu\text{g/L}$ ; and sTfR, 2.9–8.5 mg/L. Iron deficiency was defined as either SF  $<12 \mu\text{g/L}$  or sTfR  $>8.5 \text{ mg/L} + \text{ZnPP} >40 \mu\text{mol/mol}$  heme (27). Anemia was defined as Hb  $<120 \text{ g/L}$  in children aged  $\geq 12$  y and  $<115 \text{ g/L}$  in children aged 5–11 y (28). Salt color was evaluated by colorimetry (Chroma Meter CR-310; Minolta, Osaka, Japan) at the Human Nutrition Laboratory in Zürich. For DFS, measurement of iodine content by titration with thiosulfate (29) is complicated by interference from iron in the salt. We therefore used a modification of the Sandell-Kolthoff reaction (23) to measure the salt iodine content of 10-g salt aliquots dissolved in distilled water. Iron in salt and foods was measured at the Human Nutrition Laboratory in Zürich by using atomic absorption spectroscopy (Varian Techtron Pty Ltd, Mulgrave, Australia). Phytic acid in foods was measured by using a modification of the Makower method (30).  $T_{vol}$  was calculated by using the method of Brunn et al (22). The same physician (MZ) performed all ultrasound measurements during the study. To estimate intraobserver variability, duplicate  $T_{vol}$  measurements were done in 25 children at the 10- and 40-wk visits; the mean ( $\pm$ SD) intraobserver variability was  $3.7 \pm 2.0\%$ .

### Statistical analysis

Data processing and statistical analyses were done with the use of SPLUS 2000 (Insightful Corporation, Seattle), PRISM3 (GraphPad, San Diego), and EXCEL 97 (Microsoft, Seattle) software. Normally distributed data were compared by Student's  $t$  test. Variables not normally distributed were compared with the use of the Wilcoxon or Mann-Whitney tests. A two-factor repeated-measures analysis of variance was done to compare effects of time and group and time-by-group interaction for salt color (lightness), salt iodine, Hb, SF, sTfR, ZnPP, UI,  $T_4$ , and  $T_{vol}$ . 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 test. Logistic regression was done to compare effects of time and group and time-by-group interaction for the binary variables of IDA and iron deficiency without anemia. Significance was set at  $P < 0.05$ .

## RESULTS

### Salt intake and iron bioavailability

Fifty families comprising a total of 322 subjects (median age, 19 y; range, 2–74 y) kept the 3-d weighed food records. Mean ( $\pm$ SD) salt intake for adult males and females was  $12.1 \pm 2.9$  and  $9.7 \pm 2.3$  g/d, respectively. For children aged 6–15 y, mean salt intake was 7.3–11.6 g/d. Mean iron intake for adult males was  $15.4 \pm 2.7$  mg/d. For children aged 6–15 y, mean iron intake was 9.2–14.5 mg/d, 97% of which was nonheme iron. There were no significant seasonal differences in mean daily iron or salt intake. Among all family members, mean intakes of meat, fish, and poultry; phytic acid;

**TABLE 1**

Comparison of color and iodine concentration in iodized salt (IS) and dual-fortified salt (DFS) containing iodine and iron at mixing and after 4, 8, 12, and 20 wk of storage<sup>1</sup>

Length of storage (wk)	Color <sup>2</sup>		Iodine	
	IS	DFS	IS	DFS
	<i>µg/g salt</i>			
Dry season <sup>3</sup>				
0	86.4 ± 1.6	86.8 ± 2.6	25.1 ± 2.9	24.4 ± 3.1
4	85.7 ± 1.3	85.8 ± 1.3	22.7 ± 5.0	25.0 ± 2.9
8	84.3 ± 1.4	84.7 ± 2.4	24.1 ± 4.1	21.8 ± 5.3
12	85.8 ± 2.6	85.2 ± 2.0	21.2 ± 3.9	23.5 ± 2.6
20	84.3 ± 2.4	84.2 ± 1.5	22.0 ± 3.3	21.6 ± 4.2
Damp season <sup>3</sup>				
0	81.3 ± 2.4	81.0 ± 2.8	24.3 ± 3.3	25.2 ± 5.1
4	77.5 ± 2.5	76.7 ± 3.4	25.1 ± 2.7	21.9 ± 4.9
8	76.8 ± 5.4	65.6 ± 5.1 <sup>4,5</sup>	21.5 ± 4.1	23.7 ± 4.3
12	75.2 ± 3.2	65.4 ± 4.3 <sup>4,5</sup>	22.4 ± 3.2	21.8 ± 3.3
20	76.2 ± 4.3	62.0 ± 3.7 <sup>4,5</sup>	22.6 ± 2.6	21.9 ± 4.0

<sup>1</sup> $\bar{x} \pm$  SD. Both salts were fortified with potassium iodide at 25 µg I/g salt.

<sup>2</sup>Lightness scale: 1 = black, 100 = white. Significant time × fortification interaction (ANOVA),  $P < 0.001$ .

<sup>3</sup> $n = 6$  aliquots of salt at each time point.

<sup>4</sup>Significantly different from baseline in the damp season,  $P < 0.05$ .

<sup>5</sup>Significantly different from IS,  $P < 0.05$ .

and ascorbic acid [per energy intake of 4180 kJ (1000 kcal)] were 30 ± 18 g, 868 ± 90 mg, and 22 ± 11 mg, respectively. Because the diet was high in phytic acid and low in ascorbic acid, estimated nonheme iron bioavailability was only 0.4–1.3% and 1–4.3% for high and low body iron stores, respectively (21, 22).

### Stability

The color stability and iodine content of the IS and DFS at 0, 4, 8, 12, and 20 wk after mixing during both the dry and damp seasons are shown in **Table 1**. During the dry season, when the moisture content of the local salt is low (< 1%), colorimetry found no significant color difference between IS and DFS. On visual inspection, the salts were indistinguishable after 20 wk of storage. However, during the damp winter season, when the moisture content of the local salt is high (≈ 3%), the DFS developed a mild yellow color during storage, and there was a significant difference in the color of the salts at 8, 12, and 20 wk ( $P < 0.05$ ). There was no significant difference in the iodine content of the salts during the 20-wk period in either the dry or the damp season. In both seasons, the salts each lost ≈ 15% of their iodine content by 20 wk.

### Acceptability

During the baseline acceptability interviews, acceptance of the DFS was nearly unanimous (**Table 2**). Table 2 also shows the results of interviews done after 8 mo of household salt use. In response to the questions on salt use and acceptability, 14% of households in the DFS group rated the salt color unacceptable (ie, they would purchase another salt with better color if it were available) during the damp season, but 100% of the IS group households found the salt color during the same season acceptable. In the DFS group, 17% reported that the salt changed the color of foods: 15% reported a pale gray color of a cooked sauce containing milk and onion, and 2% reported the same in salads with onions.

**TABLE 2**

Acceptability of iodized salt (IS) and dual-fortified salt (DFS) containing iodine and iron at baseline and after 8 mo of salt use<sup>1</sup>

Questions	Salt	
	IS	DFS
	%	
Baseline ( $n = 400$ households)		
1. Which salt is preferable?	51	49
2. Is second-choice salt acceptable?	99	99
After 8 mo (IS group, $n = 52$ ; DFS group, $n = 52$ )		
1. What amount of salt used/mo in the household?		
≤ 1 kg	17	14
> 1 but < 2 kg	31	30
2 kg (amount distributed/mo to each study household)	44	45
> 2 kg	8	11
2. Was salt consumed daily by children?	100	98
3. Was the salt color acceptable in damp season?	100	86 <sup>2</sup>
4. Was the salt color acceptable in dry season?	100	100
5. Was salt used for all foods during cooking and at table?	98	98
6. Did salt change the color of foods?	4	17 <sup>3,4</sup>
7. Was the salt taste acceptable in all foods?	98	96
8. Was the salt acceptable overall?	98	94

<sup>1</sup>Percentages of positive answers are shown.

<sup>2,3</sup>Significantly different from IS: <sup>2</sup> $P < 0.02$ , <sup>3</sup> $P < 0.001$ .

<sup>4</sup>The affected foods were milk and onion.

### Organoleptic changes

In the triangle testing that compared IS to DFS, in both seasons there was no significant, detectable difference in color, odor, or taste between the salts in any of the 4 traditional foods. These included the 2 staples, bread and couscous, that are pale and have a mild taste.

### Efficacy trial

The treatment and control groups at baseline are compared in **Table 3**. Randomization at the household level was effective; there were no significant differences in baseline characteristics between the groups. Of the 377 children who began the study, 367 completed it; 9 children moved away (4 in IS group, 5 in DFS group) and 1 child in the IS group refused further venipuncture. In the monitoring of aliquots of salt taken at mixing at 1, 3, 6, and 9 mo ( $n = 24$ ), the mean (±SD) iodine concentrations in the IS and the DFS were 22.9 ± 3.0 and 23.4 ± 3.1 mg I/g salt, respectively. There was no significant difference in mean iodine concentration between the salts at any of the monthly mixings (data not shown). The mean iron concentration in the DFS measured at 0, 3, and 9 mo ( $n = 18$ ) was 0.9 ± 0.3 mg Fe/g salt.

As shown in **Table 4**, mean Hb at 40 wk was significantly greater in the DFS group than in the IS group ( $P < 0.02$ ). All indexes of iron status (SF, sTfR, and ZnPP) were significantly better ( $P < 0.05$ ) at 40 wk in the DFS group than in the IS group (Table 4). As shown in **Figure 1**, the prevalence of IDA and iron deficiency without anemia was sharply lower in the DFS group at 40 wk ( $P < 0.01$ ). Iron fortification had no measured effect on growth; there was no significant difference in weight, height, or body mass index (in kg/m<sup>2</sup>) between the 2 groups throughout the 40 wk (data not shown).

The changes in UI, T<sub>4</sub>, and T<sub>vol</sub> in the 2 groups are shown in **Table 5**. There were no significant differences in median UI between the 2 groups throughout the study. In both groups, median UI at 10, 20, and 40 wk was significantly increased from the

TABLE 3

Characteristics of the children in the iodized salt (IS) and dual-fortified salt (DFS) groups at baseline<sup>1</sup>

Characteristic	IS group (n = 85 F, 99 M)	DFS group (n = 89 F, 94 M)
Age (y)	10.0 ± 2.4 <sup>2</sup>	10.4 ± 2.5
BMI (kg/m <sup>2</sup> )	15.9 ± 1.7	16.2 ± 1.8
Hemoglobin (g/L)	111 ± 14	113 ± 13
Serum ferritin (μg/L)	23 ± 18	20 ± 16
Serum transferrin receptor (mg/L)	8.2 (4.4–82.6) <sup>3</sup>	8.7 (4.3–77.1)
Whole-blood zinc protoporphyrin (μmol/mol heme)	63 ± 47	63 ± 47
Prevalence of iron deficiency anemia [n (%)]	66 (36)	64 (35)
Prevalence of iron deficiency without anemia [n (%)]	26 (14)	30 (16)
Urinary iodine (μg/L)	18 (0–127)	16 (0–143)
Serum thyroxine (nmol/L)	82.1 ± 17.3	82.8 ± 19.8
Thyroid volume (mL)	8.9 ± 3.4	9.2 ± 3.7
Prevalence of goiter [n (%)]	133 (72)	128 (70)

<sup>1</sup>There were no significant differences between the 2 groups.<sup>2</sup> $\bar{x} \pm SD$ .<sup>3</sup>Median; range in parentheses.

baseline value ( $P < 0.001$ ). At 20 and 40 wk, median UI was well above the WHO/ICCIDD cutoff (100 μg/L) for risk for iodine deficiency (2). Mean serum T<sub>4</sub> in the DFS group at 20 and 40 wk was significantly higher than baseline in the DFS group ( $P < 0.02$ ) and significantly higher than the concentration in the IS group at 20 and 40 wk ( $P < 0.05$ ). Mean T<sub>vol</sub> decreased significantly from baseline in both groups ( $P < 0.05$ ). At 40 wk, mean T<sub>vol</sub> was significantly lower in the DFS group than in the IS group ( $P < 0.05$ ).

## DISCUSSION

Previous attempts to produce a stable and efficacious DFS produced mixed results. In India, Mannar et al (31) reported that the iodine content and color of a DFS containing potassium iodide and ferrous fumarate was stable for 8 wk in waterproof packing. The Indian National Institute of Nutrition (NIN) proposed a DFS containing ferrous sulfate, potassium iodide, and a stabilizer, sodium hexametaphosphate (8, 10). The mean absorption of iron from the NIN DFS consumed as part of a rice-based meal was 6.1%, and the addition of sodium hexametaphosphate increased iron absorption by ≈50% (32). The stability of iodine in the NIN DFS depended on salt quality; when magnesium chloride was present as an impurity, the salt lost significant iodine (33). In a field trial in India, the NIN DFS showed good efficacy against iodine deficiency, but there was no overall impact on Hb concentrations (10). In a second trial in Indian schoolchildren that

compared the NIN DFS to IS, the iodine again showed good efficacy. However, there was only a marginal benefit to Hb: Hb concentrations actually decreased significantly in the DFS and IS groups, but the decline in the DFS group was not as great (10). In a recent field trial of another DFS that contained iron and iodine, performed in Indian tea pickers, Hb concentrations and work output improved (34). However, the authors provided no details on the iodine and iron compounds in the DFS and no data on color stability or iodine efficacy. Mannar and Diosady (11) developed a DFS with potassium iodide coated with maltodextrin and iron as ferrous fumarate. Iron absorption from this DFS in iron-enhancing and iron-inhibiting meals was 13.5% and 4%, respectively (9). However, unpublished data from our laboratory indicate that nonencapsulated ferrous fumarate added to low-grade salt in West Africa causes unacceptable changes in color to dark brown after storage for 4 wk.

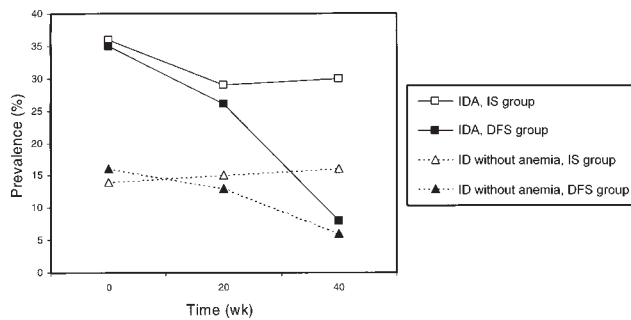
In the present study, we used microencapsulated ferrous sulfate to place a physical barrier around the iron to reduce color changes and potential losses of iodine from the salt. Stability was determined over a period of ≈5 mo in both dry and damp seasons to approximate the time required for the production, distribution, and consumption of salt in this region. Iodine stability was excellent: the iodine content of the DFS was comparable to that of the IS throughout 20 wk of storage in both the dry and the damp seasons. However, iron encapsulation did not entirely prevent color changes. The color of the DFS was indistinguishable from that of

TABLE 4

Hemoglobin, serum ferritin (SF), serum transferrin receptor (sTfR), and zinc protoporphyrin (ZnPP) concentrations in the iodized salt (IS; n = 184) and dual-fortified salt (DFS; n = 183) groups over 40 wk

	Hemoglobin <sup>1</sup>		SF <sup>1</sup>		sTfR <sup>1</sup>		ZnPP <sup>1</sup>	
	IS	DFS	IS	DFS	IS	DFS	IS	DFS
	g/L		μg/L		mg/L		μmol/mol heme	
Baseline	111 ± 14 <sup>2</sup>	113 ± 13	23 ± 18	20 ± 16	8.2 (4.4–82.6) <sup>3</sup>	8.7 (4.3–77.1)	63 ± 47	63 ± 47
20 wk	115 ± 14	116 ± 13	29 ± 20	36 ± 26 <sup>4</sup>	8.5 (4.4–78.5)	7.8 (4.5–23.9)	58 ± 43	44 ± 24 <sup>5,6</sup>
40 wk	116 ± 12	127 ± 12 <sup>5,6</sup>	17 ± 12	40 ± 25 <sup>4,7</sup>	8.9 (3.8–118.0)	6.5 (3.0–15.3) <sup>4,7</sup>	57 ± 43	35 ± 24 <sup>5,8</sup>

<sup>1</sup>Significant treatment × time interaction,  $P < 0.01$  (ANOVA).<sup>2</sup> $\bar{x} \pm SD$ .<sup>3</sup>Median; range in parentheses.<sup>4,5</sup>Significantly different from baseline: <sup>4</sup> $P < 0.05$ , <sup>5</sup> $P < 0.01$ .<sup>6–8</sup>Significantly different from IS: <sup>6</sup> $P < 0.02$ , <sup>7</sup> $P < 0.05$ , <sup>8</sup> $P < 0.01$ .



**FIGURE 1.** By logistic regression, the probability of iron deficiency anemia (IDA) and iron deficiency (ID) without anemia was significantly less in 6–15 y-old children receiving dual-fortified salt (DFS) containing iron and iodine ( $n = 183$ ) than in those receiving iodized salt (IS) ( $n = 184$ ). For both IDA and ID without anemia, the difference between the IS and DFS groups increased significantly with time ( $P < 0.01$ , comparing the time-and-group model with the time-only model).

the IS during the dry season, but it became less acceptable during the damp season. The difference between seasons was likely due to the variation in the moisture content of the stored salt ( $< 1\%$  in the dry season and  $\approx 3\%$  in the damp season). Although the partially hydrogenated vegetable oil capsule is water resistant, some loss of capsule likely occurs as a result of abrasion during mixing. When the salt moisture content is high, in the presence of water and oxygen, the ferrous iron is oxidized and precipitation of ferric hydroxide, which has a yellow-brown color, occurs.

The DFS was an effective vehicle for iodine and iron. At baseline, the children had severe IDD by all measured criteria: the median UI concentration was  $17 \mu\text{g/L}$ , the rate of goiter was 72%, and 20% had hypothyroidism. We wanted both salts to provide 150–250  $\mu\text{g}$  I/d (2). Because the salt was freshly mixed each month, we anticipated the loss of  $\approx 20\%$  of the iodine during storage and cooking (29). We therefore fortified the salts with 25  $\mu\text{g}$  I/g salt. Median UI rose rapidly in both groups to concentrations indicating iodine sufficiency. Thyroid volume and the prevalence of hypothyroidism were significantly reduced in both groups. Consistent with our previous findings (3, 6, 7), the addition of iron to IS improved the efficacy of the iodine. Compared with the IS group, the DFS group had significantly greater

reductions in thyroid size and improved thyroid hormone status. Details have been presented in a separate publication (35). To reduce the prevalence of IDA, our fortification goal was to provide  $\approx 0.5$  mg absorbed Fe/d to the children in the study. On the basis of local salt intakes of 7–12 g/d in children and an estimated iron bioavailability of 1–4%, we fortified the DFS at 1 mg Fe/g salt. This provided an additional 7–12 mg dietary Fe/d, which doubled the children's baseline iron intakes. The encapsulated ferrous sulfate had good bioavailability. In the DFS group, mean Hb increased by 14 g/L after 40 wk, and all indexes of iron status improved significantly. The prevalence of IDA was reduced from 35% to 8% (Figure 1), with the sharpest decline in IDA occurring between 20 and 40 wk. The delay in IDA response suggests that, in these children, an increase in iron intake of  $\approx 10$  mg/d for  $> 20$  wk was needed for a substantial effect on Hb. In previous trials, iron-fortified foods that have clearly improved the iron status in target populations include infant formula (36), infant cereal (37), sugar (38), fish sauce (39), and curry powder (40). It is noteworthy that, in these successful trials, the iron-fortified food was consumed with an enhancer of iron absorption (ascorbic acid or EDTA), which was added to overcome absorption inhibitors. In the present study, despite the high phytic acid content of the diet, iron fortification of salt without an enhancer significantly improved iron status. For example, our data (increases in mean Hb and serum ferritin of 14 g/L and 20  $\mu\text{g/L}$ , respectively, after 9 mo in the DFS group) compare favorably to the results of Ballot et al (40). In their study, the fortification of curry powder with sodium iron EDTA for 2 y in older children and adults increased mean Hb by 5–8 g/L and mean serum ferritin by 15  $\mu\text{g/L}$ .

Several factors may have contributed to iron efficacy in this study. Providing  $\approx 10$  mg ferrous sulfate/d to growing children with a very high prevalence of IDA may have favored higher iron bioavailability than that predicted by available algorithms. Most children ate 3 main meals, as well as midmorning and midafternoon snacks, all of which contained measurable amounts of salt. Thus, absorption was likely enhanced by the delivery of iron in repeated small doses throughout the day, as the fractional absorption of nonheme iron increases with decreases in dose (41). In addition, full liberation of iron encapsulated in partially hydrogenated vegetable oil requires digestion by lipase in the proximal duodenum. This brief delay in iron appearance may reduce the binding of iron to inhibitory substances (such as phytic acid) in

**TABLE 5**

Urinary iodine and serum thyroxine concentrations and thyroid volumes in the iodized salt (IS;  $n = 184$ ) and dual-fortified salt (DFS;  $n = 183$ ) groups over 40 wk

	Urinary iodine <sup>1</sup>		Thyroxine <sup>2</sup>		Thyroid volume <sup>2</sup>	
	IS	DFS	IS	DFS	IS	DFS
	$\mu\text{g/L}$		$\text{nmol/L}$		$\text{mL}$	
Baseline	18 (0–127) <sup>3</sup>	16 (0–143)	82.1 $\pm$ 17.3 <sup>4</sup>	82.8 $\pm$ 19.8	8.9 $\pm$ 3.4	9.2 $\pm$ 3.7
10 wk	79 (12–488)	87 (19–511)	91.4 $\pm$ 23.8	96.6 $\pm$ 24.3	8.7 $\pm$ 3.9	9.1 $\pm$ 2.8
20 wk	179 (22–432)	183 (31–529)	89.8 $\pm$ 19.9	103.5 $\pm$ 20.2 <sup>5,6</sup>	8.3 $\pm$ 2.7	7.5 $\pm$ 3.4 <sup>5</sup>
40 wk	182 (14–474)	189 (23–406)	85.3 $\pm$ 12.7	102.2 $\pm$ 17.4 <sup>5,6</sup>	7.3 $\pm$ 2.4 <sup>5</sup>	5.7 $\pm$ 2.1 <sup>6,7</sup>

<sup>1</sup>Significant main effect of time,  $P < 0.001$ .

<sup>2</sup>Significant treatment  $\times$  time interaction,  $P < 0.01$  (ANOVA).


<sup>3</sup>Median; range in parentheses.

<sup>4</sup> $\bar{x} \pm \text{SD}$ .

<sup>5,7</sup>Significantly different from baseline: <sup>5</sup> $P < 0.02$ , <sup>7</sup> $P < 0.001$ .

<sup>6</sup>Significantly different from IS,  $P < 0.05$ .

the stomach and upper duodenum and thereby potentially increase iron absorption. In Hb-repletion trials in rats, the bioavailability of ferrous sulfate was similar or better when it was encapsulated with hydrogenated soybean oil or ethyl cellulose (relative bioavailability: 114% and 133%, respectively, compared with 100% for nonencapsulated ferrous sulfate; 13). Another potential reason for the clear and significant impact on iron status is that, in this population, IDA is primarily due to low iron bioavailability and not to increased iron losses. Morocco is unlike many developing countries, in that there is no malaria in northern Morocco and the prevalence of hookworm and other intestinal parasites that cause blood loss is low (M Bousfiha, personal communication, 2001). Moreover, the low prevalence of infection and/or inflammation in this region may have increased our ability to rely on serum ferritin and other iron indexes to clearly define IDA and detect changes in iron status (27, 42).

The development of a stable and efficacious DFS containing iodine and iron could provide new opportunities for the global control of IDA and IDD. The cost-effectiveness of such an approach remains to be determined. Current cost estimates for encapsulated ferrous sulfate are 3–4 times the cost of nonencapsulated ferrous sulfate, but the price of the former could drop substantially if production were to expand to a larger scale. Our findings suggest that a DFS containing encapsulated ferrous sulfate provides an effective fortification strategy in the conditions of northern Morocco. Because the performance of DFS may vary according to climate, salt quality, and dietary habits, however, these studies will need to be repeated in other countries under local conditions. Further refinements in iron capsule design are likely to be needed to increase resistance to moisture and abrasion while maintaining bioavailability. 

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