Triple fortification of salt with microcapsules of iodine, iron, and vitamin A[1–3]

Michael B Zimmermann, Rita Wegmueller, Christophe Zeder, Nourredine Chaouki, Ralf Biebinger, Richard F Hurrell, and Erich Windhab

ABSTRACT

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

Objective: We aimed to develop a stable, efficacious salt fortified with iodine, iron, and vitamin A.

Design: A novel spray-cooling technique was used with hydrogenated palm oil to package potassium iodate, micronized ferric pyrophosphate, and retinyl palmitate into microcapsules (mean particle size: 100 μm). We used the microcapsules to create triple-fortified salt (TFS) with 30 μg I, 2 mg Fe, and 60 μg vitamin A/g salt. After storage trials, we compared the efficacy of TFS with that of iodized salt in a 10-mo, randomized, double-blind trial in goitrous schoolchildren ($n = 157$) who had a high prevalence of vitamin A deficiency and iron deficiency anemia.

Results: After storage for 6 mo, losses of iodine and vitamin A from the TFS were ≈12–15%, and color was stable. In the TFS group, mean hemoglobin increased by 15 g/L at 10 mo ($P < 0.01$), iron status indexes and body iron stores improved significantly ($P < 0.05$), and mean serum retinol, retinol-binding protein, and the ratio of retinol-binding protein to prealbumin increased significantly ($P < 0.01$). At 10 mo, prevalences of vitamin A deficiency and iron deficiency anemia were significantly lower in the TFS group than in the iodized salt group ($P < 0.001$).

Conclusion: Newly developed microcapsules containing iodine, iron, and vitamin A are highly stable when added to local African salt. TFS was efficacious in reducing the prevalence of iron, iodine, and vitamin A deficiencies in school-age children. *Am J Clin Nutr* 2004;80:1283–90.

KEY WORDS Iodine, iron, vitamin A, deficiency, triple fortification, salt, anemia, goiter, children, Morocco

INTRODUCTION

Iron deficiency anemia (IDA), vitamin A deficiency (VAD), and the iodine deficiency disorders (IDD) affect >30% of the world's population (1, 2). These deficiencies often coexist in children in developing countries (3, 4). IDA interferes with the thyroidal metabolism of iodine and may reduce the efficacy of iodine prophylaxis (3). VAD may impair iron metabolism and aggravate anemia (5). In Africa, universal salt iodization is effective against IDD (6), but the control of IDA and VAD on a national scale remains a challenge (7, 8). Finding suitable vehicles for fortification with iron and vitamin A in rural Africa is difficult. Salt may be such a vehicle, particularly in poor areas of subsistence farming, where salt is one of very few regularly purchased food items (4, 9). Triple fortification of salt could be highly effective because of the beneficial interactions of iron, iodine, and vitamin A in metabolism (5, 10, 11). The addition of all 3 micronutrients to a single foodstuff might cost less than separate fortification programs.

However, ensuring the bioavailability of iron and the stability of iodine and vitamin A in fortified salt is difficult. Water-soluble iron compounds—the compounds in which the iron is most bioavailable—react with moisture and impurities in salt, and that causes unacceptable changes in color (12, 13). In the presence of ferrous iron and moisture, losses of iodine and vitamin A from a fortified salt would be high (14, 15). Ferric pyrophosphate (FePP) is a poorly soluble iron compound that is white; its addition to salt produces negligible color change or iodine loss in low-grade salt (14). Although most commercial forms of FePP with mean particle sizes ≥ 10 μm have low relative bioavailability (7), a smaller particle size increases the absorption of FePP (16).

Encapsulation can reduce iron-mediated color change in fortified salt (4) and may increase the stability of iodine and vitamin A. Mannar and Diosady (13) reported that iodine stability in salt significantly increased when potassium iodide was coated with maltodextrin. Encapsulation of vitamin A increases its stability in salt (15) and may allow the use of a less expensive form of vitamin A than that typically used to fortify dry mixes (17). Encapsulation of iron in hydrogenated oils at substrate-to-capsule ratios of ≥ 50:50 is not likely to compromise bioavailability (4, 18). Zlotkin et al (19) showed the efficacy of microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to weaning foods.

[1] From the Human Nutrition Laboratory (MBZ, RW, CZ, RB, and RFH) and the Food Process Engineering Laboratory (EW), Swiss Federal Institute of Technology, Zürich, Switzerland, and the Ministry of Health, Rabat, Morocco (NC).

[2] Supported by the Thrasher Research Fund (Salt Lake City), the Foundation for Micronutrients in Medicine (Rapperswil, Switzerland), and the Swiss Federal Institute of Technology (Zürich, Switzerland).

[3] Address reprint requests and correspondence to M Zimmermann, MD, Human Nutrition Laboratory, Swiss Federal Institute of Technology Zürich, Seestrasse 72/Postfach 474, CH-8803 Rüschlikon, Switzerland. E-mail: michael.zimmermann@ilw.agrl.ethz.ch.

Received April 2, 2004.

Accepted for publication June 3, 2004.

IDA, VAD, and IDD are common in rural Morocco (4, 20, 21), and salt is regularly consumed there at a rate of 5–15 g/d (4). To develop a TFS for this region, we used a novel spray-cooling process to encapsulate FePP, potassium iodate, and retinyl palmitate in hydrogenated oil in a single processing step. To set the fortification level of the TFS, we measured salt, vitamin A, and iron intakes from the local diet. We then tested the stability and acceptability of our TFS and its sensory effects in local meals. Finally, we compared the efficacy of the TFS to that of iodized salt (IS) in a randomized double-blind trial in schoolchildren.

SUBJECTS AND METHODS

The study was done in villages in the Rif Mountains of northern Morocco, an area of endemic goiter (20). The villages are 500–600 m above sea level and have a temperate climate, with an 8-mo dry season (temperature range: 22–34 °C; $\bar{x}$ rainfall: 23 cm/mo), and a 4-mo damp season (temperature range: 10–22 °C; $\bar{x}$ rainfall: 77 cm/mo). The population is of mixed Berber and Arab descent. This region is isolated from commercial routes, >95% of the population is rural, and most available food is produced locally on small farms (22).

Measurement of salt consumption, iron and vitamin A intakes, and iron bioavailability

To establish the optimal fortification level for the TFS, we needed to know local salt, vitamin A, and iron intakes and the estimated bioavailability of iron from the diet. Three-day weighed food records were kept in 50 households randomly selected from local census rolls. Households were asked to continue with their usual food choices 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 foods and beverages consumed were weighed on a Kern 440–53 scale (Kern & Sohn GmbH, Albstadt, Germany) that is accurate to ± 1 g.

In this region, meals consist of 1 or 2 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 (22): for each male ≥14 y old, UC = 1.0; for each female ≥10 y old, UC = 0.8; and for each male <14 y old and each female <10 y old, UC = 0.3 + 0.05 × age, with age measured in years. Nutrient intakes were calculated from the food-composition table of the Moroccan Ministry of Agriculture (23) and from the Food and Agriculture Organization food-composition table for Africa (24). In addition, local legumes, cereals, and vegetables were directly analyzed for iron and phytic acid content. Dietary iron bioavailability was estimated by using the algorithms of Tseng et al (25) and Reddy et al (26) and adjusted for body iron stores (27). Vitamin A intakes were calculated as retinol activity equivalents (RAEs) by using a conversion factor of 12 μg β-carotene to 1 μg retinol for a mixed fruit-and-vegetable diet (28, 29).

Development of the iron, iodine, and vitamin A microcapsules

In our food engineering laboratory in Zürich, we used a newly developed, single-step, spray-cooling technique to build microcapsules containing iodine, iron, and vitamin A. The components were FePP that was micronized by being ground to a mean particle size of ≈2.5 μm (Dr Paul Lohmann, Emmerthal, Germany), potassium iodate (Riedel-de Haen, Hannover, Germany) that was micronized by being ground to a mean particle size of ≈6 μm (K8 Beadmill; Bühler, Uzwil, Switzerland), and vitamin A as liquid retinyl palmitate at 1.7 million IU/g, which was stabilized with 10 mg of butylated hydroxytoluene/1 million IU (BASF, Burgbernheim, Germany).

The 3 nutrients were coated with hydrogenated palm oil (Nutriswiss, Lyss, Switzerland) with a melting point of 63 °C and containing 1% lecithin (Loders Croklaan, Wormerveer, Holland). Palm oil was chosen over soybean oil because of palm oil's higher melting point and lower cost and because of concerns about the acceptability of genetically modified soybeans.

To produce the microcapsules, a specifically designed, stainless-steel, cold-spraying tower (Schmidlin, Affoltern, Switzerland) and specifically modified two-phase nozzles were used. The palm oil was heated to ≈85 °C, and a suspension containing the 3 nutrients was made. The suspension was then passed through a screw pump (Scheerle, Steckborn, Germany), atomized with the use of air as the second medium, and cooled with the use of partially evaporated liquid nitrogen (15, 30).

The smooth, spherical capsules are 40% substrate and 60% capsule and have a mean particle size of ≈100 μm. The microcapsule characteristics were chosen to *1*) achieve sufficiently high attraction-interaction forces to reduce segregation when the microcapsules were mixed with larger salt grains ($\bar{x}$ local salt grain size: ≈1.5 mm); *2*) optimize handling of the product; *3*) adjust the kinetics of the release of the micronutrients from the capsules; and *4*) maintain a ratio of surface to volume that was adequate to protect the nutrients. During the production of the compound, losses of vitamin A and iodine are ≈35% and 20%, respectively, because of oxidation during heating and spraying. These anticipated losses are compensated for by spraying at an iron:iodine:vitamin A ratio of 1 mg:15 μg:50 μg (an overage) to achieve the final target ratio for fortification, ie, 1 mg:≈12 μg:≈30 μg.

Fortification of salt

One local cooperative supplies nearly all salt for the villages in the study; the salt is produced in drying ponds by using water from a salty spring. The salt (95.4% NaCl) is not washed or ground, and it has a milky-white color and an average grain size of ≈1.5 mm. Its moisture content is <1% during the dry season but 3–4% during the damp season. It contains 2.5% $CaSO_4$, <0.1% $MgSO_4$, and <2 ppm iodine. Although Morocco legislated mandatory salt iodization in 1997, financial constraints have not yet allowed this local cooperative to begin iodization. To prepare the IS, iodine was added as reagent-grade potassium iodate (Sigma & Aldrich, Buchs, Switzerland) at a concentration of 25 μg I/g salt. The TFS was fortified at a concentration of 2 mg Fe, 25 μg I, and 60 μg vitamin A/g salt with the use of microcapsules. First, to prepare the IS and TFS, concentrated mixes were made by adding 840 mg KIO_3 and 480-g microcapsules, respectively, to 2-kg salt batches with the use of a small, electric, rotating-drum mixer (MINI 80; Engelsmann, Ludwigshafen, Germany) at 26 rpm for 10 min. The 2-kg mixes were then added to 18-kg batches of salt by using a large, electric, rotating-drum mixer (ELTE 650; Engelsmann) at 30 rpm for 10 min.

Stability testing

The IS and TFS were stored locally for 6 mo as 2-kg batches in closed low-density transparent polyethylene bags that were kept indoors and out of direct sunlight. A 6-mo study was done to approximate the time required for the production, distribution, and consumption of salt in this region. After storage for 0, 2, 4, and 6 mo, 30-g salt aliquots ($n = 3$) were taken for measurements of iodine concentration in the IS and TFS and of vitamin A concentration in the TFS. Color stability was determined by reflectance colorimetry as well as by panel visual inspection of unmarked samples side-by-side on white backgrounds.

Sensory testing

To evaluate potential sensory changes in local foods, IS and TFS were added to meals prepared by local women in their kitchens. Each type of salt was added in parallel in identical amounts to separate portions of 4 common foods: bread (mainly white flour), bisarra (fava bean and olive oil puree), chaaria (semolina noodles in milk), and couscous (semolina). The flavor, odor, and color of the foods were then compared by a panel of 18 local adults ($\bar{x}$ age: 33 y; 59% female) with the use of triangle tests (31). 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 indicate which sample differed from the other 2 samples and to describe how it differed.

Efficacy study

The subjects were children 6–14 y old from 2 neighboring primary schools. Informed written consent (or, if the parents were illiterate, oral consent) was obtained from the parents, and oral assent was obtained from the children. The Swiss Federal Institute of Technology in Zürich and the Ministry of Health in Rabat, Morocco, gave ethical approval for the study. All children in the 2 schools were invited to participate in the 10-mo study; all ($n = 159$) accepted and were enrolled. At baseline, weight and height were measured, and a spot urine sample was collected for measurement of urinary iodine (UI). Five milliliters of whole blood was collected by venipuncture for measurement of hemoglobin, serum ferritin (SF), whole-blood zinc protoporphyrin (ZnPP), serum transferrin receptor (sTfR), serum retinol (SR), retinol-binding protein (RBP), prealbumin (transthyretin; TTR), and C-reactive protein (CRP).

Because each participating family shared a monthly salt portion (*see* below), children were randomly divided by household into 2 groups. Group 1 (IS group) was given IS, ie, salt fortified with 25 μg I/g salt. Group 2 (TFS group) was given TFS, ie, salt triple-fortified with 25 μg I, 60 μg vitamin A, and 2 mg Fe/g salt. IS and TFS were prepared as described above. For monitoring, 30-g aliquots ($n = 6$) of the salts were taken and measured for iodine, iron, and vitamin A content at each monthly mixing. 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 mo during the 10-mo study 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 5 and 10 mo, all baseline measurements were repeated. After completion of the study, all remaining children with IDA or VAD were treated with oral iron [60 mg Fe (as ferrous sulfate) for 4 d/wk for 12 wk] or oral vitamin A (200 000 IU as a single dose; 32).

Acceptability testing

To judge the acceptability of TFS and IS after 10 mo of household salt use during the efficacy study, an interview was done in ≈50% of households in the IS ($n = 41$) and TFS ($n = 30$) groups. The female head of household, who was blind to group assignment, answered forced-choice questions on patterns of salt use, color and taste acceptability, and overall satisfaction with the salt (*see* Results).

Laboratory analyses

Whole blood was transported on ice to the local laboratory. Hemoglobin was measured in whole blood (refrigerated and measured on the same day as collection) with the use of an AcT8 Counter (Beckman Coulter, Krefeld, Germany). Anemia was defined as a hemoglobin concentration $<$ 120 g/L in children aged $\geq$ 12 y and $<$ 115 g/L in children aged 5–11 y (33). ZnPP was measured in washed red blood cells (refrigerated and measured within 24 h of collection) with the use of a hematofluorometer (Aviv Biomedical, Lakewood, NJ). Serum and urine samples were aliquoted and frozen at −20 °C until they were analyzed. UI was measured by using the Sandell-Kolthoff reaction as modified by Pino et al (34). SF and sTfR were measured by using enzyme-linked immunosorbent assays (RAMCO, Houston). CRP and TTR were measured by using nephelometry (TURBOX; Orion Diagnostica, Espoo, Finland). SR was measured by using HPLC, and RBP was measured by using an enzyme-linked immunosorbent assay (Immundiagnostik AG, Bensheim, Germany). Iron deficiency was defined either as SF $<$ 15 μg/L or as sTfR $>$ 8.5 mg/L + ZnPP $>$ 40 μmol/mol heme (35). Body iron was estimated by the method of Cook et al (36). VAD was defined as an SR concentration $<$ 0.70 μmol/L (37). Because an SR concentration $<$ 1.05 μmol/L may indicate low vitamin A status (38), this cutoff was also applied to our data. SR data were presented both as the proportion of children below these cutoffs and as distributions (8). Various cutoffs for serum RBP concentration have been proposed, but it is not yet possible to determine a cutoff that reliably reflects an SR concentration $<$ 0.70 μmol/L (39–41). Therefore, we presented our RBP data only as distributions. RBP:TTR has been proposed as an additional biochemical indicator of VAD, but there is no agreement on a cutoff (42, 43); these data were presented as distributions.

To measure salt color, 10 g salt was transferred into a glass container; color determination on the Hunter scale was established with the use of a Spectral Photometer (Chroma Meter CR-310; Minolta, Osaka, Japan) by using an illuminant D_{65} (average daylight, including ultraviolet spectra) setting and a 0° observer angle with a large reflectance spectrum. On the Hunter scale, the *L* value measures light reflection (a value of 100 is pure white; a value of zero is pure black), the *a* value is a measure of redness and greenness, and the *b* value is a measure of yellowness and blueness. Each sample was measured 3 times, and the glass container was rotated 90° after each measurement. The color of the TFS was then compared with that of the IS, and the absolute

TABLE 1
Color and iodine and vitamin A concentrations in the iodized salt (IS) and triple-fortified salt (TFS) at mixing and after 2, 4, and 6 mo of storage[1]

Length of storage (mo)	Color				Iodine[5]		Vitamin A
	Lightness[2]		ΔE[3,4]				
	IS	TFS	IS	TFS	IS	TFS	TFS
					μg/g salt		*μg/g salt*
0	80.9 ± 0.88	79.82 ± 0.26	0.88 ± 0.09	9.01 ± 1.05	27.4 ± 3.8	25.7 ± 3.0	69.7 ± 7.1
2	80.3 ± 0.45	79.87 ± 0.31	1.28 ± 0.43	9.17 ± 0.07	18.6 ± 2.1	22.2 ± 6.1	74.5 ± 3.2
4	80.7 ± 1.01	79.73 ± 0.38	0.96 ± 0.09	9.02 ± 0.28	21.0 ± 3.1	26.5 ± 3.7	69.4 ± 2.7
6	80.3 ± 0.75	79.59 ± 0.11	1.34 ± 0.73	9.02 ± 0.52	19.4 ± 1.4	20.6 ± 0.9	61.2 ± 4.5

[1] All values are $\bar{x}$ ± SD.
[2] Lightness scale: 1 = black, 100 = white.
[3] ΔE, absolute color difference between TFS or IS and an IS reference sample.
[4] Significant main effect of fortification, $P < 0.001$ (ANOVA).
[5] Significant main effect of time, $P < 0.001$ (ANOVA).

color difference was expressed by ΔE. ΔE was calculated by using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

where ΔL, Δa, and Δb describe the difference between the color of the TFS and the reference color (IS).

Iodine content in the TFS was measured by using inductively coupled plasma mass spectrometry (44). For IS, iodine content was measured in salt aliquots dissolved in distilled water by using the Pino et al–modified Sandell-Kolthoff reaction (34). At iodine concentrations of 30 μg/g salt, the CV of this assay in our laboratory is 7%. This method shows good agreement ($r^2 = 0.97$) with isotope abundance ratio measurements by mass spectrometry (44). Vitamin A content of the TFS was measured by using fluorescence spectrophotometry (Luminescence Spectrometer LS 50B; Perkin Elmer, Wokingham, United Kingdom). The iron content of foods and of the TFS was measured by using a standard addition method and flame atomic absorption spectroscopy (SpectrAA-400; Varian, Mulgrave, Australia). The particle size of the microcapsules and of their components was measured by using laser diffraction spectrometry (Malvern Mastersizer X; Renes, Lausanne, Switzerland), and microcapsule morphology determined by scanning electron microscopy (S 900; Hitachi, Tokyo).

Statistical analysis

Data processing and statistical analysis were conducted by using SPLUS (2000; Insightful Corporation, Seattle), PRISM (version 3; GraphPad, San Diego), and EXCEL (XP 2002; Microsoft, Redmond, WA) software packages. A two-factor repeated-measures ANOVA was done to compare effects of the group × time interaction on hemoglobin, CRP, SF, sTfR, ZnPP, body iron, SR, RBP, RBP:TTR, and UI and effects of the fortification × time interaction on salt color and iodine content. If the interaction effect was significant ($P < 0.05$), *t* tests between groups and paired *t* tests within groups over time were done and adjusted for multiple comparisons (Bonferroni correction). Proportions were compared by using the chi-square test. When data were not normally distributed, statistical analysis was done after log transformation. Logistic regression was used to compare effects of the group × time interaction on the binary variables of IDA, low vitamin A status, and VAD. Significance was set at $P < 0.05$.

RESULTS

Salt, iron, and vitamin A intakes 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 (±SD) salt intake for adult males and females was 12.1 ± 2.9 and 9.7 ± 2.3 g/d, respectively. For children aged 6–14 y, mean salt intake was 7.3–11.6 g/d, and mean iron intake was 9.2–14.5 mg/d, 97% of which was nonheme iron. Because the diet was high in phytic acid and low in ascorbic acid, estimated nonheme iron bioavailability was 1–4.3%. For children aged 6–8 y and 9–13 y, total vitamin A intakes were 206 ± 67 and 288 ± 71 μg RAE/d [ie, 48–52% of the recommended dietary allowance (28)], of which 69% came from retinol in animal foods and 31% from carotenes from plant foods.

Stability and sensory testing

Color stability and the iodine and vitamin A content of the IS and TFS at 0, 2, 4, and 6 mo after mixing are shown in **Table 1**. Colorimetry showed no significant difference between IS and TFS in the *L* color value and no significant color change in the IS and TFS over 6 mo of storage. The ΔE of ≈9 is due to the slight difference in color between the TFS (light beige) and the IS (milky white). There was no significant difference between TFS and IS in iodine content during storage; both salts lost ≈15% of their iodine content after 6 mo. The TFS lost ≈12% of its vitamin A content after 6 mo. In the triangle testing that compared IS with TFS, there was no significant difference in color, odor, or taste (or all three) between the fortified salts in any of the traditional foods. Three of the foods tested —bread, chaaria, and couscous—were pale and mild-tasting.

Efficacy trial

The baseline characteristics of the children in the IS ($n = 83$) and TFS ($n = 74$) groups were age of 10.6 ± 2.4 and 10.4 ± 2.1 y, sex ratio (girls:boys) of 40:43 and 33:41, weight of 31.2 ± 9.9 and 29.4 ± 8.3 kg, and height of 1.34 ± 0.14 and 1.34 ± 0.12 m, respectively. Randomization at the household level was effective; there were no significant differences between groups in these baseline characteristics or in the other characteristics presented in **Tables 2**, **3**, and **4**. Of the 159 children who began the study, 157 completed it; 2 children in the TFS group moved away. In the monitoring of

TABLE 2
Hemoglobin, transferrin receptor (TfR), zinc protoporphyrin (ZnPP), serum ferritin (SF), and body iron concentrations and the prevalence of iron deficiency anemia (IDA) in the iodized salt (IS) and triple-fortified salt (TFS) groups over 10 mo

Time (mo)	Hemoglobin[1]		TfR[1]		ZnPP[1]		SF[1]		Body iron[1]		IDA[2]	
	IS	TFS	IS	TFS	IS	TFS	IS	TFS	IS	TFS	IS	TFS
	g/L		*mg/L*		*μmol/mol heme*		*μg/L*		*mg/kg*		*n (%)*	
0	116 ± 9[3]	114 ± 9	7.5 ± 2.4	8.0 ± 3.4	50 ± 36	47 ± 27	15.2 (7.1, 25.9)[4]	15.2 (7.0, 24.7)	1.23 ± 2.64	1.07 ± 2.95	28 (34)	23 (31)
5	116 ± 11	124 ± 12[5,6]	7.0 ± 1.9[7]	5.9 ± 1.5[4,8]	49 ± 37	34 ± 20[6,7]	19.0 (7.3, 38.2)	30.7 (11.9, 80.2)[5,6]	2.21 ± 2.79[7]	4.43 ± 2.25[5,6]	23 (28)	5 (7)
10	115 ± 8	129 ± 10[5,6]	7.7 ± 2.4	5.8 ± 1.4[5,6]	52 ± 35	27 ± 19[5,6]	15.0 (6.9, 28.1)	31.2 (12.8, 79.9)[5,6]	1.08 ± 3.10	4.69 ± 2.12[5,6]	24 (29)	4 (5)

[1] Significant treatment × time interaction. $P < 0.001$ (ANOVA).
[2] Significant difference between time-and-group model and time-only model, $P < 0.001$ (logistic regression).
[3] $\bar{x} \pm$ SD (all such values).
[4] Geometric $\bar{x}$; −1 SD and +1 SD in parentheses (all such values).
[5,7] Significantly different from baseline: [5] $P < 0.01$, [7] $P < 0.05$.
[6,8] Significantly different from IS: [6] $P < 0.01$, [8] $P < 0.05$.

aliquots of salt taken at the monthly mixings, the iron and vitamin A concentrations in the TFS group were 2.2 ± 0.8 mg and 64.4 ± 19.6 μg/g salt, respectively. The iodine concentrations in the IS and the TFS groups were 22.9 ± 3.0 and 24.5 ± 4.2 μg/g salt, respectively. The prevalence of an elevated CRP was 4–6% in both groups at all timepoints, and there was no significant difference in mean CRP or in the prevalence of elevated CRP between groups (data not shown).

The changes in iron status are shown in Table 2. Mean hemoglobin at 10 mo was significantly greater in the TFS group than in the IS group ($P < 0.01$). All indexes of iron status (SF, sTfR, and ZnPP) and body iron stores were significantly improved at 10 mo in the TFS group ($P < 0.02$). The changes in vitamin A nutrition during the study are shown in Table 3. Mean SR, RBP, and RBP:TTR increased significantly in the TFS group ($P < 0.01$). At 10 mo, the prevalence of VAD and low vitamin A status was significantly lower in the TFS group than in the IS group ($P < 0.001$).

There were no significant differences in median UI between the groups throughout the study. The baseline median (range) UI in the IS and TFS groups was 12 (2–70) and 10 (4–127) μg/L, respectively, which indicated severe IDD (6). At 10 mo, the median (range) UI in the IS and TFS groups was 104 (22–1784) and 97 (23–927) μg/L, respectively. The median UI in both groups was significantly higher at 10 mo than at baseline ($P < 0.0001$), and it was near the WHO/International Council for the Control of the Iodine Deficiency Disorders cutoff (>100 μg/L) for iodine sufficiency (6).

Acceptability testing

The results of interviews done after 10 mo of household salt use are shown in Table 4. Both salts were universally used in both the IS and TFS households, and there were no significant differences in acceptability of salt color, salt taste in foods, and overall acceptability between groups. However, 16% of the IS and 32% of the TFS heads of households noted a slight color change in one or more foods (mainly egg and milk dishes) when the respective salts were added. These color changes were mild and sporadic and did not affect overall acceptability. In similar foods, color changes were not detected in the triangle sensory testing of IS and TFS done before the efficacy study.

DISCUSSION

To our knowledge, this is the first published report of successful triple fortification of salt with iron, iodine, and vitamin A. Several factors contributed to this success. First, because ferrous iron causes rapid color change and iodine losses when added to

TABLE 3
Serum retinol and retinol-binding protein (RBP) concentrations, ratio of RBP to transthyretin (TTR), and prevalence of vitamin A deficiency (VAD) and low vitamin A status in the iodized salt (IS) and triple-fortified salt (TFS) groups over 10 mo

Time (mo)	Retinol[1]		RBP[1]		RBP:TTR[1]		VAD[2] (SR < 0.07 μmol/L)		Low vitamin A status[3] (0.07 μmol/L ≥ SR < 1.05 μmol/L)	
	IS	TFS	IS	TFS	IS	TFS	IS	TFS	IS	TFS
	μmol/L		*mg/L*				*n (%)*		*n (%)*	
0	0.91 ± 0.14[4]	0.93 ± 0.17	21.1 ± 9.3	21.2 ± 11.0	0.23 ± 0.16	0.25 ± 0.18	13 (15)	9 (12)	58 (70)	42 (57)
5	0.94 ± 0.15	1.14 ± 0.13[5,6]	24.7 ± 9.1	29.6 ± 10.21	0.32 ± 0.17[5]	0.35 ± 0.17[5]	11 (13)	0 (0)	51 (61)	30 (41)
10	0.91 ± 0.15	1.18 ± 0.12[7,8]	22.4 ± 8.8	32.8 ± 11.1[7,9]	0.26 ± 0.15	0.38 ± 0.14[5,6]	14 (17)	1 (1)	53 (64)	23 (31)

[1] Significant treatment × time interaction, $P < 0.001$ (ANOVA).
[2] VAD = serum retinol < 0.07 μmol/L. Significant difference between time-and-group model and time-only model, $P < 0.001$ (logistic regression).
[3] Low vitamin A status = 0.07 μmol/L ≥ SR < 1.05 μmol/L. Significant difference between time-and-group model and time-only model, $P < 0.01$ (logistic regression).
[4] $\bar{x} \pm$ SD (all such values).
[5,7] Significantly different from baseline: [5] $P < 0.05$, [7] $P < 0.01$.
[6,8,9] Significantly different from IS: [6] $P < 0.05$, [8] $P < 0.01$, [9] $P < 0.02$.

TABLE 4
Acceptability of iodized salt (IS) and triple-fortified salt (TFS) containing iodine, iron, and vitamin A after 10 mo of salt use[1]

	Salt	
Questions	IS	TFS
	%	
1) Salt quantity used monthly in the household?		
≤1 kg	0	3
1–2 kg	24	23
2 kg (amount distributed monthly to each study household)	52	47
>2 kg	24	30
2) Salt consumed every day by children?	100	100
3) Salt used for all foods during cooking and at table?	100	100
4) Salt changed the color of foods?	16	32[2]
5) Salt color acceptable in damp and dry seasons?	100	97
6) Salt taste acceptable in all foods?	96	100
7) Salt acceptable overall?	100	100

[1] Percentages of positive answers are shown.
[2] Significantly different from IS, $P < 0.02$.

IS, we used micronized FePP, a poorly soluble, white iron compound with good bioavailability due to micronization. The absorption of FePP with a mean particle size of ≈0.5 μm is comparable to that of ferrous sulfate (16), and the relative bioavailability (RBV) of FePP with a mean particle size of 2.5 μm is ≈70% of that of ferrous sulfate (R Wegmüller, personal communication, 2004). When FePP is added to salt containing potassium iodate, the iodine's stability is comparable to that of IS (14).

Second, to reduce losses of iodine and vitamin A, a physical barrier was placed around them by microencapsulation. The use of hydrogenated palm oil as the capsule material had 2 clear advantages. The first advantage is that oil is an ideal matrix for fat-soluble vitamin A, because oil stabilizes retinol and delays oxidation of the vitamin (8). The second advantage is that it allowed us to use a less costly vitamin A compound, because vitamin A compounds needed for fortification of dry matrixes (eg, flour and sugar) are ≥4 times as expensive as are oily forms, and their stability is inferior (8). In the TFS, the vitamin A in the microcapsules showed excellent stability over 6 mo of storage: ie, losses of ≈12–15%. This compares favorably with the usual vitamin A losses during 9 mo of storage of fortified flours (30–50%) and fortified sugar (30–60%) (8, 17). However, if the TFS were to be stored at a temperature above the melting point of the capsule (63 °C), the microcapsules would likely degrade. Another disadvantage of encapsulation is the losses of iodine (20%) and vitamin A (35%) that occur during production of the microcapsules. Although we anticipated these losses by overage, further work must be done to increase the stability of iodine and, particularly, vitamin A during spraying to reduce costs.

In many African countries, diets are based mainly on cereals and legumes that are poor sources of vitamin A. Even carotenoid-rich vegetables have low vitamin A bioavailability (29), and thus plant-based dietary diversification without animal foods may not supply adequate vitamin A. Vitamin A supplementation is highly effective if it reaches infants and young children (32), but VAD in developing countries is not confined to these groups. It can affect young women, school-aged children, and adolescents. Thus, food fortification with vitamin A is an attractive option, but finding suitable food vehicles has been difficult. The biological efficacy, but not the effectiveness, of vitamin A–fortified oil (45, 46), margarine (45, 47, 48), monosodium glutamate (49, 50), and cereal flours (51–53) has been shown. The efficacy and program effectiveness of sugar fortification in Central America are well established (54, 55). However, in much of rural Africa and Southeast Asia, oils, margarine, flours, and sugar either are not widely distributed or are not consumed by the neediest persons. Salt could be a good vehicle, because, in poor regions of subsistence farming in Africa and Indonesia, it is one of the few regularly purchased food items (9, 56, 57).

However, there are several potential limitations to the use of salt as a vehicle for vitamin A and iron. First, it is important that the usual amount of salt consumed by the target population be large enough. If not, the vitamin A and iron concentrations will be too high, which will cause technical and cost problems. According to 3-d weighed food records, per capita salt consumption in rural Ivory Coast is 6–9 g/d (9); in rural Morocco, it is 6–15 g/d (4). To maximize the potential efficacy of our intervention trial, we fortified the salt with high concentrations of vitamin A (60 μg/g) and iron (2 mg/g). In a long-term fortification program, concentrations could be lower. For example, consumption in the household of 6–8 g salt fortified with 20 μg vitamin A/g would provide approximately one-third of the recommended dietary allowance for 4–6-y-old children and approximately one-fifth of the recommended dietary allowance for pregnancy (28). A second potential barrier to salt fortification is, paradoxically, its low price: fortification could lead to a large increase in its price. Although the annual cost to supply the same amount of vitamin A through salt could be similar to that for cereal flours and sugar, the margin of increase in the product price would be higher for salt. Therefore, fortified salt might not be able to compete with nonfortified salt, unless the government mandated fortification by all producers or unless prices were maintained by subsidies.

To set the fortification level for iron in the salt, 3-d weighed food records were done to measure salt intakes and the estimated bioavailability of iron from the local diet. Our measured intakes of salt may have been somewhat high, because food records tend to overestimate true salt intake (58). To reduce the prevalence of IDA, our fortification goal was to provide ≈0.5 mg absorbed Fe/d to the children in the study. On the basis of local intakes of 7–12 g salt/d by children, a relative bioavailability to ferrous sulfate of 70% of the micronized FePP, and an anticipated dietary iron absorption of 2–4%, we fortified the TFS with 2 mg Fe/g salt. The total dose of Fe delivered by the TFS during the 10-mo trial was ≈5.4 g, which is based on a study period of 300 d, mean salt intakes of 9 g/d in children, and a fortification level of 2 mg Fe/g salt. The mean increase in total-body iron in the TFS group was ≈105 mg, whereas the mean decrease in total-body iron in the IS group was ≈10 mg. On the basis of these data, the mean daily gain in total-body iron in the TFS group was ≈0.4 mg, which suggests that ≈2% of the fortification iron was absorbed during the 10-mo trial. In previous trials, iron-fortified foods that have clearly improved the iron status in target populations include infant formula and cereal (59, 60), sugar (61), curry powder (62), and fish sauce (63). 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.

Several factors may have contributed to efficacy in this study. Most children ate 3 main meals, as well as midmorning and midafternoon snacks, all of which contained measurable amounts of salt. Thus, iron absorption was likely enhanced by the repeated delivery of small doses throughout the day, because the fractional absorption of nonheme iron increases with decreases in dose (64). Another potential reason for the clear effect on iron and vitamin A status is that, in this region, there is a low rate of infection and inflammation (the prevalence of elevated CRP was only ≈5%) and no malaria, and diarrheal disease and hookworm are rare (M Bousfiha, personal communication, 2001). Therefore, IDA and VAD are mainly due to low intakes rather than to greater losses. Moreover, the low prevalence of infection or inflammation (or both) in this region increased our ability to rely on SF and SR concentrations to clearly define IDA and VAD and detect changes in status (65, 66).

The introduction of IS into an area of long-standing iodine deficiency carries some risk. The most serious and common complication of salt iodization is iodine-induced hyperthyroidism (IIH; 67). In most cases, IIH is mild and transient and does not produce clinical symptoms. The medical staff of the local health center was made aware that our introduction of IS might increase the risk of IIH among older people in the community with nodular goiters, and the staff members were instructed in managing any cases that might occur. However, no cases of IIH were reported during the study period.

Global control of deficiencies of iron, iodine, and vitamin A requires an integrated approach that includes dietary diversification, targeted supplementation, and food fortification. A stable and efficacious TFS could be a useful new fortification strategy, particularly in rural Africa and South and Southeast Asia. Our TFS meets the 3 basic requirements of a useful fortified food for these regions: *1*) salt is an indigenous commodity regularly consumed by the target population (women and young children) and produced at a few centralized sites; *2*) the TFS was nearly indistinguishable from iodized salt in sensory tests and was universally accepted; and *3*) all 3 nutrients in the TFS were stable during storage and showed good bioavailability. However, because the performance of a TFS may vary according to climate and salt quality, these studies will need to be repeated in other countries, eg, in tropical regions of Africa and Southeast Asia. Moreover, for this strategy to be useful on a national scale, the issue of the large price increment of a TFS—due to the costs of fortification, storage, and distribution as well as the costs of installing local production facilities in less developed countries—remains to be resolved.

We thank the participating children and teachers and the staff at the Brikcha Health Center. We offer special thanks to Rabie Rahmouni (Brikcha, Morocco); Mohammed El-Yazami (Chefchaouen, Morocco); and Max Haldimann (Ministry of Health, Bern, Switzerland); Fabian Rohner, Vivienne Bühr, Sibila DeLorenzi, Marie-Helene Balsat, Sabine Mattmann, Astrid Huber, and Nicholas Hurrell (Swiss Federal Institute of Technology Zürich). We thank Dr. Paul Lohmann GmbH KG (Emmerthal, Germany) for supplying the ferric pyrophosphate and the ferrous sulfate tablets; BASF (Ludwigshafen, Germany) for providing the retinyl palmitate; and Task Force Sight and Life (Basel, Switzerland) for providing the vitamin A supplements.

MBZ, RW, EW, CZ, NC, RB, and RFH contributed to the study design. The development of the microcapsules was done by EW, RW, MBZ, RB, and RFH. The fieldwork and data collection were done by MBZ, RW, NC, CZ, and RB. The statistical analysis was done by MBZ and FR. MBZ, RW, EW, CZ, RB, and RFH completed the final data analysis. The manuscript was written by MBZ and edited by RW, EW, CZ, NC, RB, and RFH. None of the authors had a financial or personal conflict of interest with regard to this study.

REFERENCES

1. WHO/UNICEF/UNU. IDA: prevention, assessment and control. Report of a joint WHO/UNICEF/UNU consultation. Geneva: World Health Organization, 1998:1–9.
2. Vitamin A deficiency and iodine deficiency disorders prevalence estimates for the global burden of disease. Micronutrient Deficiency Information System (MDIS). Geneva: World Health Organization, 2001.
3. Zimmermann MB, Adou P, Zeder C, Torresani T, Hurrell RF. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in the Côte d'Ivoire. Am J Clin Nutr 2000; 71:88–93.
4. Zimmermann MB, Zeder C, Chaouki N, Saad A, Torresani T, Hurrell RF. Dual fortification of salt with iodine and microencapsulated iron: a randomized, double blind, controlled trial in Moroccan schoolchildren. Am J Clin Nutr 2003;77:425–32.
5. International Vitamin A Consultative Group. IVACG statement: vitamin A and iron interactions. Washington, DC: ILSI Research Foundation, 1998.
6. WHO/UNICEF/ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination. WHO/NHD/01.1. Geneva: World Health Organization, 2001.
7. Hurrell RF. Fortification: overcoming technical and practical barriers. J Nutr 2002;132:806S–12S.
8. Dary O, Mora JO. Food fortification to reduce vitamin A deficiency: International Vitamin A Consultative Group Recommendations. J Nutr 2002;132:2927S–33S.
9. Hess S, Zimmermann MB, Staubli F, Tebi A, Hurrell RF. An evaluation of salt intake and iodine nutrition in the Côte d'Ivoire. Eur J Clin Nutr 1999;53:680–6.
10. Hess SY, Zimmermann MB, Adou P, Torresani T, Hurrell RF. Treatment of iron deficiency in goitrous children improves the efficacy of iodized salt in Côte d'Ivoire. Am J Clin Nutr 2002;75:743–8.
11. Zimmermann MB, Zeder C, Chaouki N, Torresani T, Saad A, Hurrell RF. Addition of encapsulated iron to iodized salt improves the efficacy of iodine in goitrous, iron-deficient schoolchildren: a randomized, double blind, controlled trial. Eur J Endocrinol 2002;147:747–53.
12. Sivakumar B, Brahmam GNV, Nair KM, et al. Prospects of fortification of salt with iron and iodine. Br J Nutr 2001;85:S167–73.
13. Mannar MGV, Diosady LL. Double fortification of salt with iron and iodine. In: Food fortification to end micronutrient malnutrition. Ottawa: The Micronutrient Initiative/International Development Research Centre, 1998:89–94.
14. Wegmüller R, Zimmermann MB, Hurrell RF. Dual fortification of salt with iodine and encapsulated iron compounds: stability and acceptability testing in Morocco and Côte d'Ivoire. J Food Sci 2003;68:2129–35.
15. Buhr V. Development and evaluation of spray-cooled microcapsules containing iron, iodine and vitamin A for use in salt fortification. Diploma (BS) Thesis. Zürich: Swiss Federal Institute of Technology, 2003.
16. Fidler MC, Walczyk T, Davidsson L, et al. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. Br J Nutr 2004;91:107–12.
17. SUSTAIN. Final Report of the Micronutrient Assessment Project. Washington, DC: US Agency for International Development, 1999.
18. Hurrell RF, Furniss DE, Burri J, Whittaker P, Lynch SR, Cook JD. Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. Am J Clin Nutr 1989;49:1274–82.
19. Zlotkin S, Arthur P, Yeboah Antwi K, Yeung G. Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods. Am J Clin Nutr 2001;74: 791–5.
20. Chaouki N, Ottmani S, Saad A, et al. The prevalence of iodine deficiency disorders in children 6–12 years old in Morocco. Bull Epidemiol Morocco 1996;1:2–23.
21. Mokhtar N. Vitamin A situation of reproductive age women in the Gharb region of Morocco: findings from a regional study: an OMNI/USAID Report. Rosslyn, VA: International Science and Technology Institute, 1998.

22. Larbi A. Iron intake, sources and bioavailability in Chefchaouen Province, Morocco. PhD dissertation. Corvallis: Oregon State University, 1991.
23. Ministry of Agriculture of Morocco. Food composition table for Morocco. Rabat: Ministry of Agriculture, 1977.
24. Food and Agriculture Organization. Food composition tables for use in Africa. Rome: Food and Agriculture Organization, 1968.
25. Tseng M, Charakraborty H, Robinson DT, Mendez M, Kohlmeier L. Adjustment of iron intake for dietary enhancers and inhibitors on population studies: bioavailable iron in rural and urban residing Russian women and children. J Nutr 1997;127:1456–68.
26. Reddy BR, Hurrell RF, Cook JD. Estimation of nonheme iron bioavailability from meal composition. Am J Clin Nutr 2000;71:937–43.
27. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 1991;54:717–22.
28. Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2001:82–161.
29. West CE, Eilander A, van Lieshout, M. Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin A deficiency in developing countries. J Nutr 2002;132:2920S–6S.
30. Wagner T. Spray chilling of single- and multiple phase fluids. PhD dissertation. Zürich: Swiss Federal Institute of Technology, 1997.
31. Meilgaard M, Civille GV, Carr BT. Sensory evaluation techniques. Boca Raton, FL: CRC Press, 1991.
32. De Benoist B, Matines J, Goodman T. Vitamin A supplementation and the control of vitamin A deficiency: conclusions. Food Nutr Bull 2001; 22:335–7.
33. UNICEF/WHO/UNU/The Micronutrient Initiative. Preventing iron deficiency in women and children: background and consensus on key technical issues and resources for advocacy, planning and implementing national programmes. Ottawa: International Nutrition Foundation, 1998:10.
34. Pino S, Fang SL, Braverman LE. Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine. Clin Chem 1996; 42:239–43.
35. Cook JD, Baynes RD, Skikne BS. Iron deficiency and the measurement of iron status. Nutr Res Rev 1992;5:189–202.
36. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359–64.
37. WHO/UNICEF. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. Geneva: World Health Organization, 1994.
38. West KP. Extent of vitamin A deficiency among preschool children and women of reproductive age. J Nutr 2002;132:2857S–66S.
39. Gamble MV, Ramakrishnan R, Palafox NA, Briand K, Berglund L, Blaner WS. Retinol binding protein as a surrogate measure for serum retinol: studies in vitamin A-deficient children from the Republic of the Marshall Islands. Am J Clin Nutr 2001;73:594–601.
40. Semba RD, Yuniar Y, Gamble MV, Natadisastra G, Muhilal N. Assessment of vitamin A status of preschool children in Indonesia using plasma retinol-binding protein. J Trop Pediatr 2002;48:84–7.
41. de Pee S, Dary O. Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. J Nutr 2002;132:2895S–901S.
42. Donnen P, Dramaix M, Brasseur D, Bitwe R, Bisimwa G, Hennart P. The molar ratio of serum retinol-binding protein (RBP) to transthyretin (TTR) is not useful to assess vitamin A status during infection in hospitalised children. Eur J Clin Nutr 2001;55:1043–7.
43. Rosales FL, Chau KK, Haskell MH, Shankar AH. Determination of a cut-off value for the molar ratio of retinol-binding protein to transthyretin (RBP:TTR) in Bangladeshi patients with low hepatic vitamin A stores. J Nutr 2002;128:1681–7.
44. Haldimann M, Wegmüller R, Zimmermann MB. Determination of iodine concentration in salt dual fortified with iron and iodine. Eur Food Res Technol 2003;218:96–8.
45. Johnson LE. Oils, fats and margarine: overview of technology. In: Food fortification to end micronutrient malnutrition: state of the art. Ottawa: The Micronutrient Initiative/International Development Research Centre, 1997:22–6.
46. Favaro RMD. Evaluation of the effect of heat treatment on the biological value of vitamin A fortified soybean oil. Nutr Res 1992;12:1357–63.
47. Solon FS. A case report on the fortification of margarine with vitamin A: the Philippine experience. In: Food fortification to end micronutrient malnutrition: state of the art. Ottawa: The Micronutrient Initiative/International Development Research Centre, 1997:32–6.
48. Sridhar KK. Tackling micronutrient malnutrition: two case studies in India. In: Food fortification to end micronutrient malnutrition: state of the art. Ottawa: The Micronutrient Initiative/International Development Research Centre, 1997:27–31.
49. Solon FS, Latham MC, Guirriec R, Florentino R, Williamson DF, Aguilar J. Fortification of MSG with vitamin A: the Philippine experience. Food Technol 1985;39:71–7.
50. Muhilal A, Murdiana A, Azis I, Saidin S, Jahari AB, Karyadi D. Vitamin A fortified monosodium glutamate and vitamin A status: a controlled field trial. Am J Clin Nutr 1988;48:1265–70.
51. Chavez JF. Enrichment of precooked corn flour and wheat flour in Venezuela: a successful experience. In: Food fortification to end micronutrient malnutrition: state of the art. Ottawa: The Micronutrient Initiative/International Development Research Centre, 1997:62–5.
52. Solon FS. The progress of wheat flour fortification with vitamin A in the Philippines. In: Food fortification to end micronutrient malnutrition: state of the art. Ottawa: The Micronutrient Initiative/International Development Research Centre, 1997:66–71.
53. USAID/UNICEF. Opportunities for micronutrient interventions. Fortification of wheat flour with vitamin A: an update. New York: US Agency for International Development/UNICEF, 1998.
54. Arroyave G, Mejia LA, Aguilar JR. The effect of vitamin A fortification of sugar on the serum vitamin A levels of preschool Guatemalan children: a longitudinal evaluation. Am J Clin Nutr 1981;34:41–9.
55. Mora JO, Dary O, Chinchilla D, Arroyave G. Vitamin A sugar fortification in Central America: experience and lessons learned. Arlington, VA: The USAID Micronutrient Program/US Agency for International Development, 2000.
56. Staubli-Asobayire F. Development of a food fortification strategy to combat iron deficiency in the Ivory Coast. PhD dissertation. Zürich: Swiss Federal Institute of Technology, 2000.
57. Melse-Boonstra A, Pee S, Martini E, Halati S, Sari M, Kosen S, Muhilal, Bloem M. The potential of various foods to serve as a carrier for micronutrient fortification: data from remote areas in Indonesia. Eur J Clin Nutr 2000;54:822–7.
58. Melse-Boonstra A, Rexwinkel H, Bulux J, Solomons NW, West CE. Comparison of three methods for estimating daily individual discretionary salt intake: 24 hour recall, duplicate-portion method, and urinary lithium-labelled household salt excretion. Eur J Clin Nutr 1999;53:281–7.
59. Walter T, Olivares M, Hertrampf E. Field trials of food fortification with iron: the experience of Chile. In: Lönnerdal B, ed. Iron metabolism in infants. Boca Raton, FL: CRC Press, 1990:127–55.
60. Walter T, Pino P, Pizarro F, Lozoff B. Prevention of iron deficiency anemia: a comparison of high and low iron formulas in term healthy infants after 6 months of age. J Pediatr 1998;132:635–40.
61. Viteri FE, Alvarez E, Batres R, Torun B, Pineda O, Mejia LA, Sylvi J. Fortification of sugar with iron sodium ethylenediaminotetracetate (NaFeEDTA) improves iron status in semirural Guatemalan populations. Am J Clin Nutr 1995;61:1153–63.
62. Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe(111)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. Am J Clin Nutr 1989;49:162–9.
63. Van Thuy P, Berger J, Davidsson L, et al. Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. Am J Clin Nutr 2003;78:284–90.
64. Skikne B, Baynes RD. Iron absorption. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron metabolism in health and disease. London: Saunders, 1993:152–87.
65. Staubli-Asobayire F, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. Am J Clin Nutr 2001;74:776–82.
66. Thurnham DI, McCabe GP, Northrop-Clewes CA, Nestel P. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. Lancet 2003;362: 2052–8.
67. Stanbury JB, Ermans AE, Bourdoux P, et al. Iodine-induced hyperthyroidism: occurrence and epidemiology. Thyroid 1998;8:83–100.