# A multicenter community study on the efficacy of double-fortified salt

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## Abstract

**Background.** *Iron and iodine deficiencies affect more than 30% of the world's population. Typical Indian diets contain adequate amounts of iron, but the bioavailability is poor. This serious limiting factor is caused by low intake of meat products rich in heme iron and intake of phytates in staple foods in the Indian diet, which inhibits iron absorption.*

**Objective.** *To test the stability of double-fortified salt (DFS) during storage and to assess its efficacy in improving the iron and iodine status of the communities.*

**Methods.** *The stability of both iodized salt and DFS during storage for a 2-year period was determined. The bioefficacy of DFS was assessed in communities covering three states of the country for a period of 1 year. This was a multicenter, single-blind trial covering seven clusters. The experimental group used DFS and the control group used iodized salt. The salts were used in all meals prepared for family members, but determination of hemoglobin by the cyanmethemoglobin method was performed in only two or three members per family, and not in children under 10 years of age (n = 393 and 436 in the experimental and control groups, respectively). The family size was usually four or five, with a male: female ratio of 1:1, consisting of two parents with two or three children. Hemoglobin was measured at baseline, 6 months (midpoint), and 12 months (endpoint). Urinary iodine was measured in only one cluster at baseline and endpoint. All the participants were dewormed at baseline, 6 months, and 12 months.*

**Results.** *The iron and iodine in the DFS were stable during storage for 2 years. Over a period of 1 year, there was an increase of 1.98 g/dL of hemoglobin in the experimental group and 0.77 g/dL of hemoglobin in the control group; the latter increase may have been due to deworming. The median urinary iodine changed from 200 µg/dL at baseline to 205 µg/dL at the end of the study in the experimental group and from 225 µg/dL to 220 µg/dL in the control group. There was a statistically significant (p < .05) improvement in the median urinary iodine status of subjects who were iodine deficient (urinary iodine < 100 µg/L) in both the experimental and the control groups, a result showing that DFS was as efficient as iodized salt in increasing urinary iodine from a deficient to sufficient status. There was a statistically significant increase (p < .05) in hemoglobin in all seven clusters in the experimental group compared with the control.*

**Conclusions.** *The iron and iodine in the DFS are stable in storage for 2 years. The DFS has proved beneficial in the delivery of bioavailable iron and iodine.*

**Key words:** Iron and iodine fortification, salt

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## Introduction

Iron is the second most abundant metal in the earth's crust, yet iron deficiency is a worldwide public health problem [1, 2]. Anemia leads to considerable morbidity, mortality, impaired immune status, and decreased productivity. Iron deficiency in infancy causes impairments in physical development and cognition that may be irreversible [3–5]. Among micronutrient deficiencies, iron and iodine deficiencies affect > 30 % of the global population [6].

Typical Indian diets contain adequate amounts of iron, but the bioavailability of iron is poor because the intake of meat products rich in heme iron is low due to low socioeconomic status and also because of the presence of phytates that inhibit iron absorption in rice and wheat, the staple cereals of Indians. Thus, only 2% to 5% of the iron intake is absorbed, and it is not surprising that iron-deficiency anemia is widespread.

Among the public health approaches advocated for the control and prevention of iron deficiency are the distribution of supplements of medicinal iron and fortification of foods with a suitable iron compound. The former is recommended as a short-term measure to correct anemia, and the latter to improve the iron balance over a period of time and build up iron reserves. Since India already has a program to supply iodized salt, double fortification of salt with iron and iodine makes eminent sense.

Anemia is also a problem in the project areas of the BAIF Development Research Foundation (BAIF is the Bharatiya Agro Industries Foundation, one of India's oldest nongovernmental organizations, which has been involved in rural development for more than four decades). To address the problem on a larger scale, a field trial of double-fortified common salt was conducted in 2002–2004 by the BAIF Development Research Foundation. The Sundar Serendipity Foundation (SSF), Chennai, provided the double-fortified salt for the study.

Although there have been other studies on double-fortified salt in India [7] and by the Swiss Federal Institute Zurich [8] in Morocco and Côte d'Ivoire, it was felt that a multicenter study performed simultaneously in different parts of a vast country like India would throw light on the challenging task of combating anemia and iodine deficiency together.

The objective was to evaluate the use of double-fortified salt (DFS) as a public health measure in the control of anemia and iodine-deficiency disorders. The specific objectives were to test the stability of DFS in storage and provide DFS to selected families for 1 year in seven clusters, to determine the efficacy of salt in decreasing anemia and iodine deficiency disorders in this population.

## Methods and materials

### Production and supply of salt

Both DFS and iodized salt were obtained from SSF, Chennai. Twenty-two batches of iodized salt and DFS were prepared by SSF before the start of the study. These batches were transported to BAIF headquarters in Pune. They were then transported to the different study sites, where they were stored centrally and distributed on a monthly basis throughout the study. Control samples of the 22 batches prepared were used for the stability tests. The color of the DFS and iodized salt was tested using a reflectance meter, and there was no difference between the color of the DFS and iodized salt.

Salt was doubly fortified with ferrous sulfate monohydrate (Hiena Pharma, Mumbai) and potassium iodate (Calibre Chemicals, Mumbai). The ferrous sulfate was chelated in the laboratory of SSF with chelating agents, and the absorption promoter was added to enhance iron absorption. Ferrous sulfate was chelated with malic acid and sodium hexametaphosphate. The acidic pH was maintained by sodium dihydrogen phosphate, which served as an absorption promoter. The resulting chelated iron complex was white in color. The DFS contains 1,000 ppm of iron and 40 ppm of iodine, or 1 mg of iron and 40 μg of iodine per gram of salt. Our studies have shown that each person consumes about 10 g of salt per day. The DFS therefore provides about 10 mg of chelated iron and 400 μg of iodine per person per day. The iodine was encapsulated to prevent decomposition. Potassium iodate, the iodine compound, was coated in the SSF laboratory with an alkaline compound, food-grade sodium bicarbonate (P.D. Fine Chem, Bangalore), to keep it in an alkaline medium for stability. The potassium iodate was further encapsulated in cellulose acetate phthalate (GM Chemicals, Mumbai) and coated with a layer of silicone (Process Chemical Company, Mumbai) to provide heat resistance during cooking. The cellulose acetate phthalate coat protects the potassium iodate from the acidic environment of iron in the salt, thereby enhancing the shelf-life of iodine in the fortified salt as well as in the acidic medium of the stomach. Iodine is bioavailable and absorbed in the alkaline pH of the intestine, where the cellulose acetate phthalate coat disintegrates. Even though the iron is chelated, the environment of the salt is at an acidic pH, since the iron is not coated. The stability of iodine therefore is dependent on the cellulose acetate phthalate coat. In India it is mandatory for salt to contain iodine at a level of 30 ppm at the manufacturer's site.

The salt was packed in 1-kg bags and supplied to the families through self-help groups. Self-help groups are examples of community-based organizations at the lowest level. They are made up of 10 to 20 individuals, generally women. The idea is to meet regularly (at least every month), inculcate the habit of regular saving, and utilize the pooled money for individual needs as credit, with the repayment of loans contributing to increasing the pool. However, the self-help groups go beyond thrift-credit. They are platforms to discuss various issues relevant to the group and the village, including health and social issues. In fact, the groups play an important role in mobilizing the community, whether

it is for village cleanliness, childhood immunization, de-addiction drives, or accessing and implementing government schemes. The enterprising self-help groups may start and manage income-generating activities, so it seemed prudent and consistent with their mission to entrust them with the distribution of the salt.

## Storage stability studies

The stability of the iron and iodine contents in DFS and the iodine content in iodized salt were assessed for a period of 2 years. The control samples of the salts were stored at 30°C and 45% humidity in the SSF Laboratory, where the stability studies were performed. We chose 30°C and 45% humidity because they represent the average weather conditions of the sites where the study was conducted. Each batch of DFS produced was tested at 4-month intervals for iron and iodine, and each batch of iodized salt produced was tested at 4-month intervals for iodine, for a period of 2 years. The method of analysis of iron and iodine was according to the specifications of the Bureau of Indian Standards [9].

## Bioefficacy study

The project areas were the seven clusters of the BAIF European Union Project. The multicenter single-blind study was carried out in three states of the country: Karnataka (three clusters—Tumkur, Uttar Kanada, and Dharwad), Gujarat (two clusters—Surat and Bharuch), and Uttar Pradesh (two clusters—Pratapgad and Gonda). The clusters are administrative units for implementing the Transfer of Technologies for Sustainable Development project, which was funded by the European Union. Each cluster comprises a group of villages (an average of 20) covered under the project. For convenience, the clusters have been named after the districts where they are located. The members of the self-help groups were informed about the trial, and volunteer families were enrolled after informed oral consent had been obtained from the heads of the families. The study was approved by the Institutional Review Board of BAIF.

## Climate of the seven clusters

Tumkur has a dry climate, with an annual rainfall of 550 mm, a maximum temperature of 35°C in the summer, and a minimum temperature of 18°–20°C in the winter. Uttar Kanada lies close to the western ghats. It is mountainous, with an annual rainfall of 2,500 mm during the monsoon season, which lasts from June to September. The maximum temperature is 30°C in the summer, and the minimum is 10°C in the winter. Dharwad is in the plains, with an annual rainfall of 1,000 mm. The summer temperature peaks to 40°C, and the winter temperature is around 18°–20°C. Gonda

and Pratapgad lie in the Gangetic plains. The summer temperature in Gonda peaks up to 45°C. Winters are very cold, with temperatures dropping to 1°–2°C. Winters are very foggy, making movement very difficult. Because of the cold conditions, finger-pricking for phlebotomy becomes very difficult. Therefore, the time of starting the study was chosen to avoid performing phlebotomy during the winter. Pratapgad has scorching summers, with temperatures soaring to 45°C and winter temperatures dropping to 4°–5°C. Surat and Bharuch lie in the Narmada basin. The annual rainfall is 800–1,000 mm, summer temperatures are 40°–42°C, and winter temperatures are 18°–20°C.

## Sample size and selection

Earlier studies had shown a rise in hemoglobin of about 0.5 to 1 g/dL after a year's consumption of DFS [10]. To calculate the sample size, we assumed an increase of hemoglobin of 0.5 g/dL, with SD of 1.0 and a 95% confidence interval, power of 80% using the formula $2*(1.96 + 0.84)^2 * ((1)^2/(0.5)^2)$ and arrived at a sample size of 63 persons per group. Sample sizes of 63 persons per cluster in the experimental group and 63 persons per cluster in the control group were calculated for measurement of hemoglobin. Two to three members (preferably adults; children less than 10 years of age were excluded due to the difficulty of phlebotomy in young children and the reservations of parents of young children in consenting to the procedure) were included from each of the 30 families in the experimental group to constitute a sample size of 63 to 90 per cluster, and similarly 30 families with two or three members per family were included to constitute a sample size of 60 to 90 per cluster for the control. A total of seven clusters were selected.

The exact number of families in the cluster depended on the geographic distribution of the households. Some clusters had more than 60 families and some less. Surat and Bharuch, which were very close together, were considered as one unit. Since isolated villages were chosen for study, the sample size for hemoglobin estimation in Bharuch and Surat was around 30. Similarly, in Uttar Kanada, an isolated village was chosen, so the sample size for hemoglobin estimation was 28 in the experimental group. In the remaining clusters, the sample size was more than 60. The DFS and the iodized salt were supplied and consumed by all the members of the households, but the hemoglobin measurements were performed on only two or three members of the household.

## Study design

Each cluster had an experimental area where DFS was supplied and a control area where plain iodized salt was supplied. In each cluster, two adjoining villages were

selected randomly, and these formed the experimental and control groups. After the random selection process, it was determined that all clusters shared a similar economic background.

For a cluster, a village was a functional unit. In each village, self-help groups of women were identified, and volunteer families were listed. The salt was not provided free but was sold at the prevailing market price for common salt in the area. BAIF subsidized the remaining cost of the salt. Self-help groups were chosen to facilitate behavior change communication, the logistics of supply, and maintenance of records. Both the experimental and the control families were educated about anemia and the approach of using fortified salt in cooking all the meals of the family.

All members of the families in the experimental and control groups were dewormed with albendazole 400 mg at baseline, 6 months (midpoint), and 12 months (endpoint). This was essential because helminths compete for micronutrient absorption, and the intestinal tract had to be clear of worms for absorption of the nutrients.

The biochemical parameters assessed were hemoglobin and urinary iodine. Blood samples for hemoglobin analysis were collected at baseline, 6 months, and 12 months. Urinary iodine analysis was performed only in the Gujarat cluster at the beginning and the end of the trial in both the experimental and the control groups.

### Blood collection, storage, and hemoglobin analysis

Fingerprick blood samples were obtained with accurately calibrated hemoglobin pipettes. Twenty microliters of blood from the fingerprick was collected and added to 5 mL of Drabkin's solution. The optical density was read with a portable field colorimeter within a few hours of collecting the blood in a central area in the village. Hemoglobin was determined by the cyanmethemoglobin method as described by Dacie and Lewis [11]. The same batch of hemoglobin standards was used to calibrate the colorimeter in all the clusters. Samples were analyzed by a study worker who was unaware of the previous results.

In all seven clusters, hemoglobin measurements were performed three times on 393 subjects in the experimental group and 436 in the control group. Twenty-six individuals in the experimental and 21 in the control group who were not present for all three rounds of hemoglobin analysis were excluded from statistical analysis.

Urinary iodine level was determined by the Pino modification of the Sandell Kolthroff reaction, using the portable colorimeter [12]. Random urine samples were collected by technicians from the households and analyzed within a few hours of collection.

### Statistical analysis

Data were analyzed with SPSS version 11 and Microsoft Excel. ANOVA and *t*-tests were performed to determine the differences in hemoglobin over time and between groups. Median urinary iodine values were tested by the Wilcoxon signed-ranks test and the Mann-Whitney test.

## Results

Benefits noted with the use of DFS were an increase in well-being and a decrease in menstrual problems in women. These changes were assessed by questionnaires administered to the heads of households and the women each family. Overall, the people would prefer to consume DFS if it were available at an affordable price.

### Characteristics and stability of iodized salt and DFS

#### *Storage quality*

No color changes in the salts were observed during transport or storage. Although the study lasted for only 1 year in each cluster, the entire study in all three states of the country took 2 years to complete because the study did not start simultaneously in all seven clusters. Hemoglobin analysis was performed with the same colorimeter in all seven clusters. Thus, the study started in each cluster after baseline hemoglobin measurements had been performed. The salt, however, was prepared before the start of the study. Since the entire study lasted for 2 years, analysis of stability for a period of 2 years was performed. The stability of iodine in iodized salt and of iron and iodine in DFS is given in **table 1.** Iron was found to be stable. ANOVA found no significant difference ($p > .05$) between the stability of iodine in DFS and in iodized salt.

#### *Taste*

There were no complaints regarding taste. People noted that the amount of salt to be added to food was slightly less than usual. The right amount was mastered over a period of time. People also observed that food turned slightly sour when kept for more than 6 hours. This may be due to the absorption promoters. The benefits of the salts were assessed by questionnaires addressed to the heads of the households and the women in the families.

### Results of efficacy study

The mean ($\pm$ SD) baseline hemoglobin was $10.34 \pm 2.56$ g/dL in the experimental group and $10.29 \pm 2.62$ g/dL in the control group, and there was no significant difference between the groups. After 6 months of

TABLE 1. Stability of iodine in iodized salt and of iron and iodine in double-fortified salt at 30°C and 45% relative humidity [a]

| Measurement | Baseline | 4 mo | 8 mo | 12 mo | 16 mo | 20 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| Iodine in iodized salt[b] | 41.30 ± 3.08 | 39.80 ± 2.48 | 37.50 ± 1.86 | 38.15 ± 2.27 | 32.60 ± 3.14 | 31.10 ± 2.89 | 29.20 ± 2.96 |
| Iodine in double-fortified salt[b] | 40.90 ± 2.35 | 38.80 ± 3.16 | 37.10 ± 2.43 | 38.01 ± 2.43 | 31.90 ± 2.98 | 30.50 ± 2.57 | 27.70 ± 3.61 |
| Iron in double-fortified salt[c] | 1,026.70 ± 35.20 | 1,014.40 ± 27.18 | 1,013.60 ± 17.60 | 1,008.86 ± 33.80 | 1,008.13 ± 32.90 | 1,006.00 ± 23.33 | 996.20 ± 35.16 |

a. The values are means ± SD of 22 batches prepared for the study. Iron and iodine concentrations are given in parts per million. Differences are considered significant if $p < .05$.
b. No significant differences between the stability of iodine in double-fortified salt and iodized salt for the entire period.
c. No significant change in iron levels throughout the study.

intervention, the mean hemoglobin in the experimental group increased to 11.30 ± 2.21 g/dL, and after 1 year it further increased to 12.32 ± 1.93 g/dL. These increases at 6 months and 1 year were statistically significant ($p < .05$). The increase of 1.98 g/dL in 1 year could be

TABLE 2. Changes in hemoglobin concentration in groups receiving double-fortified salt (experimental group) and iodized salt (control group)[a]

| Time | Experimental group ($n = 393$) | Control group ($n = 436$) |
|---|---|---|
| Baseline | 10.34 ± 2.56[b] | 10.29 ± 2.62 |
| Midpoint (6 mo) | 11.30 ± 2.21[b,c] | 10.61 ± 2.47[c,d] |
| Endpoint (1 yr) | 12.32 ± 1.93[b,c] | 11.06 ± 2.59[c,d] |

a. Hemoglobin concentrations are expressed as means ± SD (g/dL). A difference is considered significant if $p < .05$.
b. Significant improvement in the experimental group from baseline to midpoint and from midpoint to endpoint.
c. Significant improvement in the experimental group compared with control.
d. Significant improvement in the control group from midpoint to endpoint.

due to the use of DFS and deworming. In the control group, the mean hemoglobin increased from 10.29 ± 2.62 g/dL at baseline to 10.61 ± 2.47 g/dL after 6 months and to 11.06 ± 2.59 g/dL after 1 year, and the increase from 6 months to one year was statistically significant. The increase of 0.77 g/dL in the control group in 1 year could be due to deworming, since the elimination of the helminths could have resulted in better absorption of the nutrients from the diets (**table 2**).

The increase in hemoglobin of 1.98 g/dL in the experimental group is significantly ($p < .05$) greater than the increase of 0.77 g/dL in the control group. The increase of 0.77 g/dL of hemoglobin in the control group could be attributed to deworming, and if this value is subtracted from 1.98 g/dL, the increase in hemoglobin in the experimental group (due to deworming and intake of fortified salt), then the increase of hemoglobin of 1.21 g/dL could be attributed to the bioavailability of iron from the fortified salt.

The increase in hemoglobin is significantly greater ($p < .05$) in the experimental group than the control group in all seven clusters. The increase in hemoglobin

TABLE 3. Changes in hemoglobin concentration in groups receiving double-fortified salt (experimental group) and iodized salt (control group) according to cluster[a]

| Cluster | N | Baseline | Endpoint (1 yr) | Change | N | Baseline | Endpoint (1 yr) | Change |
|---|---|---|---|---|---|---|---|---|
| | | Experimental group | | | | Control group | | |
| Uttar Kanada | 28 | 9.23 ± 2.30[b] | 10.08 ± 2.26[b] | 0.85 ± 0.40[c] | 47 | 9.34 ± 2.26 | 9.32 ± 2.46 | −0.02 ± 0.60[c] |
| Dharwad | 59 | 10.63 ± 2.96[b,d] | 11.55 ± 2.65[b,e] | 0.93 ± 0.43[c] | 81 | 9.35 ± 3.10[d] | 9.40 ± 3.13[e] | 0.05 ± 0.47[c] |
| Tumkur | 90 | 9.47 ± 2.74[b] | 13.04 ± 1.21[b,e] | 3.58 ± 2.73[c] | 99 | 9.44 ± 2.31[f] | 11.97 ± 2.08[e,f] | 2.53 ± 1.86[c] |
| Pratapgad | 81 | 12.49 ± 1.40[b] | 13.68 ± 1.03[b,e] | 1.19 ± 0.67[c] | 106 | 12.39 ± 1.38[f] | 12.64 ± 1.33[e,f] | 0.25 ± 0.62[c] |
| Gonda | 88 | 9.77 ± 2.02[b,d] | 11.91 ± 1.40[b] | 2.13 ± 1.10[c] | 69 | 11.06 ± 1.98[d,f] | 11.57 ± 1.57[f] | 0.51 ± 0.84[c] |
| Surat and Bharuch | 47 | 9.31 ± 1.75[b,d] | 11.13 ± 1.36[b,e] | 1.82 ± 1.10[c] | 34 | 7.94 ± 1.90[d,f] | 8.34 ± 1.84[e,f] | 0.40 ± 0.47[c] |
| Total | 393 | | | | 436 | | | |

a. Hemoglobin concentrations are expressed as means ± SD (g/dL). A difference is considered significant if $p < .05$.
b. Endpoint hemoglobin significantly higher than baseline hemoglobin in experimental group.
c. Change in hemoglobin at endpoint significantly more in experimental group than in control group.
d. Baseline hemoglobin significantly different in experimental and control groups.
e. Endpoint hemoglobin significantly higher in experimental group than in control group.
f. Endpoint hemoglobin significantly higher than baseline hemoglobin in control group.

in the experimental groups varies from cluster to cluster, from a high of 3.58 g/dL in the Tumkur cluster to a low of 0.85 g/dL in the Uttar Kanada cluster. In the control groups, the highest increase in hemoglobin was in the Tumkur cluster ( 2.53 g/dL) and the lowest was a decrease of 0.02 g/dL in the Uttar Kanada cluster (**table 3).** Although the experimental and control groups were selected from similar communities in each cluster, with the same dietary habits and belonging to the same socioeconomic groups, there were significant ($p < .05$) differences in baseline hemoglobin status between the experimental and control groups in Dharwad, Gonda, Surat, and Bharuch. In the other three clusters, Uttar Kanada, Tumkur, and Pratapgad, there were no significant differences in mean baseline hemoglobin between the experimental and control groups (**table 3).** Baseline hemoglobin also varied from 9.23 g/dL in Uttar Kanada to 12.49 g/dL in Pratapgad. These differences may be due to wide variations in food habits and environmental sanitation between different states of India. Despite these baseline differences in different states, in all age categories and in both males and females, there was a significantly higher increase in hemoglobin in the experimental group than in the control group ($p < .05$) (**table 4).**

### Results of urinary iodine studies

Urinary iodine studies were done only in Gujarat in the Bharuch and Surat clusters. This was because analysis of urinary iodine is much more difficult than analysis of hemoglobin, and we were able to train technicians to do the urinary iodine analysis in Gujarat only. Urinary iodine analysis was performed in 47 adults in the experimental group and 34 adults in the control group

TABLE 4. Changes in hemoglobin concentration from baseline to 1 year in groups receiving double-fortified salt (experimental group) and iodized salt (control group) according to sex and age[a]

| Sex | Age (yr) | Experimental group | | Control group | |
|---|---|---|---|---|---|
| | | N | Change | N | Change |
| Female | 10–18 | 30 | 2.09 ± 1.58 | 28 | 0.56 ± 1.00 |
| | 19–30 | 79 | 2.34 ± 1.74 | 70 | 0.35 ± 1.10 |
| | 31–45 | 78 | 1.88 ± 1.87 | 130 | 0.81 ± 1.53 |
| | 46–65 | 38 | 2.31 ± 2.14 | 50 | 1.43 ± 1.88 |
| Male | 10–18 | 26 | 1.94 ± 2.17 | 40 | 0.83 ± 1.33 |
| | 19–30 | 61 | 1.49 ± 1.32 | 30 | 0.75 ± 1.50 |
| | 31–45 | 45 | 1.82 ± 1.81 | 58 | 0.66 ± 1.26 |
| | 46–65 | 36 | 1.98 ± 2.10 | 30 | 0.94 ± 1.52 |
| Total | | 393 | | 436 | |

a. Hemoglobin concentrations are expressed as means ± SD (g/dL). There was a significantly ($p < .05$) higher increase in hemoglobin concentration in the experimental group than in the control group for both males and females of all age groups.

on whom hemoglobin tests were also performed. The median urinary iodine at baseline was 200 µg/L in the experimental group and 225 µg/L in the control group. There was no significant difference ($p > .05$) between the experimental and control groups at baseline.

At endpoint, urinary iodine increased to 205 µg/L in the experimental group and was reduced marginally to 220 µg/L in the control group. Thus, median iodine values were maintained at around 200 µg/L throughout the study in both the experimental and control groups. This could imply that the iodine from the DFS is absorbed in the same manner as the iodine from iodized salt. Thus, the bioavailability of iodine from DFS and iodized salt is similar (**table 5**).

TABLE 5. Urinary iodine excretion values in groups receiving double-fortified salt (experimental group) and iodized salt (control group) in Gujarat-Surat and Bharuch clusters according to baseline iodine status[a]

| Iodine status | Experimental group | | | Control group | | |
|---|---|---|---|---|---|---|
| | N | Baseline | Endpoint (1 yr) | N | Baseline | Endpoint (1 yr) |
| Deficient | 7 | 75 (50–85)[d,h] | 225 (120–600)[e,h] | 14 | 50 (35–85)[d,h] | 220 (95–340)[e,h] |
| Sufficient | 40 | 200 (100–600)[f] | 195 (70–500)[g] | 20 | 325 (100–600)[f] | 207.5 (60–600)[g] |
| Whole group | 47 | 200 (50–600)[b] | 205 (70–600)[c] | 34 | 225 (35–600)[b] | 220 (60–600)[c] |

a. Urinary iodine excretion values are expressed as median (range) in micrograms per liter. Subjects with baseline urinary iodine values more than and less than 100 µg/L were considered iodine sufficient and deficient, respectively. Differences are considered significant if $p < .05$ according to the Mann-Whitney test or the Wilcoxon signed-ranks test.
b. No significant difference between experimental and control groups at baseline.
c. No significant difference between experimental and control groups at endpoint.
d. Significant difference in baseline iodine status between the experimental and control groups of subjects who were iodine deficient at baseline, with the control group having a lower urinary iodine concentration than the experimental group.
e. No significant difference in endpoint iodine status between the experimental and control groups of subjects who were iodine deficient at baseline.
f. Significant difference in baseline iodine status between the experimental and control groups of subjects who were iodine sufficient at baseline, with the control group having a higher urinary iodine concentration than the experimental group.
g. No significant difference in endpoint iodine status between the experimental and control groups of subjects who were iodine sufficient at baseline.
h. Significant increase from baseline to endpoint among subjects who were iodine deficient at baseline in both the experimental and the control groups.

In both the experimental and the control groups, the absorption of iodine, which is reflected by the increase in urinary iodine excretion, was significantly higher in the subgroup of those who were iodine deficient, i.e., with a median urinary iodine excretion of less than 100 µg/L at baseline. At the end of the study, in both the experimental and the control groups, median urinary iodine levels increased from the deficient level of less than 100 µg/L to the sufficient level of more than 100 µg/L (**table 5).** There was no significant difference ($p > .05$) in the endpoint status between the experimental and control groups in those subjects who were iodine deficient at baseline. Thus, iodized salt and DFS were equally effective in changing the iodine-deficient status to an iodine-sufficient status in both the experimental and the control groups.

There was a significant difference ($p < .05$) between the experimental and the control groups in the baseline status of those who were iodine sufficient at baseline, with the control group having a higher median urinary iodine than the experimental group. There was no significant difference ($p > .05$) between the experimental and control groups after 1 year of intervention in those subjects who were iodine sufficient at baseline. This means that iodized salt and DFS were equally effective in maintaining urinary iodine status in subjects who were iodine sufficient at baseline. At the endpoint, there was only one subject in the experimental group and one subject in the control group whose urinary iodine was less than the sufficient status of 100 µg/L. The reason for this is not known.

## Discussion

The literature survey on DFS showed that maintaining the stability of iodine in the presence of iron is difficult [7]. The bioavailability of iron is also in question. In the study of DFS conducted by the Indian National Institute of Nutrition, there was a drop in hemoglobin of the children in both the experimental and the control groups [7]. Earlier studies used only inorganic iron compounds without biopromoters [7, 13]. Earlier studies also used ferrous sulfate encapsulated with hydrogenated soybean oils. This DFS developed a yellow coloration when the moisture content of the salt was 3% to 4%, even though the mean hemoglobin increased by 14 g/L [14]. In another study testing the stability of 16 forms of encapsulated iron in salts of north and west Africa, the authors found that encapsulated ferrous iron caused unacceptable color changes to salt. The authors felt that this might be because current encapsulating technology uses hydrogenated plant oils that do not sufficiently prevent moisture penetration, and iron solubility and capsule integrity are further compromised by mechanical abrasion during salt mixing. The capsules also melt at 45° to 50°C and may cause unwanted sensory changes during food preparation [8]. Other studies have used iron sources such as micronized ground ferric pyrophosphate (FePP) and iodine with no biopromoter added [15]. In our study we used ferrous sulfate as the iron source, but we chelated the iron and added an absorption promoter. Because the iron is well chelated, it does not react with the salt to produce discoloration and does not react with the food preparations to produce sensory changes. If a high-quality salt is used with low calcium and magnesium content, the chelated iron compound can be directly added to the salt and no microencapsulation is required, as was done in this study. However, if iron has to be added to salt with high magnesium, calcium, or moisture content, then microencapsulating the iron compound with compounds like glyceryl stearate or edible waxes is preferable. Thus, we feel that chelation of the ferrous sulfate not only increases its bioavailability but also prevents the interaction of ferrous sulfate with the salt and the food that produces yellow colorations in the salt during storage or color changes in food preparations.

The Micronutrient Initiative developed a DFS containing potassium iodide coated with maltodextrin and ferrous fumarate [16]. However, nonencapsulated ferrous fumarate added to low-grade salt from developing countries produced unacceptable dark-brown color changes [8]. The DFS developed by the Indian National Institute of Nutrition (NIN) uses ferrous sulfate and sodium hexametaphosphate without microencapsulation, and the stability of the iodine is dependent on the quality of salt. In the presence of magnesium chloride as an impurity, the salt lost a significant amount of iodine [10]. In field trials of the NIN DFS, one study found no overall benefit on hemoglobin concentrations, whereas in a second study, the hemoglobin concentrations decreased significantly in both the DFS and the iodized salt groups, but to a lesser degree in the DFS group [7]. This may be because the iron was not chelated and no absorption promoters were added.

We used chelated ferrous sulfate with biopromoters to ensure the maximum bioavailability of iron to enable bioabsorption of iron even in the presence of phytates, which are present in abundance in the Indian diet. We have shown in our earlier bioefficacy studies of DFS fortified with iron and iodine that there is an increase in hemoglobin among tea pickers using fortified salt, along with an improvement in their productivity [17]. We feel that chelated ferrous sulfate has a higher bioavailability than ferrous sulfate but without the problems caused by ferrous sulfate, such as coloration of the salt or food during cooking.

The quantity of salt consumed by a person per day is about 10 g. The fortified salt contains 10 mg of iron per 10 g of salt. Thus, the use of DFS in all the food preparations ensured a supply of small quantities of iron throughout the day. The types of food consumed

in the three states are quite varied. In Uttar Pradesh and Gujarat, wheat is the predominant staple, whereas in Karnataka, the main staple is rice. However, there was a statistically significant improvement in hemoglobin in all the seven clusters. This may be because the iron was delivered in repeated small doses throughout the day, and fractional absorption of nonheme iron increases with decreasing dose [18]. Moreover, the salt has chelated ferrous sulfate with biopromoters that enhance the absorption of iron not only from the salt, but also from the food, as seen in other fortification studies that have used biopromoters or chelators [19–23]. Deworming alone may have caused an increase in hemoglobin by reducing blood loss and increasing iron absorption.

Malaria was not a major problem in any of the study areas, but infestation with helminths such as ascaris may be quite common, and this may be the reason for the increase in iron status in the control group where deworming alone caused an increase in hemoglobin status. The degree of infestation by helminths may be different in the seven clusters, but it might have been highest in the Tumkur cluster, where there was an increase in hemoglobin of 2.5 g/dL in the control group. This study also suggests the importance of deworming in studies, especially in rural areas, as pointed out in other studies [24, 25].

DFS is also an ideal vehicle for iodine. In our study we found no statistically significant differences between iodized salt and DFS in losses of iodine in storage. The potassium iodate in iodized salt was not microencapsulated. The potassium iodate in the DFS was microencapsulated to increase the shelf-life of iodine in the salt and to increase iodine bioavailability in the human system. The finding of no significant difference between iodized salt and DFS in loss of iodine during storage shows that the microencapsulation of iodine in DFS has done its job in protecting the potassium iodate from the harsh effects of iron, and that thus microencapsulation has prevented iodine losses. Similarly, the absorption of iodine from iodized salt and DFS was very similar, as seen from the urinary iodine changes in both the iodized salt group and the DFS group.

After use of the salt for 1 year, which included the rainy season, 98% to 100 % of the households rated the taste and color of DFS as acceptable. In fact, since the study was single blinded, the families did not know whether they were receiving iodized salt or DFS. There was no perceptible difference between iodized salt and DFS.

The cost of the chelated iron complex used in 1 kg of salt was 5 US cents. The cost of 1 kg of salt was 5 US cents. The microencapsulated potassium iodate used in 1 kg of salt cost 0.3 US cent. The total cost of the DFS per kilogram was 10.3 US cents. The cost of uncoated potassium iodate without microencapsulation in 1 kg of iodized salt is 0.1 US cent. The increase in cost of potassium iodate due to coating and microencapsulation is only 0.2 US cent.

In this study, we used salt of high purity and microencapsulated the iodine in DFS. However, the same iron complex can be used in salt of lower purity if the iron complex is also microencapsulated. We observed a significant improvement in hemoglobin status in the group using DFS among all age groups and among both males and females, which demonstrates iron availability. Iodine bioavailability is demonstrated by the improvement in urinary iodine. Thus, we feel that DFS with chelated iron compounds can be used as an effective strategy to combat micronutrient malnutrition in developing countries.

## Acknowledgments

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