Double-Fortified Salt Is Efficacious in Improving Indicators of Iron Deficiency in Female Indian Tea Pickers

Jere D. Haas, Maike Rahn, Sudha Venkatramanan, Grace S. Marquis, Michael J. Wenger, Laura E. Murray-Kolb, Annie S. Wesley, and Gregory A. Reinhart

Abstract

Poor iron status affects 50% of Indian women and compromises work productivity, cognitive performance, and reproduction. Among the many strategies to reduce iron deficiency is the commercial fortification of iodized table salt with iron to produce a double-fortified salt (DFS). The objective of this study was to test the efficacy of DFS in reducing iron deficiency in rural women of reproductive age from northern West Bengal, India. The participants were 212 women between 18 and 55 y of age who worked as full-time tea pickers on a large tea estate. Participants in the randomized, controlled, double-blind study were assigned to use either DFS or a control iodized salt for 7.5 to 9 mo. The DFS was fortified with 3.3-mg ferrous fumarate (1.1-mg elemental iron) per kg of iodized salt, whereas the control salt contained only iodine (47 mg/kg potassium iodate), and both salt varieties were distributed gratis to the families of participants at 0.5 kg/mo for each 2 household members. At baseline, 53% of participants were anemic (hemoglobin <120 g/L), 25% were iron deficient (serum ferritin <12 μg/L), and 23% were iron-deficient anemic. Also, 22% had a transferrin receptor concentration >8.6 mg/L and 22% had negative (<0.0 mg/kg) body iron stores. After 9 mo the participants receiving DFS showed significant improvements compared with controls in hemoglobin (+2.4 g/L), ferritin (+0.13 log10 μg/L), and body iron (+1.43 mg/kg), with change in status analyzed by general linear models controlling for baseline values. This study demonstrated that DFS is an efficacious approach to improving iron status and should be further evaluated for effectiveness in the general population. This trial was registered at clinicaltrials.gov as NCT01032005.

Introduction

Iron deficiency is the most common nutrient deficiency worldwide (1,2), with an estimated 30% prevalence of anemia in nonpregnant women (3). In India, where this study was conducted, an estimated 52% of nonpregnant women of reproductive age are anemic (3), with much of this attributed to iron deficiency. Iron deficiency anemia (IDA)1 and iron deficiency without anemia pose a serious public health problem because they affect human capital formation and quality of life (4). The nutritional deficiency has also been linked to impaired physical performance (5) and endurance (6), muscle fatigue (7), reduced worker productivity (8,9), impaired behavior and cognition, and suboptimal maternal-infant interactions (10,11). Affordable access to better iron nutrition is needed in marginalized, poor populations. Fortification of salt as a vehicle for iron delivery is promising because it engages existing systems for producing and distributing a widely used and affordable food (12,13). Recent technological advances—specifically, microencapsulation of ferrous fumarate with a soy stearene coat (14,15)—have resulted in a low-cost formulation of double-fortified salt (DFS) with iron and iodine that prevents the chemical interaction of iodine with iron while maintaining stability in storage under tropical conditions.

The efficacy of DFS in improving hemoglobin has been assessed in Indian female tea pickers (16) and in Ghanaian women (17); both studies demonstrated a significant increase in hemoglobin in the DFS group compared with a control group that used only iodized salt. The difference in change in hemoglobin ranged from 1 to 2 g/L, with both studies demonstrating that DFS was efficacious in improving hemoglobin. However, the studies did not measure changes in iron status.

1 Supported by the Micronutrient Initiative, Ottawa, Canada. This is a free access article, distributed under terms (http://www.nutrition.org/publications/guidelines-and-policies/license/) that permit unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

2 Author disclosures: J. D. Haas, M. Rahn, S. Venkatramanan, G. S. Marquis, M. J. Wenger, L. E. Murray-Kolb, and G. A. Reinhart, no conflicts of interest.

3 Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

4 Abbreviations used: AGP, α-1-acid glycoprotein; CRP, C-reactive protein; DFS, double-fortified salt; GLM, general linear model; IDA, iron deficiency anemia; MCV, mean corpuscular volume; MUAC, mid-upper arm circumference; SRL, Super Religare Laboratory; sTfR, soluble transferrin receptor.

5 To whom correspondence should be addressed. E-mail: jdh12@cornell.edu.

© 2014 American Society for Nutrition.
Manuscript received July 28, 2013. Initial review completed August 30, 2013. Revision accepted March 10, 2014. First published online April 17, 2014; doi:10.3945/jn.113.183228.
Participants and Methods

Participants. The project was conducted from June 2009 to August 2010 at the Panighatta Tea Estate in the Darjeeling District of India. The Darjeeling District is located in the northern part of the state of West Bengal. The tea estate is located on a flat plain (terai) 20 km outside the city of Siliguri at an altitude of 150 m above sea level. The mean monthly temperature ranges between 17 and 28°C, and the mean annual precipitation is 3266 mm, with heavy monsoon rains between May and October. Participants were female laborers 18 to 55 y of age working as full-time, experienced tea pickers on the tea estate. Women picked tea 6 d/wk for a total of 6 h/d divided equally between morning and afternoon and a 2-h lunch break. Tea production ranged from 25 to 68 kg/d per person, depending on the season. Women lived on the tea estate with their families in 9 villages (known as “lines”) of ~50 households per village, and belonged to Nepali or Adivasi ethnic groups. Most women were born and raised on the tea estate and continued a multigenerational family tradition of picking tea.

Study design. The study design was a double-blind, randomized, controlled food-fortification trial. A call for volunteers was initiated through a blood screening for anemia made available to all adult female residents of the tea estate. Of the 498 women screened, 217 were not qualified for a variety of reasons (age, pregnancy, health, residence, or occupation), and 281 were eligible for complete iron status assessment at baseline. Following the analysis of the venous blood sample, 36 participants were excluded for a variety of reasons (Fig. 1), leaving 245 women to continue with the study. All women with a hemoglobin concentration of <80 g/L were excluded and treated with 60 mg/d of ferrous sulfate for 30 d with continued treatment as needed for an additional 30 d. Anthelmintic treatment with 200 mg of albendazole was administered to all eligible participants 4 wk before the initial baseline blood collection and at the study midpoint.

The qualified women were between 18 and 55 y of age, healthy, and not pregnant. They were experienced full-time tea pickers and lived on the tea estate. To ensure approximate equal distribution of anemia in the treatment and control groups, the eligible women were stratified based on hemoglobin concentrations into a mildly-to-moderately anemic group (hemoglobin = 80 to 119 g/L; n = 133) and a non-anemic group (hemoglobin = 120 to 140 g/L; n = 112). Women from each stratum were randomly assigned into either a DFS group or control group that used only iodized salt. Loss to follow-up resulted in the final sample of 212 women, with 104 in the DFS group and 108 in the control group.

Two rounds of venous blood collection were scheduled over a 5- to 6-wk period at both baseline and end line. Every effort was made to collect blood samples at end line that corresponded to exactly 10 mo from baseline blood collection. Blood was collected at the hospital on the tea estate by trained phlebotomists from the Super Religare Laboratory (SRL). The samples were then stored on ice until delivered the same day by car to the SRL branch in Siliguri for blood sample preparation for next day air shipment to the central SRL in Kolkata.

Written informed consent was obtained from all participants at screening and again at baseline. The study was approved by the institutional review boards of Cornell University and McGill University and the ethics committee of the Child in Need Institute (India), the collaborating institution responsible for logistic support.

Salt. The treatment group consumed DFS containing 47 mg/kg of potassium iodate and 3.3 mg of microencapsulated ferrous fumarate (1.1-mg elemental iron) per gram of salt. The microencapsulated DFS premix was manufactured by the University of Toronto under a research grant from the Micronutrient Initiative, Ontario, Canada (14). Ankur Chemfoods produces consumer market iodized salt by crushing, washing, and drying raw sea salt and spraying it with potassium iodate. The DFS premix was then added to the iodized salt. The DFS premix with encapsulated ferrous fumarate was manufactured at Pam Glatt Pharma Technologies. Stability, organoleptic, and the stability testing of the DFS was previously undertaken by Andersson et al. (18) for households near Bangalore. The authors observed some discoloration and black specks, but the salt was still considered acceptable for consumption by all of the participants. The control group used only iodized salt, produced by the same salt company that produced the DFS. To mimic the DFS in appearance, a premix of sodium fumarate was added to the iodized control salt. Based on salt disappearance during a study of rural Indian households in Karnataka, India (18), mean elemental iron intake from DFS for adults was estimated to be ~10 mg/d. From this estimate of iron intake, we calculated the total sample size of 164 participants to observe a significant improvement in serum ferritin. This calculation assumed that iron absorption from DFS would be 10%, and that mean ferritin would increase in iron-depleted, non-anemic women from 15 to 26 μg/L in 4 mo when it should stabilize prior to the anticipated end of the 8-mo intervention period. We also accounted for 50% iron deficiency without anemia, 25% prevalence of inflammation [high α-1 acid glycoprotein (AGP) or C-reactive protein (CRP)] at baseline or end line, and a 25% dropout rate, which would result in a final sample size of 112 participants with potential to show a significant improvement in iron status.

To ensure blinding and improve participants’ compliance, the DFS and control salt were delivered to the tea estate in 500-g bags marked with 4 color codes (red, blue, green, and black). Two colors per type of salt were assigned by the salt manufacturer, resulting in random assignment of the 245 participants into 4 color-coded groups. Households received 1 bag for every 2 household members each month; the salt was used ad libitum in their food preparation. Women were instructed to finish any opened salt bags before opening a new one to facilitate assessment of salt consumption. Salt was also available between monthly distributions if needed. In addition, free iodized salt was distributed monthly to all nonparticipating households on the tea estate in 1-kg bags without color codes. No other salt was sold on the tea estate during the study period, and local food shops and vendors were compensated for the loss of income from salt sales. The exact content of each color-coded bag of salt, DFS or iodized control, was known only to the salt manufacturer, who retained the codes until the first round of data analysis was completed to determine the difference in iron-status response across the 4 color groups. After this the codes were revealed with green and red belonging to DFS and black and blue to the control salt. Experimental salt distribution began on October 15, 2009 and finished on August 31, 2010. Participants were exposed to the DFS or control salt between 7.5 and 9.0 mo depending on the date of the end line blood sampling.

Biochemical data. Hemoglobin, mean corpuscular volume (MCV), serum ferritin, soluble transferrin receptor (sTfR), CRP, AGP, vitamin B-12, and serum folate were measured via venous blood sample at baseline and end line. Initial hemoglobin concentrations during screening were assessed with a portable calibrated photometer (HemoCue AB) from a finger puncture. Blood hemoglobin concentration, hematocrit, and MCV were analyzed with a Coulter Counter (Beckman) and serum ferritin and CRP by chemiluminescent immunoassay on the Immulite 2000 at the Kolkata branch of the Clinical Research Services of SRL. Frozen serum samples were shipped by air to the molecular diagnostics laboratory in Lucknow, India, for analysis of sTfR by ELISA (BioVendor), folate and vitamin B-12 on the Immulite 2000, and AGP by radial immunodiffusion.
At midpoint, capillary blood hemoglobin was assessed via HemoCue. Because the equation to compute total body iron reported by Cook et al. (19) used sTfR determined by the Ramco ELISA kit (Ramco Laboratories), we converted the BioVendor-derived sTfR to Ramco-adjusted sTfR with the prediction equation derived from 35 random duplicate samples:

$$sTfR_{\text{Ramco}} = 1.821 \times sTfR_{\text{BioVendor}}^{0.739}, \quad R^2 = 0.90$$

Body iron was derived from the ratio of sTfR$_{\text{Ramco}}$ and ferritin by the equation from Cook et al. (19):

$$\text{Body Iron (mg/kg)} = \left(\frac{\log (sTfR/\text{ferritin}) - 2.8229}{0.1207}\right)^2$$

To adjust ferritin for concurrent inflammation, reflected in elevated CRP and AGP values, the correction factors developed by Thurnham et al. (20) were applied to reduce ferritin values down to noninflammatory concentrations. Analysis of ferritin and body iron response to intervention was performed using both adjusted and unadjusted values and found to not differ. Therefore, only analysis of unadjusted ferritin and body iron values are reported.

Anemia was defined as hemoglobin $<120$ g/L, and IDA was defined as anemia with ferritin $<12$ mg/L or sTfR $>8.6$ mg/L.

Salt consumption. An individual participant’s salt consumption was estimated from 1 randomly chosen day at both midpoint and end line with food weighed at lunch, the major meal, and by 24-h dietary recall. Daily salt consumption for each participant was calculated from the mean intake at midpoint and end line. Total household salt use was assessed by weighing salt bags on 3 consecutive days at 2 randomly chosen time points during the intervention period. Daily household salt consumption was calculated by averaging intake values for the 2 time points. Consumption per household member was approximated by dividing the mean daily salt use by the number of household members. Iodine excretion was assessed at baseline and end line with a single spot urine sample; urinary iodine was analyzed by the molecular diagnostics laboratory with a fast colorimetric method (21).

Anthropometry and demographics. Age, height, weight, and mid-upper arm circumference (MUAC) were measured at baseline, midpoint, and end line. Height was taken to the nearest millimeter with a field stadiometer, weight was taken on a digital scale to the nearest 0.5 kg, and a constant for the weight of clothing, determined to be 0.465 kg, was subtracted to calculate net weight. MUAC was measured with a flexible tape to the nearest millimeter. BMI was calculated as weight (kg)/height (m$^2$). Ethnicity (Adivasi or Nepali), diet (vegetarian or nonvegetarian), and socioeconomic indicators (marital status, literacy, schooling, income, number of children and adults in household, quality of housing) were assessed with questionnaires.

Statistical analysis. Statistical analysis was performed with SAS, version 9.3 (SAS Institute). A difference-in-difference analysis was used to assess the efficacy of the intervention with unadjusted means of hemoglobin, ferritin, sTfR, and body iron. Significance of differences in iron status indicators between intervention groups was assessed with 2-sample $t$ tests or chi-square tests. For comparisons between baseline and end line, a paired Student’s $t$ test or McNemar’s test was used. Continuous ferritin has a highly skewed distribution and was therefore normalized with a log$_{10}$ transformation. When skewed variables could not be transformed successfully, Wilcoxon two-sample and signed rank nonparametric tests were used. Values are means $\pm$ SDs, and statistical significance for all analysis is $\alpha < 0.05$.

In addition, the efficacy of the intervention was assessed with linear regression analysis. Outcome variables were the changes (end line minus baseline) in iron status indicators, specifically hemoglobin, log$_{10}$ ferritin, sTfR, and body iron. All regression models controlled for their respective continuous baseline iron measure to adjust for the potential confounding effects of differences in mean baseline values between groups. The main independent variable of interest was treatment (DFS compared with...
control. Covariates that were assessed as potential confounders were diet (vegetarian vs. nonvegetarian), ethnicity (Adivasi vs. Nepali), BMI, MUAC, and socioeconomic indicators. The potential effect of village was assessed by controlling for village as a random factor in a mixed regression model. Because the random effect of village did not have interclass correlation coefficients of 10% or greater, the more parsimonious solution, general linear models (GLMs) with only fixed effects, was used.

Plausibility analysis included assessment of potential to benefit over the continuous range of iron status indicators. Participants with more severe iron deficiency were expected to show a greater change in iron status indicators than participants with less severe or no iron deficiency. This was examined through a test of the significance of an interaction between the intervention group and baseline iron status that should indicate a significantly greater change of iron indicators in the DFS group because of iron fortification compared with controls. A second plausibility analysis assessed the dose response of DFS by associating the amount of iron consumed from DFS with the change in iron status indicators while controlling for baseline iron status. Participants who consumed higher amounts of DFS throughout the study period were expected to exhibit a greater change in iron status indicators than participants who consumed smaller amounts of DFS.

Results

The participants who completed this study had a mean age of 39.5 y, mean height of 150.5 cm, mean BMI of 19.6 kg/m², and mean MUAC of 24.0 cm. There were no significant differences at baseline between the DFS and control group participants for these measures. Likewise, the 2 groups did not differ in iron status or inflammation at baseline (Tables 1 and 2). A high percentage (53%) of women were anemic at baseline, although only 23% of the total sample had IDA. Mean baseline hemoglobin was <120 g/L even though women with hemoglobin of <80 g/L had been excluded from participation (Fig. 1). Indicators of iron deficiency at baseline showed that 25% of the full sample had ferritin values <12 µg/L and 22% had sTfR values >8.6 mg/L. There were no differences between those who dropped out and those who completed the study for any of these measures.

Difference-in-difference analysis with unadjusted means indicated a significantly greater change in all iron measures in the DFS group than in the control group (Table 1). Adjusting for inflammation with multipliers of Thornham et al. (20) did not substantially change the difference-in-difference analysis results, even though 23% of the sample had elevated AGP at baseline and 21% at end line (Table 2). There are multiple nutritional deficiencies that can contribute to the high prevalence of anemia and prevent an increase in hemoglobin from consuming the DFS. These include folate deficiency and vitamin B-12 deficiency, which can contribute to the high prevalence of macrocytic anemia represented by the 25% prevalence of high MCV. Median urinary iodine increased from 119 µg/L (9.38 µmol/L) at baseline to 181 µg/L (14.30 µmol/L) in the whole sample (Supplemental Table 1), and there was no difference in baseline urinary iodine or change in urinary iodine between the DFS and control groups. Although 80% of the 93 participants who were iodine deficient at baseline saw a resolution of their iodine deficiency at end line, there was no difference between the treatment groups.

Mean daily consumption of elemental iron from salt estimated from 24-h recall was 8.6 ± 5.7 mg in the DFS group and 0.03 ± 0.01 mg in the control group, with a respective range of 1.9 to 23.3 mg and 0.009 to 0.09 mg, respectively. The mean daily salt consumption per household member based on disappearance from bags was 12.4 ± 7.9 g in the DFS group and 15.0 ± 13.6 g in the control group (t = 1.69, P = 0.09), whereas daily salt consumption by participants estimated from 24-h diet recall was 7.4 ± 4.8 g and 7.9 ± 4.9 g for the DFS and control group, respectively (t = 0.75, P = 0.45). The DFS group showed significant improvements in prevalence of iron deficiency and iron depletion compared with the control group. Specifically, as shown in Table 2, the prevalence of ferritin <20 µg/L declined significantly from 45 to 22% in the DFS group, whereas among the control group the prevalence dropped from 44 to 35%. The DFS group showed a significant decline in prevalence of participants with negative body iron from 25 to 9%, whereas the control group did not change from the baseline of 18%.

To test for the effects of the DFS intervention on change in iron status after controlling for baseline values, we performed a GLM analysis. Table 3 shows that the DFS group, compared with the control group, significantly improved its iron status assessed by hemoglobin, ferritin, body iron, and sTfR after adjusting for the respective baseline iron status measure. The effect of DFS on change in ferritin was 0.134 log_{10} units, which means that the change in ferritin (µg/L) was 34% higher in the DFS group after controlling for baseline ferritin and length of exposure to the intervention. Change in other indicators of iron status demonstrated similar significant positive responses to the intervention, with increases of 2.4 g/L for hemoglobin and 1.43 mg/kg for body iron in the DFS compared with the control group. The DFS group had a decline in sTfR of 0.59 mg/L compared with the control group (P = 0.005).

Analyses of the biologic plausibility are shown in Figures 2 and 3. The modifying effect of the intervention on the relation of baseline iron status to change in body iron was tested with an interaction between treatment group and baseline body iron (Fig. 2). Iron-deficient non-anemic women with negative body iron measures

| TABLE 1 | Baseline, end line, and change in iron status of DFS and control groups |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|
|          | **Baseline**    | **End line**    | **DFS**         | **Control**     | **DFS**         | **Control**     | **Difference-in-difference** |
|          | **DFS**         | **Control**     | **DFS**         | **Control**     | **DFS**         | **Control**     | **DFS**         | **Control**     | **DFS**         | **Control**     | **DFS**         | **Control**     | **DFS**         | **Control**     | **DFS**         | **Control**     |
| **Hb, g/L** | 116 ± 12      | 117 ± 11       | 117 ± 12      | 115 ± 11*       | 0.8 ± 98        | −1.9 ± 6.7**    | 2.7**          |
| **Serum ferritin** |             |                |                |                |                |                |                |
| Mean, µg/L   | 29.4 ± 25.8   | 36.2 ± 38.7    | 44.6 ± 30.2*  | 39.7 ± 34.6    | 15.2 ± 18.2*   | 3.5 ± 27.0**   | 11.6**         |
| Log_{10}, µg/L | 1.30 ± 0.42   | 1.36 ± 0.43    | 1.54 ± 0.33*  | 1.45 ± 0.37*   | 0.24 ± 0.25*   | 0.08 ± 0.24**  | 0.15**         |
| Median, µg/L | 23.0 (9.0, 58.0) | 22.0 (7.0, 76.0) | 36.5 (12.0, 81.0) | 29.5 (8.0, 87.0) | 12.0 (1.0, 41.0) | 4.0 (−9.0, 28.0)** | 8.0**         |
| sTfR, mg/L   | 7.11 ± 3.30   | 6.57 ± 3.00    | 6.42 ± 2.09*  | 6.79 ± 1.95    | −0.68 ± 2.57*  | 0.22 ± 2.14**  | −0.90**        |
| Body iron, mg/kg | 2.60 ± 4.52   | 3.36 ± 4.43    | 4.76 ± 3.29*  | 3.79 ± 3.57    | 2.16 ± 2.97*   | 0.43 ± 2.51**  | 1.74**         |

1 Values are means ± SDs, or median (10th, 90th percentile). Control (n = 108) is the group consuming iodine-fortified salt; DFS (n = 104) is the group consuming DFS with iron and iodine; sTfR was adjusted to Ramco values for use in the calculation of body iron. *Significantly different within the intervention group over time, P < 0.05; **Significantly different between intervention groups, P < 0.05. DFS, double-fortified salt; Hb, hemoglobin; sTfR, soluble transferrin receptor.

2 End line minus baseline.
TABLE 2 Baseline and end line prevalence values of nutritional status and inflammation indicators of DFS and control group

| Time of blood sample | Baseline | End line |
|----------------------|----------|----------|
| DFS                  | Control  | DFS      | Control  |
| Hb < 120 g/L, % anemic | 53       | 54       | 54       | 68*     ** |
| Ferritin < 12 μg/L, % iron deficient | 26       | 23       | 9**     | 18      |
| Ferritin < 20 μg/L, % iron depleted | 45       | 44       | 22**     | 35*     |
| sTfR > 6 mg/L, % | 25       | 19       | 13**     | 16      |
| Body iron < 0.0 mg/kg, % | 25       | 18       | 9**     | 18      |
| MCV > 95 fl, % | 18       | 25       | 14       | 14**    |
| Vitamin B-12 < 203 μg/L, % | 37       | 39       | 46**     | 48**    |
| Folate < 5 mg/L, % | 85       | 85       | 73**     | 75**    |
| CRP > 5 mg/L, % | 24       | 7        | 5        | 3       |
| AGP > 1 g/L, % | 22       | 24       | 21        | 22      |
| Urinary iodine < 100 μg/L, % | 43       | 44       | 26**    | 31**    |

1 Control (n = 108) is the group consuming iodine-fortified salt; DFS (n = 104) is the group consuming DFS with iron and iodine; sTfR was adjusted to Ramco values for use in the calculation of body iron. SI unit conversions: vitamin B-12 μg/L = 0.738 pmol/L; folate mg/L = 2.266 nmol/L; urinary iron mg/L = 78.80 nmol/L.

2 Significantly different within intervention group over time, P < 0.05. 3 Significantly different within intervention group over time, P < 0.001. **Significantly different within intervention group over time, P < 0.0001.

TABLE 3 Effect of intervention on change of iron status indicators in GLMs, controlling for baseline iron status (n = 212)

| Regression models with outcome change (end line minus baseline) | Hb (g/L) | Ferritin (log10 μg/L) | sTfR2 (mg/L) | Body Iron3 (mg/kg) |
|---------------------------------------------------------------|----------|----------------------|--------------|--------------------|
| Intercept                                                     | 1.14 (±0.65, 2.39) | -0.06 (±0.42, 0.30) | 5.88 (3.16, 8.59)* | -5.39 (±0.97, -1.71)** |
| Baseline Hb (g/L)                                             | -2.6 (±-3, -1.7)* | -0.12 (±-0.39, -0.26)* | -0.57 (±-0.64, -0.51)* | -0.39 (±-0.45, -0.33)* |
| Baseline ferritin (log10 μg/L)                                | -0.01 (±0.001, 0.01)** | -0.002 (±0.001, 0.004)** | -0.008 (±-0.02, 0.003) | 0.03 (±0.01, 0.04)* |
| Baseline sTfR (mg/L)                                          | -0.7 (±0.4, 4.5)** | 0.13 (±0.08, 0.19)* | -0.59 (±-1.00, -1.19)** | 1.43 (±0.87, 1.99)* |

1 Values are regression coefficients (95% CIs). *P < 0.001; **P < 0.01; ***P < 0.05. DFS, double-fortified salt; GLM, general linear model; Hb, hemoglobin; sTfR, soluble transferrin receptor.
2 sTfR and body iron with adjustment to Ramco values.
3 DFS vs. control (reference).

Discussion

This study demonstrated significant improvements in all measures of iron status as a result of consuming DFS during an intervention trial in a population at risk of iron deficiency. All of the advantages of the randomized, double-blind, controlled intervention trial were confirmed. With 50% prevalence of iron deficiency, participants in the high-iron DFS group and the iodized salt control group were similar at baseline for iron status and all measured potential confounding factors. The DFS and control groups consumed similar amounts of salt, although the DFS group consumed a sufficient amount of a biologically available elemental iron to account for the observed improvement in iron status. The mean change in urinary iodine excretion did not differ significantly between groups, confirming that the consumption of DFS and control salts was approximately comparable. The intervention trial was of sufficient length to allow for detectable transfer of iron to body stores in the DFS group. The statistically significant difference-in-difference analysis for change in iron status was confirmed with GLM analysis that controlled for basic confounders, indicating that there was indeed sufficient statistical power to show the hypothesized effects of DFS on iron status. Finally, 2 tests of plausibility

increased. Moreover, 22% of the sample had macrocytic anemia at baseline and might not have been responsive to iron fortification. Regression analysis (not shown) performed to test the effect of the intervention on change in log10 ferritin in the non-anemic group corroborated the findings of Figure 2; the slope of the relation between change in log10 ferritin and baseline log10 ferritin was -0.43 (P < 0.001) in the DFS group compared with -0.14 (P = 0.09) in the control group (P = 0.02 for interaction).

A second set of plausibility analyses assessed the magnitude of change in iron status with increasing mean daily amounts of salt consumed from the DFS or control salt per household member (Fig. 3). Women in the DFS group who consumed below the median (10 mg/d) amount of iron from salt over the course of the study had a marginally greater increase in body iron compared with women in the control group; these women also demonstrated intermediate change in body iron compared with women in the control group and women in the DFS group who consumed above average amounts of iron from salt. Only the difference in body iron change between the high consumers of DFS and the control group was statistically significant. The average change of log10 ferritin showed a similar association with iron intake from control and DFS salt (not shown).

Efficacy of double-fortified salt
confirmed the basic difference-in-difference results and suggest that consumption of the DFS was the source of the change in iron status.

Nevertheless, several factors may have affected the magnitude and direction of the results. Concurrent inflammation, reflected in elevated AGP and CRP values, can affect the interpretation of serum ferritin and body iron as indicators of iron status (22). Although 23% of participants had elevated AGP values and 5% had elevated CRP values at baseline, adjustments for inflammation to ferritin and body iron by regression or using multipliers of Thurnham et al. (20) did not affect the results. Hemoglobin and sTfR values also did not vary by inflammation status. The amount of elemental iron delivered by DFS was about 8.6 mg/d, which is considered adequate to meet the daily estimated average requirement (8.1 mg/d) for >50% of women of reproductive age (23). This is based on the estimated daily salt consumption of ~7.4 g in the DFS group. Although it was not possible to measure precise individual participant salt consumption, the estimates based on disappearance of monthly salt rations and periodic diet recalls suggest that sufficient iron differential should have been achieved between the DFS group and control group. However, it is likely that the women in the DFS group consumed some of the control salt and that the control group consumed some DFS because of food sharing among households and individual participants. Participants from both the DFS and control groups were documented to have shared their food during the mid-day meal. We estimate that the net effect of this food sharing, and hence salt sharing, resulted in a difference in elemental iron intake between DFS and control groups that may have been reduced to about 5.0 mg/d. The small amount of additional iron consumed by women in the control group (up to 3.0 mg/d) who received food from women in the DFS group may have contributed to a modest but significant improvement in ferritin and body iron in the control group. However, even considering the reduction in the amount of iron intake difference between groups, it was not sufficient to prevent the DFS group from achieving significant improvements in all iron status indicators relative to the control group.

Confirmation of the plausibility and internal validity was demonstrated in several ways. Women with moderate to severe iron deficiency at baseline experienced a greater change in iron status than women with milder or no iron deficiency. Moreover, the DFS group had a significantly steeper change in response to increasing baseline iron status than the control group ($P < 0.05$ for the interaction). In other words, although both groups showed a decline in the rate of change in body iron with increasing baseline iron status, the DFS group demonstrated a significantly faster return to iron sufficiency than the control group. Additional confirmation that the iron from DFS was accounting for the difference in iron status between groups comes from an analysis of the positive relation between the amount of total iron from salt and the change in body iron (Fig. 3). This effect was likely attenuated by the food sharing experienced between groups.

Two other studies have assessed the effect of DFS on hemoglobin in women. In a 1-y study, Rajagopalan and Vinodkumar (16) determined a significantly different 0.72-g/dL change in hemoglobin between intervention and control groups of female tea pickers in southern India, and Asibey-Berko et al. (17) found a significant difference in change in hemoglobin of 0.4 g/dL compared with a control group in Ghanaian women. The current study demonstrated a significant difference in change in hemoglobin of 0.27 g/dL between intervention groups (Table 2). The lower magnitude of the effect in the present study may be due to the high prevalence of macrocytic anemia, which does not respond to iron fortification if it is caused by other factors like folate and vitamin B-12 deficiency. Another possibility may be the shorter intervention length in the present study, and the aforementioned food sharing that would have reduced the group differences in iron intake. To our knowledge, the current study is the first to demonstrate the effects of DFS in women using multiple indicators of iron status. A study with Indian children found significant increases of similar magnitude (0.2 g/dL) in hemoglobin in a DFS iron-fortified group when compared with a control group. Andersson et al. (18) tested a ferrous fumarate–fortified DFS and also assessed measures of iron status in addition to hemoglobin in primary school children from Bangalore, India. A comparison confirms the substantial impact of the intervention on iron status measures in the present study. Andersson et al. related change in the DFS group to that in the control group and reported that ferritin increased by 5 μg/L, sTfR decreased by 0.4 mg/L, and body iron increased by 1.2 mg/kg. The present study found a significant 11.6-μg/L increase in ferritin, a 0.90-mg/L decrease in sTfR, and a 1.74-mg/kg increase in body iron. The smaller
change in iron status outcomes reported by Andersson et al. may be due to lower baseline prevalence of anemia (12.3%) and IDA (10.2%). In contrast, 53% of female tea pickers were anemic, and 23% had IDA, suggesting a greater potential to respond.

It is noteworthy that this study sample also benefitted from the additional iron provided by both the DFS and control salt. Median urinary iodine concentrations increased significantly in both groups and 80% of the iodine deficiency seen at baseline was resolved by the end of the study. Although iodized salt was readily available on the tea estate prior to this study, 44% of the study sample had baseline urinary iodine concentrations <100 mg/L. One could surmise the provision of iodine in the study table salt was of a consistently higher quality and had a higher iodine content compared with the commercially available salt sold in the community.

This research raises several cautionary issues that should be considered in further efforts to improve the iron status of rural Indian women. Although the focus of this efficacy study was on iron deficiency, it is clear that other nutrient deficiencies need to be addressed. Specifically, the high prevalence of folate and vitamin B-12 deficiencies, and the persistence of anemia and iron and iodine deficiencies even after 9 mo of a DFS intervention, suggests a large burden of nutritional inadequacy in this rural population of tea pickers. A clearer picture of the impact of DFS on iron deficiency and IDA requires an analysis of the role that DFS plays in resolving and preventing iron deficiency and anemia. Although mean hemoglobin concentrations improved in the DFS group compared with the control group, anemia prevalence did not improve in response to the DFS intervention. It is noteworthy that other markers of iron deficiency prevalence did improve in the DFS group, suggesting that failure to improve anemia prevalence may be due to the persistence of vitamin B-12 and folate deficiencies and possible homocystinopathies, which are known to cause anemia.

Widespread introduction of DFS into the food system is currently underway at several locations in India. This provides a valuable opportunity to evaluate the effectiveness of DFS as a public health measure that has potentially important implications for the physical and economic health of Indians throughout the country. Daily ingestion of a relatively inexpensive and widely available product like DFS that is stable in tropical climates has the potential for a sustainable solution to iron and iodine deficiency. This needs to be evaluated for effectiveness after a scaled-up introduction of DFS into the food supply. Also, this may be the time to examine salt as a potential fortification vehicle for other nutrients, especially those known to be deficient in less-developed countries. However, along with the apparent benefits of introduction of DFS, we must also consider the potential problems that accompany any manipulation of the food supply. These include the extra costs to the consumer to purchase DFS over iodized salt, the potential for overdose of the fortificants through lapse in safety and quality control of salt production, and unintended promotion of excess salt consumption with its attendant health consequences.

In conclusion, DFS with iron and iodine improved hemoglobin, ferritin, sTfR, and body iron concentrations in a population that ranged from moderately iron deficient anemic to iron sufficient. It also substantially decreased the prevalence of iron deficiency at end line. To our knowledge, this study is the first to use multiple measures of iron status to assess the efficacy of DFS in improving iron status in women. Further research from this study will investigate whether improvements in functional outcomes and the economic impact of improved iron status can be attributed to DFS consumption. Moreover, assessment of the effectiveness of DFS in a variety of community settings and cost-effectiveness in comparison with other strategies to reduce iron deficiency should be considered.

Acknowledgments

The authors thank Eric Przybylszewski, John Rheo, Julie Hammons, and Francoise Vermeylen for assistance in the early stages of data preparation and analysis, and Kim Harding for her helpful comments on the manuscript. The authors also thank Anand Lakshman from the Micronutrient Initiative, Pasang Bhutia and Nickhil Naskar from the Child in Need Institute (CINI) in Siliguri for logistic support, and A. Mallick, T. Dasgupta, D.R. Chaudhury and R. Sharma of the Panighatta Tea Estate for permission to carry out the study. J.D.H. developed the proposal, supervised data collection and analysis, contributed to writing the manuscript, and had primary responsibility for the final content; M.R. conducted the data analysis and contributed to the writing of the manuscript; S.V. supervised data collection and contributed to part of the data analysis; G.S.M. supervised the dietary data collection and analysis; M.J.W. contributed to the study implementation in the field and data analysis; L.E.M.-K. contributed to the study design and data analysis; G.A.R. provided input to the study design and interpretation of the results; and A.S.W., project manager at the Micronutrient Initiative, provided valuable assistance in reviewing the protocols and setting up the study in India and provided comments on the final manuscript. All authors read and approved the final manuscript.

Literature Cited

1. WHO. Iron deficiency anaemia: assessment, prevention and control. Geneva, Switzerland: World Health Organization; 2001. p. 1–114.
2. Coordination UNACO. Fourth report of the world nutrition situation. Geneva, Switzerland: ACC/SCN; 2000.
3. WHO. Worldwide prevalence of anaemia, 1993–2005. de Benoist B, McLean E, Egli I, Cogswell M, editors. Geneva, Switzerland: World Health Organization; 2008. p. 1–51.
4. Horton S, Ross J. The economics of iron deficiency. Food Policy. 2003;28:51–75.
5. Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nutr. 2001;131:676S–90S.
6. Zhu YI, Haas JD. Iron depletion without anemia and physical performance in young women. Am J Clin Nutr. 1997;66:334–41.
7. Brutsaert TD, Hernandez-Cordero S, Rivera J, Viola T, Hughes G, Haas JD. Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women. Am J Clin Nutr. 2003;77:441–8.
8. Li R, Chen X, Yan H, Deurenberg P, Garby L, Hautvast JG. Functional consequences of iron supplementation in iron-deficient female cotton mill workers in Beijing, China. Am J Clin Nutr. 1994;59:908–13.
9. Edgerton VR, Gardner GW, Ohira Y, Gunawardena KA, Senewiratne B. Iron-deficiency anaemia and its effect on worker productivity and activity patterns. BMJ. 1979;2:1546–9.
10. Murray-Kolb LE. Iron status and neuropsychological consequences in women of reproductive age: what do we know and where are we headed? J Nutr. 2011;141:747S–55S.
11. Perez EM, Hendricks MK, Beard JL, Murray-Kolb LE, Berg A, Tomlinson M, Isacq W, Njengele T, Sive A, et al. Mother-infant interactions and infant development are altered by maternal iron deficiency anaemia. J Nutr. 2005;135:850–5.
12. Horton S, Wesley A, Mannar MGV. Double-fortified salt reduces anaemia, benefit:cost ratio is modestly favorable. Food Policy. 2011;36:581–7.
13. UNICEF, UNO, WHO, MI. Preventing iron deficiency in women and children. New York: International Nutrition Foundation; 1999.
14. Diosady LL, Alberti JO, Mannar V. Microencapsulating for iodine stability in salt fortified with ferrous fumarate and potassium iodide. Food Res Int. 2002;35:635–42.
15. Oshinowo T, Diosady LL, Yusufali R, Wesley A. An investigation of the stability of double fortified salt during storage and distribution in Nigeria. Int J Food Eng. 2007;3:1–22.

16. Rajagopalan S, Vinodkumar M. Effects of salt fortified with iron and iodine on the haemoglobin levels and productivity of tea pickers. Food Nutr Bull. 2000;21:323–9.

17. Asibey-Berko E, Zlotkin S, Yeung G, Nti-Nimako W, Ahunu B, Kyei-Faried S, Johnston JL, Tondeur MC, Mannar V. Dual fortification of salt with iron and iodine in women and children in rural Ghana. East Afr Med J. 2007;84:473–80.

18. Andersson M, Thankachan P, Muthayya S, Goud RB, Kurpad AV, Hurrell RF, Zimmermann MB. Dual fortification of salt with iodine and iron: a randomized, double-blind, controlled trial of micronized ferric pyrophosphate and encapsulated ferrous fumarate in southern India. Am J Clin Nutr. 2008;88:1378–87.

19. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2003;101:3359–64.

20. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010;92:546–55.

21. Gnat D, Dunn A, Chaker S, Delange F, Vertongen F, Dunn J. Fast colorimetric method for measuring urinary iodine. Clin Chem. 2003;49:186–8.

22. Northrop-Clewes CA. Interpreting indicators of iron status during an acute phase response—lessons from malaria and human immunodeficiency virus. Ann Clin Biochem. 2008;45:18–32.

23. Institute of Medicine. Iron. In: Dietary reference intakes. Washington: National Academy Press; 2001. p. 344.