# Predicting potential to benefit from an iron intervention: a randomized controlled trial of double-fortified salt in female Indian tea pluckers

Julie EH Nevins[1],*, Sudha Venkatramanan[1,2], Saurabh Mehta[1] and Jere D Haas[1]

[1]Division of Nutritional Sciences, Cornell University, Savage Hall, 244 Garden Avenue, Ithaca, NY, 14853, USA: [2]Department of Dietetics and Human Nutrition, McGill University, Ste Anne de Bellevue, Quebec, Canada

## Abstract

*Objective:* The present study examines characteristics of those who benefited from a dietary Fe intervention comprised of salt double-fortified with iodine and Fe (DFS).

*Design:* Data from a randomized controlled trial were analysed to identify predictors of improved Fe status and resolution of Fe deficiency (serum ferritin (sFt) < 12 µg/l) and low body Fe (body Fe (BI) < 0·0 mg/kg) using non-parametric estimations and binomial regression models.

*Setting:* A tea estate in West Bengal, India.

*Participants:* Female tea pluckers, aged 18–55 years.

*Results:* Consuming DFS significantly ($P = 0.01$) predicted resolution of Fe deficiency (relative risk (RR) = 2·31) and of low BI (RR = 2·78) compared with consuming iodized salt. Baseline sFt ($\beta = -0.32$ (SE 0·03), $P < 0.001$) and treatment group ($\beta = 0.13$ (SE 0·03), $P < 0.001$) significantly predicted change in sFt. The interaction of baseline BI with treatment group ($\beta = -0.11$ (SE 0·06), $P = 0.08$) predicted the change in BI. DFS did not significantly predict change in Hb and marginally predicted resolution of anaemia (Hb < 120 g/l).

*Conclusions:* Baseline Fe status, as assessed by sFt and BI, and consumption of DFS predict change in Fe status and resolution of Fe deficiency and low BI. Anaemia prevalence and Hb level, although simple and inexpensive to measure, may not be adequate to predict resolution of Fe deficiency in response to an intervention of DFS in similar populations with high prevalence of Fe deficiency and multiple nutritional causes of anaemia. These findings will guide appropriate targeting of future interventions.

Fe deficiency is a highly prevalent public health concern, affecting more than one-third of the world's population[1]. Functional consequences of Fe deficiency include anaemia and reductions in physical work capacity[2,3], immune function[4] and cognitive performance[5,6], as well as increased risk of depression[7]. These outcomes may be long-lasting and can occur before the development of overt anaemia, defined for women as Hb < 120 g/l[8].

Women of reproductive age are at heightened risk of Fe deficiency, particularly in low-income countries[9] where diets are low in Fe-rich foods and high in plant foods that can inhibit Fe absorption[7,10]. Fe supplementation is an efficacious method for ameliorating Fe deficiency, but financial and physical barriers often preclude its use in low-resource settings[11]. Widespread Fe supplementation also increases the risk that Fe-replete individuals will consume too much Fe, which could exacerbate morbidities from infections, notably malaria, hookworm and schistosomiasis[12,13].

Fortifying ubiquitous foods has resulted in large-scale decreases in other micronutrient deficiencies; folate-fortified flour and vitamin D-fortified milk are two well-known examples[14–17]. Salt iodization has reduced iodine deficiency to <11 % in the Americas, which has greater iodized salt consumption compared with Europe, where iodized salt consumption is low and >50 % prevalence of iodine deficiency has been reported[18]. Sustained salt iodization is expected to reduce goitre prevalence to

<5 %[18], which by WHO estimates would eliminate iodine deficiency as a significant public health problem. Salt is a potentially successful fortification vehicle for delivering additional micronutrients, in part, because it is consumed daily by most of the world, is produced in many nutritionally at-risk areas and can be fortified using low-cost technology[18–23].

Previous research has demonstrated the efficacy of consuming salt double-fortified with iodine and Fe (DFS) to improve both iodine and Fe status in children[19,22,23] as well as Fe status in women of reproductive age[24]. Although these well-designed randomized controlled trials provide evidence for a causal relationship between consumption of DFS and improved Fe status, little work has been done to examine the magnitude of impact and the potential effectiveness of programmes to reduce Fe deficiency with widespread DFS distribution[22,23,25–27]. To maximize the success of such programmes, there must first be evidence from efficacy trials of the behavioural and biological mechanisms through which the intervention results in an outcome of interest[25,26,28,29]. Inherently, randomized controlled efficacy trials can provide only limited information about generalizability, but are often useful to identify predictors of potential to benefit which aid in targeting populations for future interventions[30].

The present analyses examine characteristics of those who benefited from an Fe intervention of DFS among female tea pluckers in West Bengal, India. Benefit is assessed with three methods: both as the absolute (i) and relative (ii) improvements in Fe biomarkers as well as the resolution of Fe deficiency (iii) over a 10-month intervention. The results presented here are of particular interest given the high economic and public health burdens of Fe deficiency and anaemia and the need to efficiently and effectively target DFS interventions to address these burdens.

## Study population and methods

### Study site and participants

Details of the study population and efficacy study are described in Haas et al.[24]. Briefly, participants were female tea pluckers (aged 18–55 years) who lived and worked on the Panighatta Tea Estate in West Bengal, India. Women were not eligible to participate if they were pregnant, had Hb concentration <80 g/l or >150 g/l, were not residents of Panighatta or were not employed full-time on the tea estate. The population included two ethnic groups: the Nepali, whose ancestors emigrated from Nepal, and the Adivasi, an indigenous people considered to be of lower social and socio-economic status than the Nepali.

### Intervention

Eligible women (n 248) were enrolled between July and September 2010, and were randomly assigned to receive salt fortified with potassium iodate 50 µg/g (IS) or salt double-fortified with potassium iodate 50 µg/g and Fe 1000 µg/g as encapsulated ferrous fumarate (DFS) for seven to ten months. All other types of commercially available salt were removed from the tea estate to promote adherence. There were no differences in loss to follow-up between groups: 108 and 104 women completed the study in the IS and DFS group, respectively. Adherence to the intervention was high and similar between treatment groups[24].

### Follow-up procedures

Participants were given deworming medication (albendazole, 200 mg) one month before the baseline (enrolment) blood draw and after five months of follow-up (midpoint). Venous blood samples were collected at baseline and endline (after ten months of follow-up). Single casual urine samples were collected at baseline and endline to assess iodine excretion. Height (cm) and weight (kg) were measured by trained anthropometrists at baseline and endline. BMI was calculated as weight (kg)/height$^2$ (m$^2$). Participant age, ethnicity and socio-economic indicators were assessed via questionnaires, as detailed in Haas et al.[24].

### Laboratory analyses

Venous blood samples were analysed for Hb, serum ferritin (sFt) and soluble transferrin receptor (TfR). Inflammation was assessed via measurement of C-reactive protein (CRP) and $\alpha_1$-acid glycoprotein (AGP). Hb was assessed with a Coulter Counter (Beckman). sFt and CRP were analysed by chemiluminescent immunoassay (Immulite 2000) and AGP by radial immunodiffusion (Kent Laboratories). ELISA was used to assess TfR (BioVendor, Asheville, NC, USA), and a subgroup of blood samples (n 35) were run in duplicate with an ELISA kit from Ramco Laboratories (Stafford, TX, USA) to calculate Ramco-adjusted TfR values by linear regression[24]. The resulting prediction equation was: $TfR_{Ramco} = 1{\cdot}821 \times TfR_{BioVendor}^{0{\cdot}739}$. Total body Fe (BI) was estimated with Cook's equation[31], which required Ramco-adjusted TfR values, as: $BI = -\,[\log(TfR_{Ramco}/sFt) - 2{\cdot}8229]/0{\cdot}1207$. Serum folate, vitamin $B_{12}$ and urinary iodine were also measured at baseline and endline with the Immulite 2000. The Hb, sFt, CRP and iodine tests were completed at the Clinical Research Services of Super Religare Laboratory, Kolkata branch, India. Analyses of TfR, AGP, folate and vitamin $B_{12}$ were performed at the Molecular Diagnostics Laboratory in Lucknow, India.

### Statistical analyses

Data processing and statistical analyses were performed using the statistical software package SAS version 9.4. Where applicable, non-parametric analyses were used for non-normally distributed measures. sFt and BI were examined both without adjustment and with Thurnham's correction factor for inflammation[32]. Values for sFt, and

thus for BI, were corrected only if a participant had CRP > 3·0 mg/l, AGP > 1·0 g/l, or both. The Bonferroni method[33] was used to correct for multiple comparisons when necessary.

The resolution of deficiency is often used as a metric in clinical settings to determine the efficacy of nutrition interventions. While this is a useful method, it may not account for false negatives; that is, individuals who were severely deficient or who had low but technically sufficient status before the intervention and who nevertheless demonstrate large increases in status at endline. To address this, we used three different assessments of response: absolute change, relative change and resolution of deficiency. The first assessment utilized an epidemiological approach, which examined the absolute change in Fe biomarkers. For this assessment, multivariate linear regression models were developed to identify predictors of change in Hb, sFt and BI across the intervention, which replicates the results of Haas *et al.*[24]. We chose these indicators to assess multiple components of Fe status: Hb for anaemia, sFt for Fe stores, and BI for a composite estimate of Fe in both tissues and stores. A negative BI value indicates Fe deficiency[31]. The second assessment, used in public health evaluations, measured response to the intervention as a relative change in Fe biomarkers[34]. Responders were defined as those whose sFt, BI or Hb increased more than 1 SD above the mean change for the control group. We selected this cut-off for response because it is outside the known day-to-day biomarker variation[35] and also captures sample-specific response. Finally, we studied the resolution of deficiency. Resolvers were defined as women who had Fe deficiency (defined as sFt < 12·0 μg/l), low BI (BI < 0·0 mg/kg) or anaemia at baseline and were Fe replete at endline. These are standard cut-off values for deficiency[36]. We also examined a less stringent definition of Fe depletion (sFt < 15·0 μg/l)[36,37]. Non-resolvers were deficient at baseline and remained deficient at endline. For both the relative change and deficiency resolution methods, predictors of response to the intervention were identified using Hodges–Lehmann–Sen estimations[38], $\chi^2$ tests and binomial regression models (to estimate relative risk).

The following variables were considered as covariates as well as potential predictors of benefit: age, measures of baseline Fe status (Hb, anaemia (Hb < 120 g/l; yes or no) and TfR) and anthropometry (weight, height and BMI). Baseline serum folate and vitamin $B_{12}$ were evaluated because deficiencies in these nutrients can result in anaemia[39]. Women were considered deficient in folate or vitamin $B_{12}$ with folate < 5 ng/ml or vitamin $B_{12}$ < 200 pg/ml[39]. Baseline urinary iodine was included because all of the intervention salt was iodized. Ethnicity (Adivasi or Nepali) was included as a random effect.

As the study was not designed to identify predictors of Fe deficiency or low BI resolution, a less stringent requirement of significance (P < 0·1) was used for initial univariate

regression analyses and interaction terms in multivariate analyses. Significant variables from the univariate models were retained in the multivariate binomial regression models to predict resolution of Fe deficiency and low BI if the P value was <0·05 or <0·1 for interactions. All tests were two-tailed.

## Results

The baseline characteristics were the same in the present sub-sample as in Haas *et al.*[24] and did not differ between treatment groups (see online supplementary material, Supplemental Table S1). We first examine those who benefited from the intervention using the three methods of assessing response described above and then identify baseline characteristics that predict potential to benefit from the DFS intervention.

### Absolute change in biomarkers across the intervention

We included change in sFt, BI and Hb as continuous dependent variables in multivariate linear regression models (Table 1). Both baseline log(sFt) and treatment group predicted change in log(sFt) (P < 0·001). The interaction term did not reach statistical significance (Table 1). Although lower baseline log(sFt) concentrations resulted in greater change in log(sFt), the effect was more pronounced in the DFS *v.* IS group (Fig. 1). Women who received DFS had greater increases in BI relative to those who received IS and baseline BI was negatively associated with change in BI (P < 0·001) when the interaction between baseline BI and treatment group was not included in the model (data not shown). The interaction between baseline BI and treatment group was significant (P = 0·08; Table 1). Ethnicity and baseline Hb predicted the change in Hb, but treatment group did not (Table 1). The interaction between ethnicity and treatment did not predict change in sFt, BI or Hb (data not shown).

Figure 1 shows that the effect of treatment on the change in both log(sFt) and BI was statistically significant only among women in the lowest quartile of sFt and the lowest half of the BI distribution at baseline, respectively. Change in Hb did not vary by treatment group at any level of baseline Hb and therefore was not included in later regression analyses.

### Relative change in biomarkers across the intervention

We next evaluated responders as those whose sFt or BI increased more than 1 SD above the mean change for the control group: >0·31 μg/l change in log(sFt) and >2·76 mg/kg change in BI, respectively. Treatment with DFS (P < 0·01) and baseline sFt < 12·0 μg/l (P < 0·001), BI < 0·0 mg/kg (P < 0·001) and TfR > 8·6 mg/l (P < 0·001)
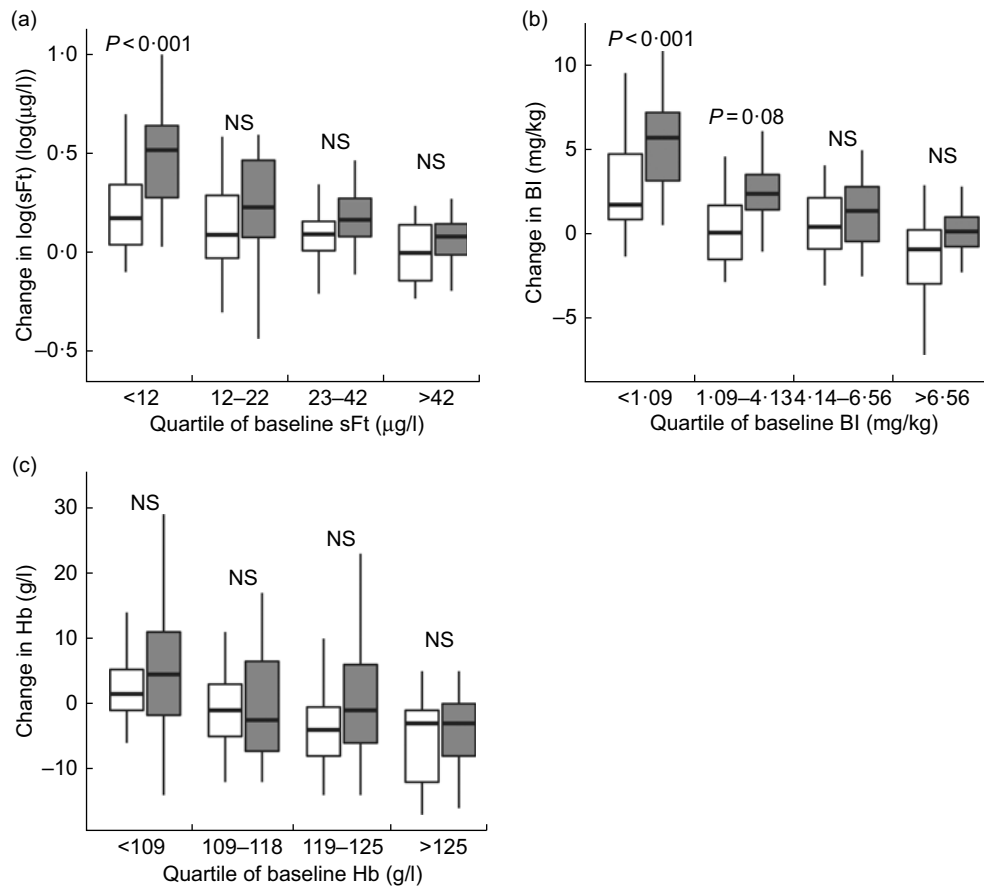
**Table 1** Multivariate baseline predictors of changes in serum ferritin, body iron and Hb in response to an intervention of double-fortified salt in female Indian tea pluckers

| Independent variable | Change in log(sFt) (log(μg/l); *n* 212) | | Change in BI (mg/kg; *n* 211) | | Change in Hb (g/l; *n* 212) | |
|---|---|---|---|---|---|---|
| | Estimate | SE | Estimate | SE | Estimate | SE |
| Intercept | 0.66*** | 0.05 | 3.85*** | 0.43 | 5.05*** | 0.77 |
| Baseline log(sFt) (log(μg/l)) | −0.32*** | 0.03 | – | – | – | – |
| Baseline BI (mg/kg) | – | – | −0.47*** | 0.05 | – | – |
| Baseline Hb (g/l) | – | – | – | – | −0.04*** | 0.01 |
| Treatment (DFS)‡ | 0.13*** | 0.03 | 1.95*** | 0.32 | 0.90 | 1.06 |
| Ethnicity (Adivasi)‡ | – | – | – | – | −0.51*** | 0.12 |
| Baseline log(sFt) × Treatment (log(μg/l))‡ | 0.10 | 0.07 | – | – | – | – |
| Baseline BI × Treatment (mg/kg)‡ | – | – | −0.11† | 0.06 | – | – |
| Baseline Hb × Treatment (g/l)‡ | – | – | – | – | 0.006 | 0.009 |

sFt, serum ferritin; BI, estimated total body Fe; DFS, double-fortified salt.
†$P < 0.1$; *$P < 0.05$; ***$P < 0.001$.
‡Reference values for independent variables: treatment, iodized salt; ethnicity, Nepali.



**Fig. 1** Box-and-whisker plots showing the change in iron indicators in female Indian tea pluckers by treatment group (□, iodized salt; ▨, double-fortified salt) and baseline quartile of iron status. Boxplots indicate the lower quartile, median and upper quartile, and whiskers indicate minimum and maximum values; *P* values indicate significance of differences between treatment groups (sFt, serum ferritin; BI, estimated total body iron)

**Table 2** Baseline predictors of relative response‡ in iron status to an intervention of double-fortified salt in female Indian tea pluckers: univariate analysis

| Independent variable | sFt (n 212) | | BI (n 211) | |
|---|---|---|---|---|
| | RR‡ | 95 % CI | RR‡ | 95 % CI |
| Age (years) | 0·98 | 0·95, 1·01 | 0·97† | 0·95, 1·00 |
| Ethnicity§ | 0·93 | 0·56, 1·54 | 1·10 | 0·64, 1·60 |
| Height (m) | 0·98 | 0·94, 1·03 | 0·99 | 0·95, 1·04 |
| Weight (kg) | 1·00 | 0·97, 1·03 | 0·99 | 0·96, 1·02 |
| BMI (kg/m$^2$) | 1·01 | 0·93, 1·09 | 0·97 | 0·89, 1·05 |
| Treatment§ | 2·28** | 1·32, 3·95 | 2·32** | 1·40, 3·84 |
| Hb (g/l) | 0·67** | 0·53, 0·85 | 0·76* | 0·62, 0·94 |
| sFt (µg/l) | 0·94*** | 0·91, 0·96 | 0·96*** | 0·94, 0·98 |
| TfR (mg/l)‖ | 1·02* | 1·00, 1·05 | NC | NC |
| BI (mg/kg) | NC | NC | NC | NC |
| Folate (ng/ml) | 0·97 | 0·84, 1·13 | 0·90 | 0·77, 1·05 |
| Vitamin B$_{12}$ (pg/ml) | 1·00 | 1·00, 1·00 | 1·00 | 1·00, 1·00 |
| Iodine (µg/l) | 1·00 | 1·00, 1·00 | 1·00 | 1·00, 1·00 |
| Height < 150 cm | 1·29 | 0·78, 2·12 | 1·32 | 0·84, 2·08 |
| BMI < 19 kg/m$^2$ | 1·11 | 0·68, 1·83 | 1·34 | 0·86, 2·12 |
| sFt < 12·0 µg/l | 3·64*** | 2·26, 5·84 | 2·75*** | 1·79, 4·23 |
| TfR > 8·6 mg/l | 3·05*** | 1·92, 4·86 | 3·01*** | 1·98, 4·57 |
| BI < 0 mg/kg | 4·03*** | 2·54, 6·39 | 3·58*** | 2·37, 5·41 |
| Anaemia (Hb < 120 g/l) | 1·46 | 0·87, 2·45 | 1·13 | 0·71, 1·79 |
| Folate < 5·0 ng/ml | 1·84 | 0·71, 4·76 | 2·14 | 0·83, 5·48 |
| Vitamin B$_{12}$ < 203 pg/ml | 1·38 | 0·84, 2·26 | 1·08 | 0·68, 1·72 |
| Iodine < 100 µg/l | 0·77 | 0·46, 1·29 | 0·92 | 0·58, 1·46 |

sFt, serum ferritin; BI, estimated total body Fe; RR, relative risk; TfR, soluble transferrin receptor; NC, model did not converge.
†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
‡Relative response defined as increase in Fe biomarkers more than 1 SD above the mean of the change in the control group; change in log(sFt) > 0·31 µg/l; change in BI > 2·76 mg/kg.
§Reference values for independent variables: ethnicity, Adivasi; treatment, iodized salt.
‖Corrected by Ramco assay.

predicted response both in sFt and BI (Table 2). Every 1·0 g/l decrease in baseline Hb ($P < 0.01$) and 1·0 µg/l decrease in baseline sFt ($P < 0.001$) predicted response for both sFt and BI. Each 1·0 mg/l increase in baseline TfR (relative risk (RR) = 1·02, $P < 0.05$) predicted response in sFt. Models for baseline BI did not converge.

### Resolution of deficiency

Resolution of deficiency is commonly used to evaluate the success of nutrition interventions, e.g.[40,41]. We examined several definitions of the resolution of Fe deficiency, low BI and anaemia by treatment group with univariate binomial regression models (Table 3). Only the RR for resolution of baseline sFt < 12·0 µg/l (RR = 2·31 in DFS v. IS, $P = 0.01$) and BI < 0·0 mg/kg (RR = 2·78 in DFS, $P = 0.01$) differed significantly by treatment group, although RR for resolution of baseline anaemia approached significance ($P = 0.07$). Some studies use a less stringent definition of Fe deficiency; thus, we also examined the resolution of sFt < 15 µg/l[37]. This cut-off had some discriminatory value; the RR for resolution approached statistical significance in this sample ($P = 0.06$).

### Baseline characteristics of resolution

Results were similar for sFt and BI, and thus only BI results are presented here. Baseline characteristics of participants are shown in Tables 4 and 5 by resolution group: non-deficient women with baseline BI ≥ 0·0 mg/kg and deficient women with baseline BI < 0·0 mg/kg. Women with low baseline BI were further divided into resolvers (BI > 0·0 mg/kg at endline) or non-resolvers. All baseline Fe biomarkers were lower in those with baseline low BI compared with those with normal baseline BI status, but only sFt and BI differed between resolvers and non-resolvers: 7·0 µg/l v. 4·0 µg/l for sFt and –1·6 mg/kg v. –4·8 mg/kg for BI, respectively ($P < 0.05$). CRP was elevated in those with normal baseline Fe status compared with resolvers ($P < 0.05$). Resolvers were shorter than women with normal baseline Fe status ($P < 0.05$; Table 4).

Table 5 shows that a greater proportion of resolvers were in the DFS group compared with the non-resolvers and those with normal baseline BI status. Prevalence of anaemia was also greater in the resolvers and non-resolvers compared with those with normal baseline Fe status ($P < 0.05$). Baseline folate, vitamin B$_{12}$ and iodine did not differ between groups, and there was a high prevalence of folate (>85 %) and vitamin B$_{12}$ (>34 %) deficiencies in this sample. Participants with normal baseline Fe values were somewhat more likely to have baseline AGP > 1·0 g/l compared with non-resolvers ($P < 0.09$) after accounting for multiple comparisons.

Change in Fe biomarkers from baseline to endline differed between resolvers and non-resolvers (Table 6). Change in sFt, TfR and BI was significantly different between BI resolution groups ($P < 0.01$). Change in Hb did not differ between BI resolvers and non-resolvers ($P = 0.1$). Urinary iodine changed similarly between resolution groups, and folate and vitamin B$_{12}$ concentrations did not change from baseline to endline in either resolution group.

Univariate binomial regression models revealed DFS treatment and baseline BI predicted resolution of BI < 0·0 mg/kg ($P = 0.01$, Table 7). No multivariate models significantly predicted resolution of sFt < 12·0 µg/l or BI < 0·0 mg/kg (data not shown).

### Discussion

In order to respond to DFS, one must have a potential to benefit from the intervention. Fe status is controlled by absorption rather than excretion mechanisms. Thus, women with low Fe status at baseline are expected to absorb more Fe, resulting in a greater increase in Fe concentration than in women with higher baseline Fe status[42].

Using as a foundation the biological plausibility analyses presented in Haas et al.[24], which reported greater increases in the change in BI with lower baseline Fe status, we aimed to identify distinguishing characteristics of

**Table 3** Resolution of deficiency by various measures of iron status and anaemia in female Indian tea pluckers by treatment group

| Baseline marker of deficiency | N | Resolution in IS group | | Resolution in DFS group | | RR‡ | 95 % CI |
|---|---|---|---|---|---|---|---|
| | | n | % of IS | n | % of DFS | | |
| sFt < 12·0 µg/l | 52 | 8 | 32·0 | 20 | 74·1 | 2·31* | 1·25, 4·27 |
| sFt < 15·0 µg/l | 65 | 12 | 36·4 | 19 | 59·4 | 1·63† | 0·96, 2·79 |
| Hb < 120 g/l | 113 | 5 | 8·6 | 12 | 21·8 | 2·53† | 0·95, 6·72 |
| BI < 0·0 mg/kg | 45 | 5 | 26·3 | 19 | 73·1 | 2·78* | 1·26, 6·10 |

IS, iodized salt; DFS, double-fortified salt; RR, relative risk; sFt, serum ferritin; BI, estimated total body Fe.
†$P < 0.1$; *$P < 0.05$.
‡Risk of resolution, reference group: IS.

**Table 4** Baseline median haematological characteristics of Indian female tea pluckers grouped according to resolution of low body iron after a double-fortified salt intervention

| | Baseline BI ≥ 0 mg/kg (n 167) | | Baseline BI < 0 mg/kg | | | |
|---|---|---|---|---|---|---|
| | | | Non-resolvers‡ (n 21) | | Resolvers‡ (n 24) | |
| Baseline variable | Median | IQR | Median | IQR | Median | IQR |
| Age (years) | 40 | 35, 45 | 40 | 34, 45 | 40 | 30, 45 |
| Weight (kg) | 43·8 | 38·9, 49·1 | 40·2 | 37·6, 46·7 | 40·4 | 37·7, 48·75 |
| Height (cm) | 150·8 | 147·1, 154·2 | 148·0 | 146·2, 152·3 | 148·3 | 146·1, 150·8 |
| BMI (kg/m$^2$) | 19·4 | 17·4, 21·2 | 17·9 | 17·3, 20·6 | 19·0 | 17·1, 21·3 |
| Hb (g/l) | 120[a] | 111, 126 | 107[b] | 102, 112 | 109[b] | 102, 122 |
| sFt (µg/l) | 30·0[a] | 18, 51 | 4·0[b] | 4·0, 5·0 | 7·0[c] | 6, 10 |
| TfR (mg/l)§ | 4·65[a] | 2·95, 6·40 | 10·9[b] | 8·4, 12·4 | 10·6[b] | 7·1, 13·9 |
| BI (mg/kg) | 5·2[a] | 3·43, 7·70 | −4·8[b] | −6·6, −2·9 | −1·6[d] | −4·8, −1·1 |
| Folate (ng/ml) | 3·41 | 2·46, 4·22 | 4·10 | 2·58, 4·78 | 3·44 | 2·07, 4·52 |
| Vitamin B$_{12}$ (pg/ml) | 223 | 185, 311 | 237 | 197, 316 | 225 | 164, 294 |
| Iodine (µg/l) | 115 | 66, 225 | 165 | 72, 234 | 127 | 81, 186 |
| CRP (mg/l) | 0·8[b] | 0·5, 1·3 | 0·7[b,c] | 0·5, 1·2 | 0·5[c] | 0·5, 0·7 |
| AGP (g/l) | 0·67 | 0·55, 1·02 | 0·70 | 0·53, 0·88 | 0·70 | 0·52, 1·00 |

BI, estimated total body Fe; IQR, interquartile range; sFt, serum ferritin; TfR, transferrin receptor; CRP, C-reactive protein; AGP, α$_1$-acid glycoprotein.
[a,b,c,d]Median values within a row with unlike superscript letters were significantly different by the Hodges–Lehman–Sen test; $P$ values for differences are <0·01 unless marked as b v. c ($P < 0.05$).
‡Non-resolvers, BI < 0·0 mg/kg at endline; resolvers, BI ≥ 0·0 mg/kg at endline.
§Corrected by Ramco assay.

individuals whose Fe status improved after the intervention $v$. individuals with no change – either absolute or relative – in Fe status. We replicated the findings of Haas *et al.* that consuming DFS predicted a greater increase in BI. We also observed a significant change in sFt in response to DFS consumption[24].

Unexpectedly, we found only a marginal increase in Hb among women consuming DFS $v$. IS who were anaemic at baseline. Although anaemia is often used as an inexpensive measure of low Fe status[43,44], Hb does not decrease to anaemic status until Fe stores are nearly completely depleted[11] and a recent meta-analysis found that, worldwide, less than 40 % of anaemia in women of reproductive age is due to Fe deficiency[37]. Anaemia can also be caused by a variety of nutrient deficiencies, including folate and vitamin B$_{12}$[39], which were not affected by this Fe intervention. Consequently, Hb may not be an appropriate measure for predicting responsiveness to DFS in populations with a similarly high prevalence of folate and vitamin B$_{12}$ deficiencies.

In addition to the analyses of continuous change, we studied predictors of relative change in Fe biomarkers. This technique identified similar predictors of response as in the absolute change analyses and may be useful for targeting interventions using baseline Hb, as this was the only analysis in which baseline Hb predicted response in sFt or BI (see Table 2). As with any method that relies on a cut-off, this approach has the limitation of potentially misclassifying true responders as non-responders because women with marginally low to normal Fe status at baseline would be expected to have only small increases post-intervention.

To examine the greater public health and clinical implications of DFS, we also analysed predictors of resolution of Fe deficiency and low BI. By definition, baseline Fe biomarkers were higher in those with normal baseline Fe status. Thus, those individuals had less potential to benefit from the intervention $v$. those with low baseline Fe concentrations. We expected those with lower Fe status to have the greatest response to the intervention. However, women

**Table 5** Baseline prevalence of demographic and haematological status of Indian female tea pluckers grouped according to resolution of low body iron after a double-fortified salt intervention

| | Baseline BI $\geq$ 0 mg/kg (*n* 167) | | Baseline BI < 0 mg/kg | | | |
| | | | Non-resolvers‡ (*n* 21) | | Resolvers‡ (*n* 24) | |
| Baseline variable | *n* | % | *n* | % | *n* | % |
|---|---|---|---|---|---|---|
| Treatment: DFS | 78 | 46·7[a] | 7 | 33·3[a] | 19 | 79·2[b] |
| Ethnicity: Adivasi | 91 | 54·5 | 15 | 71·4 | 14 | 58·3 |
| Ethnicity: Nepali | 76 | 45·5 | 6 | 28·6 | 10 | 41·7 |
| Height < 150 cm | 68 | 40·7[a] | 12 | 57·1[a,b] | 17 | 70·8[b] |
| BMI < 19·0 kg/m$^2$ | 72 | 43·1 | 13 | 61·0 | 11 | 45·8 |
| Hb < 120 g/l | 79 | 47·3[a] | 18 | 85·7[b,c] | 16 | 66·7[b] |
| Folate < 5·0 ng/ml | 142 | 86·1 | 17 | 85·0 | 20 | 83·3 |
| Vitamin B$_{12}$ < 203 pg/ml | 61 | 36·8 | 8 | 40·0 | 11 | 45·8 |
| Iodine < 100 µg/l | 74 | 44·3 | 9 | 42·9 | 10 | 41·7 |
| CRP > 5·0 mg/l | 9 | 5·4 | 1 | 4·8 | 0 | 0·0 |
| AGP > 1·0 g/l | 42 | 25·5 | 1 | 5·0 | 5 | 20·8 |

BI, estimated total body Fe; DFS, double-fortified salt; CRP, C-reactive protein; AGP, $\alpha_1$-acid glycoprotein.
[a,b,c]Percentage values within a row with unlike superscript letters were significantly different by the $\chi^2$ test; a *v.* b, $P < 0·05$; a *v.* c, $P < 0·01$.
‡Non-resolvers, BI < 0 mg/kg at endline; resolvers, BI $\geq$ 0 mg/kg at endline.

**Table 6** Changes in Hb and multiple micronutrient status indicators in the resolvers and non-resolvers of low body iron over the 10-month intervention of double-fortified salt among Indian female tea pluckers

| | Baseline BI < 0 mg/kg | | | |
| | Non-resolvers‡ (*n* 21) | | Resolvers‡ (*n* 24) | |
| | Median | IQR | Median | IQR |
|---|---|---|---|---|
| Hb (g/l) | −0·1 | −0·3, 0·4 | 0·7 | −0·1, 1·2 |
| sFt (µg/l) | 2·0[a] | 1·0, 4·0 | 15·5[c] | 10·5, 23·0 |
| TfR (mg/l)§ | −1·3[a] | −2·9, −0·6 | −4·1[b] | −6·6, −2·2 |
| BI (mg/kg) | 1·7[a] | 1·0, 3·3 | 6·3[b] | 4·8, 7·6 |
| Folate (ng/ml) | 0 | 0, 0 | 0 | 0, 0 |
| Vitamin B$_{12}$ (pg/ml) | 0 | 0, 0 | 0 | 0, 0 |
| Iodine (µg/l) | 15 | −39, 119 | 58 | 2, 165 |

BI, estimated total body Fe; IQR, interquartile range; sFt, serum ferritin; TfR, transferrin receptor.
[a,b,c]Median values within a row with unlike superscript letters were significantly different by the Hodges–Lehman–Sen test: a *v.* b, $P < 0·01$; a *v.* c, $P < 0·001$.
‡Non-resolvers, BI < 0 mg/kg at endline; resolvers, BI $\geq$ 0 mg/kg at endline.
§Corrected by Ramco assay.

with very low sFt and BI at baseline may not have been able to absorb sufficient Fe from DFS over ten months to resolve Fe deficiency, and thus the non-resolvers had lower baseline sFt than the resolvers in the univariate analyses (Table 4).

The body responds to inflammation by sequestering Fe in ferritin, resulting in elevated sFt concentrations that may mask Fe deficiency[32] and affect prevalence estimation of deficiency[45]. This phenomenon may explain why the group with normal sFt and BI at baseline had elevated baseline CRP values relative to those with Fe deficiency or low BI at baseline. If true, inflammation explains a similar relationship in BI resolution because the calculation of BI requires both TfR and sFt[31].

There were no significant multivariate models of the resolution of Fe deficiency or low BI. However, the study was designed to identify a significant difference in the mean change in sFt concentrations between DFS and IS, and may not be sufficiently powered to detect differences in currently measured predictors of resolution. While resolution analyses are useful for identifying predictors of response and can be used to target future interventions in similar populations, they are limited because they do not account for the positive change in Fe biomarkers in those with an increased potential to benefit unless the change causes Fe concentration to cross a threshold marked by a given cut-off of deficiency. Further, such analyses do not account for increases in Fe status among women who had low but technically sufficient Fe status at baseline. Future interventions and programmes should consider the limitations of relying solely on resolution of deficiency as a benchmark for success and may benefit from using other or additional models of response, including the absolute and relative change models we present here.

Our goal was to understand the multiple underlying predictors of response to dietary Fe interventions. BI provides a composite measure of Fe status from severe deficiency to repletion, but is limited in some settings because it requires assessment of both sFt and TfR. Large public health interventions with limited resources and may require a smaller number of indicators. Although BI serves as a gold standard by permitting assessment across the range of resolution, our results show that measuring sFt, perhaps in combination with Hb, is a reasonable alternative to BI which will still adequately predict response to DFS interventions.

The present study was a randomized controlled trial, with successful randomization at baseline, appropriate sample size to test efficacy and reasonable compliance. The strong study design supports the robust secondary

**Table 7** Baseline univariate predictors of low body iron resolution to an intervention of double-fortified salt in female Indian tea pluckers

| Independent variable | BI (*n* 45) | |
|---|---|---|
| | RR‡ | 95 % CI |
| Age (years) | 0·99 | 0·96, 1·00 |
| Ethnicity§ | 1·29 | 0·76, 2·21 |
| Height (m)‖ | 0·99 | 0·93, 1·00 |
| Weight (kg) | 1·00 | 0·97, 1·00 |
| BMI (kg/m²)‖ | 1·00 | 0·94, 1·10 |
| Treatment§ | 2·78* | 1·26, 6·10 |
| Hb (g/l) | 1·10 | 0·88, 1·40 |
| sFt (µg/l) | 1·00 | 0·98, 1·10 |
| TfR (mg/l)¶ | 0·98 | 0·92, 1·00 |
| BI (mg/kg) | 1·10* | 1·00, 1·20 |
| Folate (ng/ml) | 0·92 | 0·77, 1·10 |
| Vitamin B₁₂ (pg/ml) | 1·00 | 1·00, 1·00 |
| Iodine (µg/l) | 1·00 | 1·00, 1·00 |
| sFt < 12·0 µg/l | NC | NC |
| TfR > 8·6 mg/l | 0·75 | 0·44, 1·28 |
| Anaemia (Hb < 120 g/l) | 0·65† | 0·39, 1·08 |
| Folate < 5·0 ng/ml | 0·95 | 0·47, 1·92 |
| Vitamin B₁₂ < 203 pg/ml | 1·11 | 0·65, 1·91 |
| Iodine < 100 µg/l | 0·98 | 0·56, 1·70 |

BI, estimated total body Fe; RR, relative risk; sFt, serum ferritin; TfR, transferrin receptor; NC, model did not converge.
†$P < 0.1$; *$P < 0.05$.
‡BI < 0 mg/kg at baseline and BI ≥ 0 mg/kg at endline.
§Reference values for independent variables: ethnicity, Adivasi; treatment, iodized salt.
‖Height < 150 cm and BMI < 19 kg/m² were also tested; results were not different from continuous variables.
¶Corrected by Ramco assay.

analyses presented here. Although the randomized controlled trial design provides strong internal validity, the generalizability of the results is limited to other similar populations[25,27,29,42]. Thus, our findings can be applied to other populations of South Asian women of reproductive age, with high prevalence of Fe deficiency and of non-Fe deficiency anaemia, consuming DFS.

The results presented here are secondary analyses and cannot be interpreted with the same assumptions of causality as an intention-to-treat analysis. However, the results were confirmed with multiple types of analyses that defined response to the intervention by different criteria. The study may not have been adequately powered to identify all covariates which predict response to a DFS intervention. For example, although height at baseline differed slightly between the responders and those with no potential to benefit, height did not significantly predict response in the regression analyses.

## Conclusion

DFS is increasing in popularity as a vehicle to resolve and prevent Fe deficiency, particularly among rural populations in low- and middle-income countries with limited access to dietary supplements or Fe-rich foods, and among populations with low adherence to dietary supplements[19,21–23,46].

The present analyses demonstrate that salt double-fortified with microencapsulated ferrous fumarate and potassium iodate is a viable method for resolving Fe deficiency and low BI in this population. Importantly, we demonstrate that low concentrations of BI and sFt at baseline are the best predictors of response to a DFS intervention, regardless of whether individuals are clinically deficient at baseline. Further, measuring Hb alone may not be a sufficient marker of response, nor of potential to benefit from an intervention. Future studies evaluating DFS and programmes targeting similar interventions should: (i) replicate these findings in other populations; (ii) examine changes in sFt and BI, in addition to Hb, to better target Fe interventions, especially if there is a high prevalence of non-Fe deficiency anaemia in the population; and (iii) use these methods to evaluate the impact of DFS interventions on functional outcomes. Additionally, effectiveness studies are needed to test the ability of DFS to increase resolution of Fe deficiency in community settings *v.* an experimental field setting.

and formally recorded. *Clinical trial registry:* This trial was registered at clinicaltrials.gov as NCT01032005.

## Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1368980019001800

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