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Efficacy of iron-supplement bars to reduce anemia in urban Indian women: a cluster-randomized controlled trial[1,2]

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ABSTRACT

Background: India's high prevalence of iron-deficiency anemia has largely been attributed to the local diet consisting of nonheme iron, which has lower absorption than that of heme iron.

Objective: We assessed the efficacy of the consumption of iron-supplement bars in raising hemoglobin concentrations and hematocrit percentages in anemic (hemoglobin concentration <12 g/dL) Indian women of reproductive age.

Design: The Let's be Well Red study was a 90-d, pair-matched, cluster-randomized controlled trial. A total of 361 nonpregnant women (age 18–35 y) were recruited from 10 sites within Mumbai and Navi Mumbai, India. All participants received anemia education and a complete blood count (CBC). Random assignment of anemic participants to intervention and control arms occurred within 5 matched site-pairs. Intervention participants received 1 iron-supplement bar (containing 14 mg Fe)/d for 90 d, whereas control subjects received nothing. CBC tests were given at days 15, 45, and 90. Primary outcomes were 90-d changes from baseline in hemoglobin concentrations and hematocrit percentages. Linear mixed models and generalized estimating equations were used to model continuous and binary outcomes, respectively.

Results: Of 179 anemic participants, 136 (76.0%) completed all follow-up assessments (65 intervention and 71 control participants). Baseline characteristics were comparable by arm. Mean hemoglobin and hematocrit increases after 90 d were greater for intervention than for control participants [1.4 g/dL (95% CI: 1.3, 1.6 g/dL) and 2.7% (95% CI: 2.2%, 3.2%), respectively]. The anemia prevalence at 90 d was lower for intervention (29.2%) than for control participants (98.6%) (OR: 0.007; 95% CI: 0.001, 0.04).

Conclusions: The daily consumption of an iron-supplement bar leads to increased hemoglobin concentrations and hematocrit percentages and to a lower anemia prevalence in the target population with no reported side effects. This intervention is an attractive option to combat anemia in India. This trial was registered at clinicaltrials.gov as NCT02032615. *Am J Clin Nutr* doi: 10.3945/ajcn.115.127555.

Keywords: hematocrit, hemoglobin, Indian diet, iron-deficiency anemia, iron-supplement bar, Let's be Well Red, women

INTRODUCTION

Iron deficiency is the most common nutritional disorder in the world, with the highest prevalence in children (40%), women of reproductive age (30%), and pregnant women (38%) (1, 2). Iron deficiency is the leading cause of anemia, with women of reproductive age being particularly vulnerable to acquiring iron-deficiency anemia (IDA)[8] because of the increase in blood volume and muscle mass that occurs around puberty followed by regular menstruation, which increases the body's demand for iron (3, 4).

India has the highest prevalence of IDA worldwide, which has been estimated to affect 70% of children, 55% of women of reproductive age, and 85% of pregnant women (5). This high prevalence has mainly been attributed to inadequate iron intake coupled with the poor bioavailability of iron (6–8). The Recommended Daily Allowance for iron is 15–18 mg/d for women aged 14–50 y (9, 10). However, a typical Indian woman consumes only 9.5 mg Fe/d, which is derived largely from nonheme, inorganic [ferric iron(III) or ferrous iron(II)] iron sources (11) including grains, plants, cereals, lentils, vegetables, and iron supplements, such as iron pills and iron-fortified foods. The cost of meat coupled with the vegetarian culture result in nonheme-iron sources that make up ~91% of a traditional Indian diet (11).

Heme-iron sources, which are present in meat and fish, have a higher absorption rate (15–35%) (12) than that of nonheme-iron sources (2–20%) (13) because human intestinal enterocyte cells have heme-specific binding proteins that enable the efficient receptor-mediated endocytosis of heme iron. In contrast, nonheme iron in ferrous [iron(II)] form is taken up from the lumen by nonspecific divalent metal transporter 1 receptors, which work to absorb all divalent metals and, thus, absorb nonheme iron with decreased specificity. The ferric [iron(III)] form is also

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[2] Supplemental Tables 1–5 are 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://ajcn.nutrition.org.

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[8] Abbreviations used: CBC, complete blood count; CDM, covariate-dependent missingness; c-RCT, cluster-randomized controlled trial; GEE, generalized estimating equation; IDA, iron-deficiency anemia.

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transported by divalent metal transporter 1 after reduction to iron(II) by duodenal cytochrome b (14).

Strategies to combat IDA in India (**Supplemental Tables 1** and **2**) have largely focused on the distribution of iron tablets and iron-fortified foods that are derived from inorganic bivalent iron salts with low absorption rates (10–15%) (15). Iron tablets have had limited impact because of poor compliance and gastrointestinal side effects (16–18). Fortified foods provide a more sustainable alternative, but better implementation strategies are required to improve uptake (19, 20).

To complement these national efforts, in 2012, the Let's be Well Red nongovernmental organization introduced locally produced iron-rich supplement bars that contained 14 mg nonheme iron (21). Although 100,000 bars/mo are currently provided to children, young women of reproductive age, and pregnant women through schools, nongovernmental organizations, and hospitals in 3 Indian states (21), there has been no formal evaluation of their efficacy. We conducted a 3-mo, pair-matched cluster-randomized controlled trial (c-RCT) to test the hypothesis that daily intake of iron-supplement bars increases hemoglobin concentrations and hematocrit percentages in a clinically meaningful way compared with the effect of a regular diet in nonpregnant anemic women of reproductive age residing in urban India.

METHODS

Iron-supplement bar design

The nonheme iron–supplement bars (GudNeSs bars) that were evaluated in this trial were locally manufactured by Rajdhan Pvt. Ltd. To ensure palatability, cultural compatibility, and compliance, the composition of the iron-supplement bars was based on a popular Indian delicacy called chikki and contains 31.23 g jaggery (sugarcane extract), 18.37 g black and white sesame seeds, 9.18 g flax seeds, 9.18 g barley, 9.18 g nachani and ragi millet, and 12.96 g peanuts with a nutritional content of 14.22 mg elemental Fe, 23.53 g total fat (0 mg cholesterol/dL and 0 *trans* fats), 11.30 g protein, 49.23 g carbohydrates, 0.24 g Ca, 90 IU vitamin A, and 18.09 g sugar [analyses were conducted at independent Equinox Laboratories; August 2015 (a report is available upon request)]. No iron fortificants were added. Although the natural ingredients in the bar contained high amounts of folate, which are known to increase iron absorption, the heat-labile nature of folate and the 250–300°F temperatures required during preparation caused the folate to be lost.

Because the mechanism of absorption of iron is similar across nonheme-iron sources, we would not expect significant differences in rates of absorption between our iron-supplement bars and other nonheme interventions such as iron pills and iron-fortified foods.

Study design and participants

Let's be Well Red (clinicaltrials.gov; NCT02032615) was a 2-phase, pair-matched c-RCT that was conducted between March 2014 and August 2014 in Mumbai and Navi Mumbai, India. Clusters were 10 sites that agreed to provide access to their facilities for anemia education and testing camps and for the provision of daily iron-supplement bars. Sites were selected that were adjacent to residential areas across a range of socioeconomic levels (low, middle, and upper-middle incomes) and were easily accessible to women who were living in those surrounding areas. All sites were geographically separated to avoid contamination and food sharing as have been reported in previous studies (19). The 5 pairs of sites that were selected were 2 pairs of hospitals (low income), 1 pair of banks (middle income), and 2 pairs of hotels (upper-middle income). Middle- and higher-income sites were located in central Mumbai city. Because of the higher living costs in central Mumbai, lower-income neighborhoods are on the outskirts of the city of Mumbai. Therefore, our lower-income sites were selected from the suburb Navi Mumbai, which is 50 km north of central Mumbai. Sites within pairs were closer together (mean ± SD: 2.0 ± 1.5 km in Mumbai and 3.3± 2.5 km in Navi Mumbai) than were those from other pairs (3.7 ± 1.7 km for Mumbai and 9.2 ± 0.5 km for Navi Mumbai) to minimize within-pair differences while avoiding contamination.

Participants

Recruitment was performed in 2 phases. In the first phase, anemia-education and -testing camps were advertised in local print media to recruit women to screen for anemia. For this first phase, eligible participants were women aged 18–35 y who were not pregnant. Participants were asked to provide written consent (in English, Hindi, or Marathi) to participate in the study, which included both anemia education and screening (phase 1) and a subsequent intervention if the subjects were anemic (phase 2). Women who reported a nut allergy or who were taking iron supplements were excluded. Only anemic (hemoglobin concentration $<$12 g/dL) (22) women from phase 1 were invited to participate in phase 2 (the c-RCT).

Random assignment and masking

We selected cluster randomization for logistical (the daily administration of iron-supplement bars), contamination (the sharing of bars within and between sites), and equity reasons. Each individual site was considered 1 cluster. We used cluster-level matching to maximize the statistical efficiency; if clusters were well matched, the between-cluster variation within matched pairs should have been smaller than the variation between different pairs of clusters (23). Clusters were matched in pairs on the basis of income level and geography. Within each matched pair, clusters were randomly assigned to either the intervention or control arm. Specifically, clusters in each pair were first listed in a fixed order with the first cluster assigned to the intervention arm and the second cluster assigned to the control arm. A virtual coin flip was generated for each cluster pair with the use of a pseudorandom number generator with the Mersenne twister algorithm in scripting software (Python Software Foundation, version 3.3.5cr1), whereby each cluster pair was uniformly assigned a number between 0 and 1. Pseudorandom-number generators have the advantage of approximating random-number generation while offering the benefit of reproducibility through the use of a seed value (i.e., the use of the same seed for the algorithm will produce the same outcome although the seed itself may be random). For a cluster-pair value $\leq$0.5, the intervention-arm assignment was retained, whereas the assignment was swapped for cluster-pair values $>$0.5.

Qualified site managers, who were assigned by the local Independent Ethics Committee in Mumbai, led the site selection, which involved going through a list of sites that had previously shown interest in participating in the study. To maintain independence from the producers of the iron-supplement bars, study coordinators who collected data and supervised the daily consumption of bars were employed by Mulliben Dullabhai Trust, which is a nonprofit organization in Mumbai. Because of the nature of the intervention, study personnel could not be masked to allocation. The statistical analysis was conducted by the Research Design & Analysis Core of the Duke Global Health Institute at Duke University. Study statisticians conducted all analyses according to a prespecified plan and were not masked to the arm allocation during analysis.

Procedures

In the first phase of the study, an education and testing camp was held at each of the 10 study sites. All participants completed a survey questionnaire that captured information on demographics, socioeconomic status, diet, health status, and medical history. Participants attended a 1-h information session that consisted of a 2-min animated clip and an interactive presentation about anemia, its consequences, and the importance of timely treatment. After the education session, certified phlebotomists drew 1 mL blood through a venipuncture in the antecubital vein for a complete blood count (CBC) test. Blood draws were collected in labeled vials, and samples were run on an automated cell counter at Suburban Diagnostics in Mumbai, which is a center that is certified by the International Organization for Standardization, Indian Statistical Institute, and Bureau of Indian Standards.

Participants at the 5 sites who were randomly assigned to the intervention arm were provided 1 iron-supplement bar/d for 90 d. Participants at these sites were instructed to visit the site daily to receive their iron-supplement bar where study personnel supervised their consumption. Participants were instructed to promptly report any side effects (including nausea, vomiting, diarrhea, and constipation) to study personnel so that they could be referred to a clinic for follow-up. When a participant did not attend a daily visit, a member of the study team tried to contact her by phone $\leq$3 times on the same day and conducted $\leq$2 home visits the next day to encourage further participation. Despite these attempts, individuals who did not attend the daily bar distribution $>$2 d were considered to be lost to follow-up. Because bars were distributed only at the study sites, individuals who were lost to follow-up received no additional bars. If an intervention-arm participant discontinued the consumption of bars, she was still requested to provide a blood draw at the follow-up sessions. In practice, participants who stopped taking the bars also refused to participate in the blood testing. Control participants did not receive any placebo and were simply given surveys and CBCs at each of the regular intervals in the same manner as for intervention participants. A follow-up survey and CBC visits were planned for all participants at 15, 45, and 90 d postbaseline. At the end of the 90-d follow-up period, control participants were given a 3-mo supply of iron-supplement bars to use as they chose. No additional follow-up measurements were undertaken as part of the study.

Clinical assessments

Prespecified primary outcomes were the change in hemoglobin concentrations and hematocrit percentages from baseline to the 90-d follow-up. The secondary outcome was anemia at 90 d. For the primary outcomes, we prespecified secondary analyses in the subgroup of women who were classified as having moderate or severe anemia on the basis of hematocrit $<$32% at baseline measurement (22). Both hemoglobin and hematocrit were chosen for their ability to provide complementary and complete information on anemia. Although hemoglobin concentrations are used to define anemia status, hematocrit is calculated by automated cell counters by multiplying the red cell number (in millions per cubic millimeter) by the mean cell volume (in femtoliters) and, thus, give information about the underlying cause of anemia. The mean cell-volume value helps to differentiate between microcytic, macrocytic, and normocytic anemias. IDA is the most common cause of microcytic anemia (22).

Ethics

The study was approved by the Duke University Health System Institutional Review Board and the local Independent Ethics Committee in Mumbai, India (IORG0006104). All participants gave written informed consent.

Statistical analysis

We estimated the sample size with the use of a 2-sample *t* test for a pair-matched cluster-randomized trial with 5 pairs (23). On the basis of previous studies, we estimated a clinically meaningful effect of a 1-g/dL greater mean change (baseline to 90 d) in the hemoglobin concentration in the intervention arm than in the control arm (24). We used a conservative estimate of the associated SDs of change for both arms of 1.5 g/dL and an interclass correlation coefficient of 0.025. To achieve 80% power and a 2-sided significance level of 0.05 for a total of 10 clusters of equal cluster sizes, we needed to recruit $\geq$11 anemic women/cluster (total n = 110). With the anticipation of dropouts in both arms as high as 40%, we targeted the recruitment of 16 anemic women/cluster (total n = 160). With a conservative estimate of an anemia prevalence of 50% in urban women aged 18–35 y, we planned to recruit $\geq$320 women into the first phase of the study to meet the target sample size that was needed for the second-phase c-RCT.

To summarize results from the c-RCT, we provide a description of the analysis of data from all eligible anemic women who participated in the c-RCT. We compared baseline covariates to examine the balance between intervention and control arms with the use of counts and percentages for categorical variables and means $\pm$ SDs for continuous variables. Mean hemoglobin concentrations and hematocrit percentages and the prevalence of anemia were summarized by study arm at baseline and at each follow-up visit (at 15, 45, and 90 d). Cluster-level summaries of baseline characteristics of participants were calculated to compare cluster sites. Similarly, cluster-level summaries of all outcome variables were obtained at baseline and at each follow-up. All available outcome data were analyzed in the intention-to-treat framework.

Cluster-level analyses are usually recommended for c-RCTs with small number of clusters ($<$15 clusters/study arm) because

distributional assumptions of model-based approaches cannot be reliably estimated (23, 25). In our study, because the Moulton-corrected (25) intraclass correlation coefficients of baseline hemoglobin and hematocrit for the site and for site pair indicated a negligible correlation within clusters (results not shown), we used a model-based approach for our primary analyses and used a cluster-level approach in sensitivity analyses.

We estimated our primary outcomes of the changes from baseline hemoglobin concentrations and hematocrit percentages by modeling the concentrations at baseline (as an outcome) and at each of the 3 follow-up time points with the use of linear mixed models with a random intercept for each participant to account for correlations that were due to repeated measurements over time. The random intercepts and residual error terms were assumed to be independent and zero-mean normally distributed. This assumption implied a within-person correlation that was constant for all pairs of measurements of the same individual. We included indicator variables for the site pair to account for any fixed variation in hemoglobin concentrations and hematocrit percentages by pair (26). The models included time (as a factor), intervention arm (as an indicator), and the interaction of the intervention arm by time to allow for differing effects of the intervention by time. Because of the small number of clusters, we were concerned about a chance imbalance in baseline hemoglobin concentrations and hematocrit percentages. Our model accounted for this possibility because it modeled the baseline as an outcome and specified different intercepts for each arm (i.e., the model allowed for different mean concentrations of the outcome at baseline).

The intervention effect was estimated as the difference between arms in the change from baseline to 90 d, which was estimated according to the appropriate contrast from the linear mixed model. Such an approach allowed us to estimate the intervention effect while accounting for the possibility of secular trends in hemoglobin and hematocrit changes in both groups that were due to anemia education or other outside factors. Results are reported as point estimates with 95% CIs and P values for all women and for the subgroup of women with moderate or severe anemia (hematocrit $<32\%$) at baseline (22). We evaluated the sensitivity of our results with the use of the recommended cluster-level summary method of a pair-matched t test separately at each time period by analyzing cluster-level mean changes (from baseline) of hemoglobin and hematocrit (27).

An analysis of our secondary outcome of anemia status enabled us to examine the extent to which women transitioned to being nonanemic at each follow-up time point. We used the same form of the model as for the primary outcomes by modeling the intervention arm (as a factor), time (as a factor), and the interaction of the intervention arm by time with fixed effects for the cluster pair. Because all participants were anemic at baseline, we analyzed anemia status starting at the 15-d follow-up time point. We used a generalized estimating equations (GEEs) approach with the assumption of a binomial distribution, logit link function, and an exchangeable working correlation for repeated outcomes over time for the participant (28). An exchangeable working correlation was selected according to the best model fit on the basis of the quasilikelihood under the independence model criterion (29). Our main intervention effect for the secondary outcome of anemia status was the OR of intervention odds compared with control odds of being anemic at 90 d, which was obtained by taking the relevant contrast from the model.

Our analytic approach for all outcomes analyzed all available data so that, even for participants who were lost to follow-up, all available data up until the time of dropout were included in the linear mixed models and binary GEE models. Both of these models provided valid estimates of the intervention effect under the assumption that the missing outcome-data mechanism was covariate-dependent missingness (CDM) (30) if the baseline predictors of missing outcomes were included as covariates in the model. In CDM, the probability of missing an outcome does not depend on the level of the outcome and is often referred to as a special case of missing at random (30). To confirm the validity of the CDM assumption, we compared baseline characteristics by study-completion status (i.e., completed all follow-up rounds compared with did not) to identify predictors of dropout. The Wilcoxon's rank-sum test was used to test differences for continuous variables. The chi-square test or Fisher's exact test was used for categorical variables, and Fisher's exact test was used for all comparisons that involved <10 frequencies in any one category. We did not account for clustering by site or site pair in these tests, which was not problematic because we would have been more likely to detect predictors of completion status when not accounting for clustering due to inflated type I error.

In practice, only the study arm was identified as a predictor of dropout, with greater dropout in the intervention arms than in the control arm. We prespecified that any identified predictors would be included in our models in missing-data sensitivity analyses. As such, because all models included the study arm as the primary predictor, our primary analysis models accounted for the dropout mechanism, and we did not provide additional sensitivity analyses (26, 28). If we correctly identified the dropout mechanism, our analyses with the use of all available data would have provided unbiased estimates of the intervention effect, which was the effect of complete adherence to a 90-d daily consumption of the iron-supplement bars. Although unmeasured covariates may have been predictive of dropout, because we measured the most-relevant covariates related to anemia, we had no evidence to suggest that we had misspecified the missing-data mechanism. All analyses were performed with the use of Statistical Analysis System version 9.4 (SAS Institute Inc.). All reporting was done in accordance with the Consolidating Standards of Reporting Trials extended guidelines for c-RCTs.

RESULTS

Study participation

A total of 361 women attended the anemia-education, -testing, and -treating camps (**Figure 1**). Of 361 women, 182 women were not eligible to participate in the c-RCT for a single reason (n = 159) or for multiple reasons (n = 23), which included not being anemic (n = 159), taking iron pills or supplements (n = 21), being out of the specified age range (n = 13), or not having hemoglobin available (one blood sample could not be processed correctly because of hypercoagulation and blood clots). After exclusions, 179 women were eligible to take part in the c-RCT to test the efficacy of the daily consumption of iron-supplement bars in nonpregnant, anemic women of reproductive age. The 10 study sites were randomly assigned within 5 matched pairs with 84 women in the control arm (mean $\pm$ SD cluster

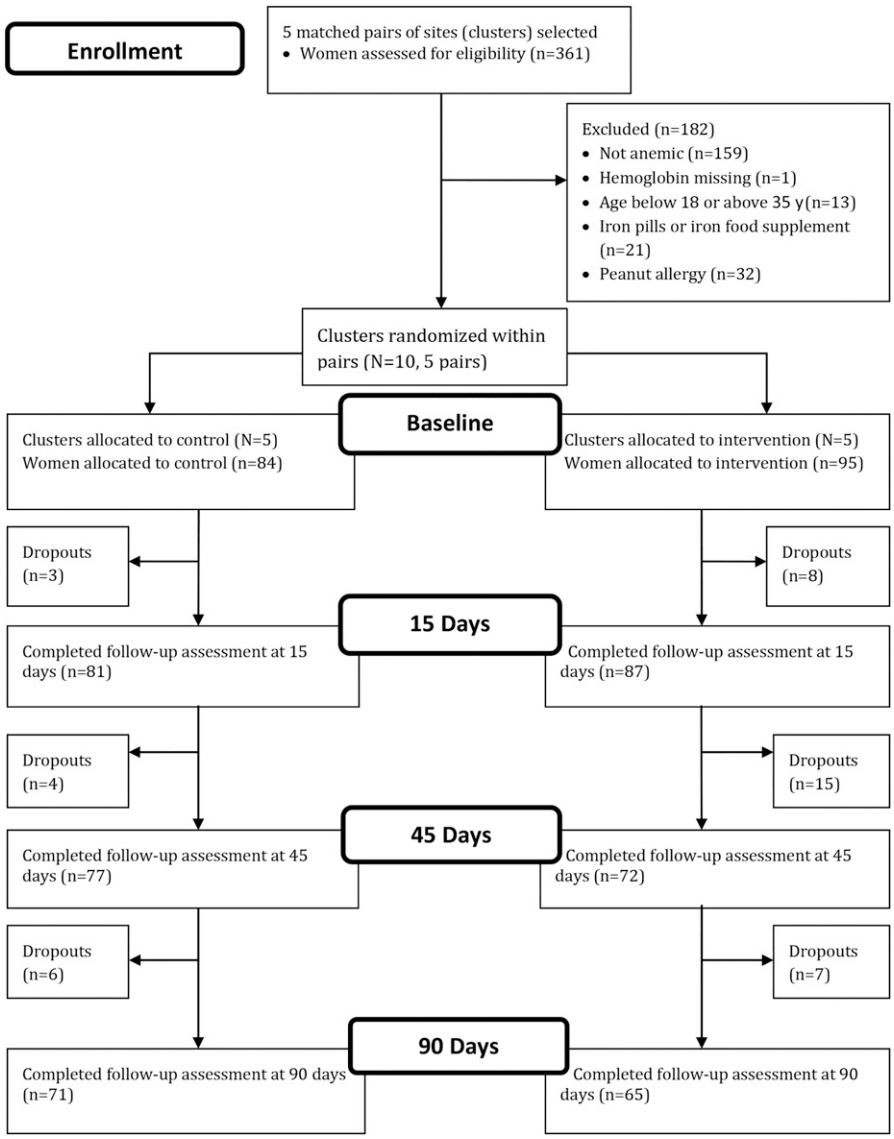

FIGURE 1 Study profile. During the screening process, 182 women were ineligible for the cluster-randomized controlled trial; of those, 159 women had a single reason and 23 women had multiple reasons.

size: n = 16.8 ± 14.4) and 95 women in the intervention arm (cluster size: n = 19.0 ± 16.6). Of 179 enrolled women, 168 women (93.9%), 149 women (83.2%), and 136 women (76.0%) completed the 15-, 45-, and 90-d follow-ups, respectively. Although the study protocol prespecified that outcomes would be measured in women who missed intermediate time points or missed the daily distribution of the nutritional bars (in the intervention arm), in practice, we only observed a complete study dropout and nonintermittent patterns of missingness when a woman might have missed a follow-up measurement but was present at a later measurement time point. None of the participants in the intervention arm reported experiencing any gastrointestinal side effects from the consumption of the iron-supplement bars.

Baseline characteristics

Baseline characteristics by study arm showed that the study arms were reasonably balanced (**Table 1**). The mean ± SD hemoglobin concentration was 10.5 ± 1.3 g/dL in the control group and 10.5 ± 1.2 g/dL in the intervention group, and hematocrit percentages were 32.7% ± 3.3% and 32.8% ± 3.1% in the control and intervention arms, respectively. Mean ± SD ages were 28.6 ± 5.4 and 28.9 ± 5.2 y in the control and intervention arms, respectively. Because of the small sample size (n = 55) at baseline of the subgroup of women with moderate or severe anemia (hematocrit <32%), baseline characteristics by arm were less-well balanced than for the whole sample (**Supplemental Table 3**). Baseline hemoglobin, which was expected to be the most-predictive covariate, was well balanced and analyzed as an outcome in the statistical model, which allowed for different mean baseline values in each arm. Therefore, we had no reason to believe that the estimated intervention effect in this subgroup was biased. Characteristics that are summarized by site pair for the whole study population are shown in in **Supplemental Table 4**.

TABLE 1
Baseline characteristics by arm of 179 anemic study participants[1]

Characteristic	Control (n = 95)	Intervention (n = 84)
Hemoglobin, g/dL	10.5 ± 1.3[2]	10.5 ± 1.2
Hematocrit, %	32.7 ± 3.3	32.8 ± 3.1
Moderate or severe anemia, n (%)	28 (33.3)	27 (28.4)
Age, y	28.6 ± 5.4	28.9 ± 5.2
Children, n (%)		
Missing data	13 (—)	11 (—)
0	21 (29.6)	21 (25.0)
1	21 (29.6)	23 (27.4)
2	16 (22.5)	27 (32.1)
≥3	13 (18.3)	13 (15.5)
Per cluster by type, n (%)		
Middle income	4 (4.8)	7 (7.4)
Upper middle income 1	8 (9.5)	9 (9.5)
Upper middle income 2	21 (25.0)	21 (22.1)
Low income 1	11 (13.1)	11 (11.6)
Low income 2	40 (47.6)	47 (49.5)
Diet score[3]	34.4 ± 24.4	31.0 ± 22.1
Anemia symptoms,[4] n (%)		
None	5 (6.0)	7 (7.4)
1–2	28 (33.3)	23 (24.2)
≥3	51 (60.7)	65 (68.4)
Medical conditions,[5] n (%)		
0	60 (71.4)	71 (74.7)
1	18 (21.4)	20 (21.1)
≥2	6 (7.1)	4 (4.2)
Menstruation, n (%)		
Missing data	3 (—)	2 (—)
Regular	67 (82.7)	74 (79.6)
Irregular	14 (17.3)	19 (20.4)
Pregnancies, n (%)		
Missing data	6 (−)	7 (−)
0	25 (32.1)	20 (22.7)
1	20 (25.6)	19 (21.6)
2	13 (16.7)	23 (26.1)
3	12 (15.4)	14 (15.9)
≥4	8 (10.3)	12 (13.6)
Miscarriages, n (%)		
Missing data	11 (—)	7 (—)
None	56 (76.7)	64 (72.7)
≥1	17 (23.3)	24 (27.3)

[1] Percentages are of the total participants observed.
[2] Mean ± SD (all such values).
[3] Iron rich–diet scores were calculated as the sum of food items eaten per week whereby iron-rich foods were scored with twice the value of non–iron rich foods.
[4] Included tiredness, weakness, breathlessness, decreased attention span, excessive sleeping, and fainting.
[5] Included the history of worm infestation, malaria, tuberculosis, and blood transfusion.

Intervention effects on hemoglobin concentrations

From a baseline mean concentration of 10.5 g/dL, 90-d mean ± SD hemoglobin concentrations were 11.9 ± 0.7 compared with 10.5 ± 1.0 g/dL in the intervention arm and control arm, respectively, with no change in the control arm (P = 0.94) (**Tables 2** and **3**). There was an overall effect of the intervention at all follow-up time points (P-arm-by-time interaction $<$ 0.001). The regression-estimated mean 90-d change in hemoglobin in the intervention arm was 1.4-g/dL (95% CI: 1.3-, 1.6-g/dL) greater ($P < 0.001$) than in the control arm. This amount represented a clinically meaningful difference between groups that was defined previously as a >1.0-g/dL greater change. The effect of the intervention was even greater (2.2 g/dL; 95% CI: 1.9, 2.4 g/dL; $P < 0.001$) for women with moderate or severe anemia at baseline (hematocrit <32%) (Table 3). These effects were clearly represented by examining the model-estimated means (**Figure 2**, solid lines corresponding to the left vertical axis), which are shown by time point and arm for all women (Figure 2A) and for those with moderate or severe anemia at baseline (Figure 2B).

Intervention effects on hematocrit percentages

A similar pattern was observed for hematocrit with an overall effect of the intervention at all follow-up time points (P-arm-by-time interaction < 0.001) and a 90-d increase in the intervention arm from a mean ± SD of 32.8% ± 3.1% to 35.6% ± 1.8% compared with no change in the control arm (P = 0.83) (**Table 4**). At a mean of 35.6% at 90 d, intervention participants were in the normal range for hematocrit (34.9–44.5%) (22). The regression-estimated mean 90-d change in hematocrit in the intervention arm was 2.7-percentage points (95% CI: 2.2-, 3.2-percentage points) greater ($P < 0.001$) than in the control arm (**Table 5**). Again, the intervention effect was larger for women with moderate or severe anemia at baseline (4.5%; 95% CI: 3.5%, 5.4%; $P < 0.001$). As for hemoglobin, we represented the model-based estimated effects on hematocrit (Figure 2, dashed lines corresponding to the right vertical axis) by time point and arm for all women (Figure 2A) and for women with moderate or severe anemia (Figure 2B).

Intervention effects on anemia prevalence

As for hemoglobin and hematocrit, there was an overall effect of the intervention at all follow-up time points (P-arm-by-time interaction < 0.001). At day 15, odds of being anemic were already significantly lower (OR: 0.2; 95% CI: 0.05, 0.5; $P < 0.001$) in the intervention arm than in the control arm (**Tables 6** and **7**). A greater intervention effect was achieved at 90 d when the ratio of odds of anemia for the intervention arm compared with the control arm was estimated to be <1% of that in the control arm (OR: 0.007; 95% CI: 0.001, 0.04; $P < 0.001$), which corresponded to observed prevalences of 29.2% compared with 98.6% in the intervention and control arms, respectively (Tables 6 and 7).

Sensitivity analyses

Paired cluster-level t tests (results not shown) confirmed the individual-level results, which showed significant differences between mean changes in hemoglobin and hematocrit for the intervention arm compared with the control arm. As reported in Statistical Analysis, only the study arm was identified as a predictor of study dropout, and therefore, we have not presented additional sensitivity analyses for missing data because all models already accounted for this sole predictor of dropout. Consequently, our primary analyses should have provided valid estimates of the efficacy of the bars, namely of complete adherence to the 90-d daily consumption of the iron-supplement bars.

TABLE 2
Outcomes for hemoglobin in 179 anemic study participants and in the subgroup of 55 participants with moderate or severe anemia[1]

	Full sample			Subgroup with moderate or severe anemia at baseline		
Arm	*n* (%)	Change,[2,3] g/dL	g/dL[2]	*n* (%)	Change,[2,3] g/dL	g/dL[2]
Outcome						
Absolute level at baseline						
Control	84 (100.0)	—	10.5 ± 1.3	28 (100.0)	—	9.0 ± 1.0
Intervention	95 (100.0)	—	10.5 ± 1.2	27 (100.0)	—	8.9 ± 1.1
Absolute level at 15 d						
Control	81 (96.4)	−0.06 ± 0.4	10.4 ± 1.2	28 (100.0)	0.09 ± 0.3	9.0 ± 1.0
Intervention	87 (91.60)	0.6 ± 0.4	11.1 ± 1.3	25 (92.6)	0.6 ± 0.4	9.5 ± 1.1
Absolute level at 45 d						
Control	77 (91.7)	−0.08 ± 0.4	10.4 ± 1.3	26 (92.9)	0.03 ± 0.3	9.0 ± 1.0
Intervention	72 (75.8)	1.1 ± 0.7	11.6 ± 1.03	20 (74.1)	1.7 ± 0.5	10.6 ± 1.2
Absolute level at 90 d						
Control	71 (84.5)	−0.02 ± 0.5	10.5 ± 1.0	24 (85.7)	0.19 ± 0. 5	9.4 ± 0.9
Intervention	65 (68.4)	1.42 ± 0.8	11.9 ± 0.7	17 (63.0)	2.4 ± 0.6	11.2 ± 1.0
Total observations, *n*	632	—	—	195	—	—

[1] Moderate or severe anemia was defined as having baseline hematocrit <32%.
[2] All values are means ± SDs of change.
[3] Differences were computed as follow-up values minus baseline values.

DISCUSSION

This study shows that the daily consumption of iron-supplement bars led to a clinically significant increase in hemoglobin and hematocrit in nonpregnant anemic women of reproductive age (18–35) over 90 d. Increases in hemoglobin were obtained as early as 15 d postbaseline with 93.1% of women in the intervention arm (compared with 34.6% of women in the control arm) showing positive increases in hemoglobin over that time. Women in our study started out with depleted stores of iron with a mean hematocrit value of 32.7% at baseline, which was equivalent to the hematocrit percentage in a healthy woman in the Western world during her third trimester of pregnancy (31). Despite this low baseline, we showed that, after consumption of iron-supplement bars for 90 d, 70.8% of the anemic women in the intervention arm had normal hemoglobin concentrations of 12.0 g/dL (22) and were no longer anemic. Of these subjects, 82.1% of women reached normal hematocrit percentages (34.9–44.5%) (22), with an average of 35.6%. Both

TABLE 3
Regression estimates for hemoglobin in 179 anemic study participants and in the subgroup of 55 participants with moderate or severe anemia[1]

	Full sample		Subgroup with moderate or severe anemia at baseline	
Variable	Estimate	*P*	Estimate	*P*
Regression estimate, g/dL				
Baseline				
Control	10.5 (10.2, 10.8)	<0.001	8.9 (8.4, 9.4)	<0.001
Intervention minus control	0.004 (−0.3, 0.4)	0.98	−0.02 (−0.6, 0.6)	0.94
Changes from baseline to 15 d				
Control	−0.06 (−0.2, 0.05)	0.27	0.09 (−0.07, 0.3)	0.25
Intervention change compared with control change	0.7 (0.5, 0.8)	<0.001	0.5 (0.3, 0.7)	<0.001
Changes from baseline to 45 d				
Control	−0.08 (−0.2, 0.04)	0.18	0.03 (−0.1, 0.2)	0.76
Intervention change compared with control change	1.2 (1.0, 1.4)	<0.001	1.7 (1.4, 1.9)	<0.001
Changes from baseline to 90 d				
Control	−0.005 (−0.1, 0.1)	0.94	0.2 (0.1, 0.4)	0.009
Intervention change compared with control change	1.4 (1.3, 1.6)	<0.001	2.2 (1.9, 2.4)	<0.001
Overall *P*-arm-by-time interaction	—	<0.001	—	<0.001
Observations used, *n*	632	—	195	—

[1] All values are model-estimated means (95% CIs). Moderate or severe anemia was defined as having baseline hematocrit <32%. All regression estimates were determined with the use of a linear mixed-effects regression with individual-level random intercepts and a fixed effect for the site pair.

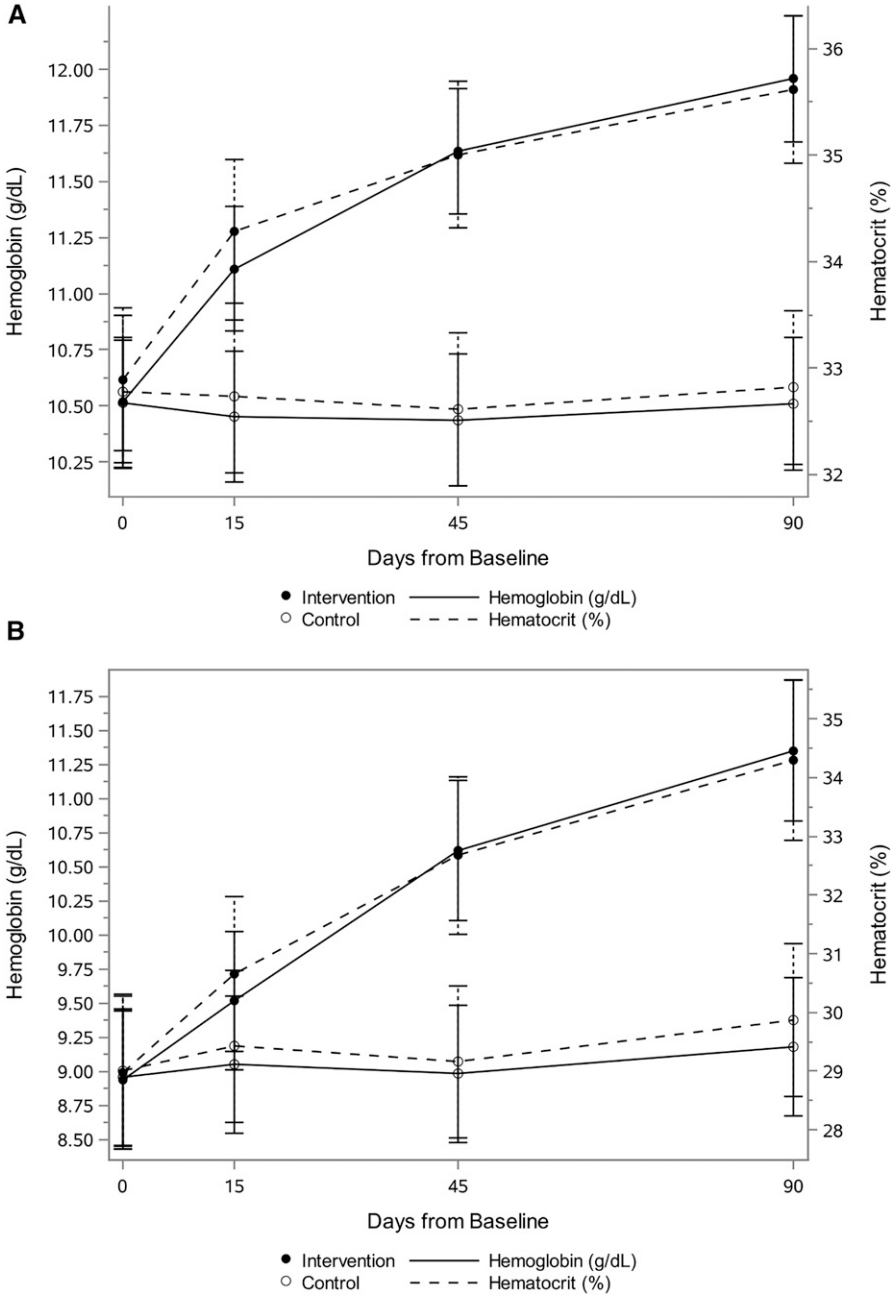

FIGURE 2 Linear mixed-effects model-estimated means (95% CIs) of hemoglobin (grams per deciliter) and hematocrit (percentage) for n = 84, 81, 77, and 72 control participants and n = 95, 87, 72, and 65 intervention participants at baseline and 15, 45, and 90 d, respectively (A), and for individuals with moderate or severe anemia at baseline including n = 28, 28, 26, and 24 control participants and n = 27, 25, 20, and 17 intervention participants at baseline and 15, 45, and 90 d, respectively (B).

hemoglobin and hematocrit are expected to rise slowly once treatment has started, which usually begins after 1 to 2 wk of treatment and reaches normal levels by 6–8 wk (32). Evidence of any intervention in our target population of nonpregnant Indian women of reproductive age has been sparse, and to our knowledge, our study is the first study to examine changes in hematocrit as an additional intervention outcome in an Indian population.

Previous studies have evaluated whether daily intake of iron tablets or iron-fortified foods lead to increased hemoglobin concentrations and improved iron status in Indian women (**Supplemental Table 5**). Both types of interventions have improved hemoglobin concentrations to varying degrees, but adverse effects, such as constipation, diarrhea, nausea, and vomiting, were widely reported with the use of iron tablets (17, 33). Although iron-fortified foods are better tolerated, it may be difficult to implement effectively in India where the production of many staple foods, such as rice milling or flour processing, is not centralized (18). Furthermore, compliance rates have been low, and treatment has often taken longer with rises in hemoglobin concentrations shown only after ≥6 mo of consumption (18, 20). The double fortification of salt has shown some

TABLE 4
Outcomes for hematocrit in 179 anemic study participants and in the subgroup of 55 participants with moderate or severe anemia[1]

	Full sample			Subgroup with moderate or severe anemia at baseline		
Arm	*n* (%)	Change,[2,3] %	%[2]	*n* (%)	Change,[2,3] %	%[2]
Outcome						
Absolute level at baseline						
Control	84 (100.0)	—	32.7 ± 3.3	28 (100.0)	—	28.9 ± 2.6
Intervention	95 (100.0)	—	32.8 ± 3.1	27 (100.0)	—	28.8 ± 2.6
Absolute level at 15 d						
Control	81 (96.4)	−0.04 ± 1.0	32.5 ± 3.0	28 (100.0)	0.4 ± 1.1	29.3 ± 2.7
Intervention	87 (91.6)	1.4 ± 1.3	34.2 ± 2.9	25 (92.6)	1.7 ± 1.6	30.6 ± 2.4
Absolute level at 45 d						
Control	76 (90.5)	−0.1 ± 0.9	32.4 ± 3.1	26 (92.9)	0.2 ± 0.7	29.0 ± 2.7
Intervention	72 (75.8)	2.2 ± 1.8	34.9 ± 2.4	20 (74.1)	3.8 ± 1.8	32.5 ± 3.1
Absolute level at 90 d						
Control	71 (84.5)	0.01 ± 1.4	32.8 ± 2.6	24 (85.7)	0.8 ± 1.7	30.2 ± 2.6
Intervention	65 (68.4)	2.7 ± 2.1	35.6 ± 1.8	17 (63.0)	5.5 ± 1.7	34.1 ± 2.9
Total observations, *n*	632	—	—	195	—	—

[1] Moderate or severe anemia was defined as having baseline hematocrit <32%.
[2] All values are means ± SDs of change.
[3] Differences were computed as follow-up values minus baseline values.

promise of raising hemoglobin concentrations by 1.1–2.0 g/dL for women (anemic and nonanemic combined) of reproductive age when consumed over 1 y according to one study (34). Compared with previous interventions involving fortification, the daily consumption of the iron-supplement bar was associated with a clinically significant average rise in hemoglobin and hematocrit that occurred in a relatively short amount of time with no reported side effects.

Despite these benefits, because the bars were largely composed of grains and peanuts, the exposure to aflatoxins from stored grains and peanuts must be considered. The preparation of the bars required heating to temperatures as high as 250–300°F, and the grains are roasted and processed before use to reduce possible risk of aflatoxin exposure (35). The use of fresh grains and regular testing should be instated to monitor exposure to aflatoxins.

There are several limitations to this study that must be noted when interpreting the results. In all cases, we had carefully preplanned analytic strategies, and we have provided a description of what study characteristics should have mitigated any negative effects. The limitations could be broadly classified as those related

TABLE 5
Regression estimates for hematocrit in 179 anemic study participants and in the subgroup of 55 participants with moderate or severe anemia[1]

	Full sample		Subgroup with moderate or severe anemia at baseline	
Variable	Estimate	*P*	Estimate	*P*
Regression estimate, %				
Baseline				
Control	32.6 (31.8, 33.4)	<0.001	28.6 (27.3, 30.0)	<0.001
Intervention minus control	0.1 (−0.8, 1.0)	0.80	−0.04 (−1.6, 1.5)	0.96
Changes from baseline to 15 d				
Control	−0.05 (−0.4, 0.3)	0.77	0.4 (−0.2, 1.0)	0.17
Intervention change compared with control change	1.4 (1.0, 1.9)	<0.001	1.3 (0.4, 2.1)	0.0042
Changes from baseline to 45 d				
Control	−0.2 (−0.5, 0.2)	0.31	0.2 (−0.5, 0.8)	0.64
Intervention change compared with control change	2.3 (1.8, 2.7)	<0.001	3.55 (2.6, 4.5)	<0.001
Changes from baseline to 90 d				
Control	0.04 (−0.3, 0.4)	0.83	0.9 (0.2, 1.5)	0.0080
Intervention change compared with control change	2.7 (2.2, 3.2)	<0.001	4.5 (3.5, 5.4)	<0.001
Overall *P*-arm-by-time interaction	—	<0.001	—	<0.001
Observations used, *n*	632	—	195	—

[1] All values are model-estimated means (95% CIs). Moderate or severe anemia was defined as having baseline hematocrit <32%. All regression estimates were determined with the use of a linear mixed-effects regression with individual-level random intercepts and a fixed effect for the site pair.

TABLE 6
Sample proportions for anemia in 179 anemic study participants and in the subgroup of 55 participants with moderate or severe anemia[1]

	Full sample		Subgroup with moderate or severe anemia at baseline	
Arm	With anemia data[2]	Anemic[3]	With anemia data[2]	Anemic[3]
Sample proportion, *n* (%)				
Absolute level at 15 d				
Control	81 (96.4)	77 (95.1)	28 (100.0)	28 (100.0)
Intervention	87 (91.6)	66 (75.9)	25 (92.6)	25 (100.0)
Absolute level at 45 d				
Control	77 (90.5)	76 (98.7)	26 (92.9)	26 (100.0)
Intervention	72 (75.8)	39 (54.2)	20 (74.1)	19 (95.0)
Absolute level at 90 d				
Control	71 (84.5)	70 (98.6)	24 (85.7)	24 (100.0)
Intervention	65 (68.4)	19 (29.2)	17 (63.0)	12 (70.6)
Total observations, *n*	632	—	195	—

[1] Anemia was defined as having a hemoglobin concentration <12 g/dL. Moderate or severe anemia was defined as having baseline hematocrit <32%.
[2] Percentages are of 84 control subjects and 95 intervention subjects who were enrolled at baseline.
[3] Percentages are of total subjects with anemia data at baseline.

to features of the study design and those related to the nature of our intervention and of the associated data collection.

There were 2 limitations related to features of the study design and data collection, both of which we have addressed with the use of primarily statistical approaches. First, because we recruited from only 10 sites (clusters) in total, we could not properly estimate the effects of higher-level clustering within the individual-level mixed effects and GEE models that were chosen. However, the estimation of the baseline intraclass correlation coefficient revealed that there was negligible higher-level clustering both because of paired sites and because of sites themselves. Further, we complemented our individual-level analysis with a cluster-level analysis, which confirmed that results were significant and robust to various analytic approaches. Second, in practice, we were not able to blind data collectors or study statisticians to treatment conditions. This limitation may have been ameliorated by the objective nature of our main outcomes and primary predictors; however, a lack of blinding introduced the possibility of bias to our study.

There are 2 limitations related to the nature of the intervention. First, we evaluated a tightly controlled intervention in which the daily consumption of iron-supplement bars was supervised and recorded by study personnel in the intervention arm. In contrast, the control arm did not receive a placebo and did not have daily contact with study personnel. Although we structured the study to be analyzed in an intention-to-treat framework, in practice, intervention participants represented only those subjects who adhered to the daily consumption, and control participants had no

TABLE 7
Regression-estimated ORs of anemia for 168 anemic study participants at 3 follow-up time points[1]

	Full sample	
Variable	OR (95% CI)	*P*
Regression estimate		
15 d		
Control[2]	Reference	—
Intervention	0.16 (0.05, 0.5)	0.0015
45 d		
Control[2]	Reference	—
Intervention compared with control	0.02 (0.003, 0.1)	<0.001
90 d		
Control[2]	Reference	—
Intervention compared with control	0.007 (0.001, 0.04)	<0.001
Overall *P*-arm-by-time interaction	—	<0.001
Observations used, *n*	453	—

[1] Anemia was defined as having a hemoglobin concentration <12 g/dL. All regression estimates were determined with the use of generalized estimating equation modeling for the binomial outcome with logit link and a fixed effect for the site pair.
[2] With estimated odds of 17.98 (95% CI: 6.5, 49.6) at 15 d, 60.5 (95% CI: 10.1, 363.4) at 45 d, and 51.0 (95% CI: 10.1, 258.6) at 90 d.

means by which to access the bars. For this reason, the results should be interpreted as representing a best-case scenario for the effect of the daily consumption of the iron-supplement bars on hemoglobin and hematocrit. In reality, the consumption of the bars could be expected to be less than daily or less than the full-recommended amount because food sharing is common, and perfect adherence in the absence of food sharing may also not be realistic in this setting. We expect the effects of the consumption of iron-supplement bars on hemoglobin and hematocrit to be more muted in an uncontrolled, real-world setting.

The nature of the intervention may have led to a second associated limitation of a higher dropout rate in the intervention arm than in the control arm. This higher dropout rate was possibly due to a higher burden of participation on the intervention group, which may have been counterbalanced by an increased motivation to comply because of the receipt of food on a daily basis and because of daily contact with study personnel. In contrast, the control group did not receive a placebo per study design, and, thus, did not have daily contact with study personnel. A delay in the receipt of the bars may have lessened the motivation to comply with data collection and, coupled with more-intermittent contact with study staff, may have increased the dropout rate. However, if the dropout rate in the study was mainly caused by a higher time burden of compliance, this effect may explain why less dropouts occurred in the control group.

We recognize that IDA is a social, political, and health problem with several factors that need to be considered while comparing results and interpreting the efficacy of our intervention. Although the arm assignment was not randomized at the individual level, the 2 arms were comparable at baseline, which allowed us to attribute the beneficial effect to the intervention itself rather than to any selection bias.

In conclusion, the iron-supplement bars that we evaluate in this study provide an iron-rich, locally sourced alternative to the use of iron pills and iron-fortified foods to alleviate IDA in Indian communities. Further research is needed to evaluate the effectiveness of this source in a more pragmatic setting and in other vulnerable populations such as children and pregnant women. The cost effectiveness should be examined compared with that of other interventions to determine whether the bars present a viable option for combatting iron deficiency in India on a larger scale.

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The authors' responsibilities were as follows—RM and ACP: wrote several sections of the manuscript and critically revised the final version of the manuscript; RM and DC: led the development of the study design and methodology and supervised the baseline data collection; ACP and XS: performed all statistical analyses and designed the tables; MD: supervised the remainder of the study and data collection and provided ethical consultation for ongoing interventions; DC and ELT: supervised the quality control of the data collection and data analysis; ELT: designed the tables, performed detailed editing and structuring of drafts of the manuscript and of the final manuscript, oversaw the development of the statistical analysis plan, performed the quality control of the statistical analyses, had full access to all study data, and had the final responsibility for the decision to submit the manuscript for publication; and all authors: critically revised, read, and approved the final manuscript before submission. RM founded Let's be Well Red in 2011. Since 2013, and before the start of the Let's be Well Red study, she has not been actively involved with the operations of the organization and is currently pursuing her medical education at Duke University School of Medicine. Although RM initiated the study, led the design of the study, and oversaw the baseline data collection, she was not involved with any data collection after random assignment and did not implement or oversee any statistical analysis. All statistical analyses were conducted according to a prespecified analysis plan. The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript. The Mulliben Dullabhai Trust is a nonprofit organization that supports efforts to evaluate sustainable interventions in the areas of nutrition and health in India. ACP, XS, MD, DC, and ELT reported no conflicts of interest related to the study.

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