Original Investigation

Iron Supplementation in Iron-Replete and Nonanemic Pregnant Women in Tanzania
A Randomized Clinical Trial

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IMPORTANCE Anemia is common in pregnancy and increases the risk of adverse outcomes. Iron deficiency is a leading cause of anemia in sub-Saharan Africa, and iron supplementation is the standard of care during pregnancy; however, recent trials among children have raised concerns regarding the safety of iron supplementation in malaria-endemic regions. There is limited evidence on the safety of iron supplementation during pregnancy in these areas.

OBJECTIVE To evaluate the safety and efficacy of iron supplementation during pregnancy in a malaria-endemic region.

DESIGN, SETTING, AND PARTICIPANTS We conducted a randomized, double-blind, placebo-controlled clinical trial among pregnant women presenting for antenatal care in Dar es Salaam, Tanzania, from September 28, 2010, through October 4, 2012. Iron-replete, nonanemic women were eligible if they were uninfected with human immunodeficiency virus, primigravidae or secundigravidae, and at or before 27 weeks of gestation. Screening of 21 316 women continued until the target enrollment of 1500 was reached. Analyses followed the intent-to-treat principle and included all randomized participants.

INTERVENTIONS Participants were randomized to receive 60 mg of iron or placebo, returning every 4 weeks for standard prenatal care, including malaria screening, prophylaxis with the combination of sulfadoxine and pyrimethamine, and treatment, as needed.

MAIN OUTCOMES AND MEASURES The primary outcomes were placental malaria, maternal hemoglobin level at delivery, and birth weight.

RESULTS Among 1500 study participants (750 randomized for each group), 731 in iron group and 738 in placebo group had known birth outcomes and 493 in iron group and 510 in placebo group had placental samples included in the analysis. Maternal characteristics were similar at baseline in the iron and placebo groups, and 1354 (91.7%) used malaria control measures. The risk of placental malaria was not increased by maternal iron supplementation (relative risk [RR], 1.03; 95% CI, 0.65-1.65), and iron supplementation did not significantly affect birth weight (3155 vs 3137 g, $P = .89$). Compared with placebo, iron supplementation significantly improved the mean increase from baseline to delivery for hemoglobin (0.1 vs −0.7 g/dL, $P < .001$) and serum ferritin (41.3 vs 11.3 µg/L, $P < .001$). Iron supplementation significantly decreased the risk of anemia at delivery by 40% (RR, 0.60; 95% CI, 0.51-0.71) but not severe anemia (RR, 0.68; 95% CI, 0.41-1.14). Iron supplementation significantly reduced the risk of maternal iron deficiency at delivery by 52% (RR, 0.48; 95% CI, 0.32-0.70) and the risk of iron deficiency anemia by 66% (RR, 0.34; 95% CI, 0.19-0.62).

CONCLUSIONS AND RELEVANCE Prenatal iron supplementation among iron-replete, nonanemic women was not associated with an increased risk of placental malaria or other adverse events in the context of good malaria control. Participants receiving supplementation had improved hematologic and iron status at delivery compared with the placebo group. These findings provide support for continued administration of iron during pregnancy in malaria-endemic regions.

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Anemia affects 1 in 4 people globally, with a higher prevalence among women and children in sub-Saharan Africa.[1] Iron deficiency (ID) is the leading cause of anemia during pregnancy. World Health Organization guidelines recommend iron supplementation for all pregnant women in areas with high anemia prevalence (≥20%-40%).[2-4] However, in areas of high malaria burden, iron supplementation increases the supply of iron to host pathogens, increasing the risk for malaria and other perinatal infections.[5] In a randomized clinical trial in Pemba, Tanzania, children receiving iron supplementation were more likely to be hospitalized or die.[6] In response, the World Health Organization recommends caution in iron supplementation of children in areas of high malaria burden, targeting supplementation only to children with anemia or high risk of ID.[7,8]

The safety of routine prenatal iron supplementation in malaria-endemic regions has not been rigorously assessed. An estimated annual 25 million women in sub-Saharan Africa are at risk for malaria infection during pregnancy.[9] Placental malaria may be decreased in iron-deficient relative to iron-replete women.[10,11] *Plasmodium falciparum* infection is more frequent and of higher parasite density in pregnant than nonpregnant women[12] and is associated with increased risks of maternal anemia, maternal death, prematurity, stillbirth, and low birth weight (LBW).[9] We therefore conducted a randomized, double-blind, placebo-controlled clinical trial to test the hypothesis that prenatal iron supplementation may be associated with increased risk of malaria but beneficial in improving hematologic status and infant birth weight among nonanemic, iron-replete women in Tanzania.

At a Glance

- The safety of routine prenatal iron supplementation in malaria-endemic regions has not been rigorously assessed.
- We performed a randomized, double-blind, placebo-controlled clinical trial to evaluate the safety and efficacy of recommended daily 60-mg prenatal iron supplements among 1500 iron-replete, nonanemic women in a malaria-endemic region with optimal standard-of-care malaria control.
- Compared with placebo, iron had no increased risk of placental malaria (relative risk, 1.03; 95% CI, 0.65-1.65) but resulted in improved hemoglobin ($P < .001$) and iron ($P < .001$) status and reduced the risk of anemia at delivery by 40% (relative risk, 0.60; 95% CI, 0.51-0.71).
- Our findings support continued universal administration of prenatal iron supplements when good malaria control is present.

Methods

Pregnant women presenting at 3 large antenatal clinics in Dar es Salaam, Tanzania, from September 28, 2010, through October 4, 2012, were screened. Eligible participants were primigravidae or secundigravidae women uninfected with human immunodeficiency virus (HIV) at or before 27 weeks of gestation (evaluated by date of last menstrual period) who were not severely anemic (hemoglobin, >8.5 g/dL [to convert to grams per liter, multiply by 10]), were not iron deficient (serum ferritin, >12 µg/L [to convert to picomoles per liter, multiply by 2.247]), and intended to stay in Dar es Salaam until 6 weeks post partum. Women who were excluded were provided with iron supplementation or other treatment per Tanzanian standards of care. Consenting eligible women were randomized (n = 1500) and were administered a background questionnaire and a full clinical examination.

The study was approved by the institutional review boards at Harvard T. H. Chan School of Public Health, Muhimbili University, and Tanzania's National Institute for Medical Research. The Tanzania Food and Drug Authority approved the use of study regimens. Written informed consent was obtained from participating women. Study progress was monitored by a data safety monitoring board. For unmasking, the data safety monitoring board used an efficacy stopping rule of $P < .001$ for primary end points and $P < .05$ for safety end points. The trial protocol can be found in the Supplement.

Participants were individually randomized to receive a daily oral dose of 60 mg of elemental iron (as ferrous sulfate) or placebo from enrollment until delivery. The active and placebo tablets and packaging were indistinguishable. A computer-generated randomization sequence in blocks of 20 was created by a scientist not involved in data collection. Study clinics were issued prelabeled regimen bottles, and at enrollment, a participant was assigned to the next numbered bottle in the regimen. At subsequent visits, supplements were dispensed with the participant's identification number from labeled regimen bottles prepared by research pharmacists. All participants and study personnel were masked until analyses were completed. Participants were instructed to consume the supplement with a meal and screened for adverse effects at each visit.

Participants attended a clinic monthly until delivery and were provided with standard prenatal care, including 5 mg/d of folic acid and intermittent preventive treatment of malaria during pregnancy using 1500 mg of sulfadoxine and 75 mg of pyrimethamine in the second and third trimesters. Peripheral blood tests for malaria parasites were performed as needed, and incident malaria cases were managed according to guidelines from the United Republic of Tanzania Ministry of Health and Social Welfare.[13] Vouchers for insecticide-treated nets (ITNs) were issued through a governmental program. At each visit, participants were administered a health questionnaire, underwent an obstetric examination, and provided a monthly supply of study regimen. Study staff collected used regimen bottles at each visit and counted remaining pills to assess adherence. On-call study midwives attended participants at delivery; collected, examined, and sampled placentas; and obtained blood samples. At the 6-week postpartum clinic visit, study staff ascertained survival status of mothers and infants.

The HIV testing at screening used 2 rapid assays (Alere Determine HIV-1/2 Ag/Ab Combo; Alere; and Uni-Gold Recombigen HIV-1/2; Trinity Biotech Plc), with confirmation of discrepant results using an enzyme-linked immunosorbent assay

(Enzygnost Integral II; Siemens Healthcare GmbH) at the research laboratory in Tanzania. Screening for hemoglobin (HemoCue AB Hb201; HemoCue AB) and ferritin (colloidal gold rapid assay; Glory Science Co and Victory Medicine Inc) was performed. Women with a hemoglobin level of 11 g/dL or higher and screening ferritin results greater than 20 µg/L were eligible for immediate randomization, whereas those with screening ferritin results in the 10- to 20-µg/L range required confirmation using the Cobas Integra 400 plus (Roche Diagnostics) at the research laboratory. A peripheral blood sample was taken from women at enrollment and delivery and tested at the research laboratory for a complete blood cell count (Ac-T 5diff AL; Beckman Coulter), serum ferritin level, and C-reactive protein (CRP) level (Cobas Integra 400 plus).

Placental malaria was evaluated using histopathologic analysis and polymerase chain reaction (PCR). The fresh placenta was sampled[14] and the tissue divided for use in histopathologic examination (microscopic infection) and nucleic acid studies (submicroscopic infection). For placental histopathologic analysis, tissue was formalin fixed, embedded, sectioned, and stained and examined by light microscopy and under polarized light for the presence of malaria pigment and parasitized erythrocytes; infections were classified by a placental histopathologist (D.R.).[15] A subset of 100 slides was submitted for external confirmation of diagnoses. To prepare placental tissue for nucleic acid studies, tissue was stabilized in RNAlater (Qiagen) and homogenized, and genomic DNA was extracted using DNeasy (Qiagen). Taqman quantitative real-time PCR (Life Technologies) was used for amplification using published primer and probe sets (*P falciparum*–specific[16] and general *Plasmodium* [18S ribosomal RNA genes[17]]). Tissue was tested for PCR inhibitors, and positive and negative controls were included on each plate for quality assurance.

Significant effort was made to reduce loss to follow-up, including limiting enrollment to women who intended to deliver within the study area and close follow-up of all enrolled women, especially in the last weeks of pregnancy, with an on-call, round-the-clock nurse. Nonetheless, some participants later left Dar es Salaam for cultural reasons (eg, at the request of their mothers or in-laws) or delivered at a nonstudy facility. We had no expectation of collecting placentas from fetal losses that were experienced at home. Therefore, for primary outcomes, although birth outcomes were obtained for 1469 of 1500 births (97.9%) and birth weights were obtained for 1363 of 1368 live birth deliveries (99.6%) (**Figure**), we were not always able to gather samples from mothers at delivery. Of 1469 women with delivery outcomes, it was not possible to collect placentas from 342 women who had early fetal loss (n = 59), delivered at a nonstudy hospital (n = 167), delivered outside Dar es Salaam (n = 98), or withdrew from the study (n = 18). Of the remaining 1127 women, 1003 placenta samples (89.0%) were obtained.

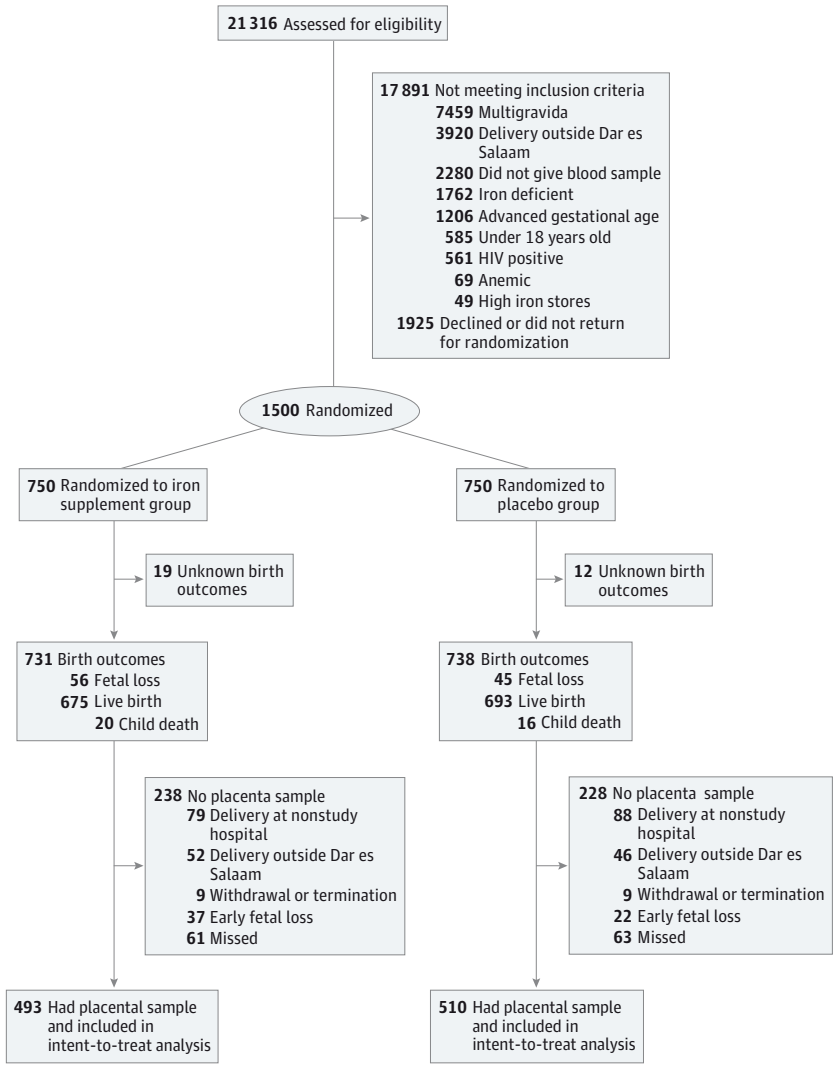
The primary outcomes were placental malaria (by histopathologic analysis or PCR), maternal hemoglobin at delivery, and infant birth weight. Secondary outcomes included prevalence of maternal anemia (hemoglobin, <11 g/dL) at delivery, LBW (<2500 g), and maternal peripheral malaria. We examined the effect of iron on the risks of secondary outcomes, including placental weight, small for gestational age (<10th percentile for gestational age based on the Alexander et al[18] and International Fetal and Newborn Growth Consortium for the 21st Century [INTERGROWTH-21st] standards[19]), preterm birth (<37 weeks of gestational age), very LBW (<2000 g), fetal loss, neonatal death, maternal hospitalization, maternal ferritin level, ID (ferritin, <12 µg/L), and maternal death. Finally, ID in the presence (ferritin, <70 µg/L; CRP, >8.2 µg/mL) or absence (ferritin, <30 µg/L; CRP, ≤8.2 µg/mL) of inflammation at delivery was assessed using published cutoffs.[20] Although the study was powered based on primary outcomes, these secondary outcomes maximize our understanding of safety and efficacy.

The sample size of 1500 was determined to provide at least 80% power at a 5% significance level to detect a 35% or higher effect of the intervention on placental malaria at a background rate of 20%, assuming 10% loss to follow-up. Analyses followed the intent-to-treat principle and included all randomized participants. Differences in baseline measures and outcomes between the 2 treatment groups were assessed with χ^2 tests for categorical variables and Wilcoxon rank sum tests for continuous variables. Differences between the treatment groups in binary outcomes measured repeatedly on participants were assessed using log-binomial models with an exchangeable correlation structure.[21,22] Results are unadjusted given the balance achieved across regimens by randomization. Twin pregnancies (n = 28 pairs) were analyzed as a single outcome in which the final birth weight used was the mean of the 2 twin birth weights. For binary outcomes, at least 1 of the twins had the outcome. To evaluate potential selection bias due to loss of follow-up, the main analysis examining placental malaria risk between the iron and placebo groups was repeated with marginal structural models using inverse probability of treatment weighting.[23] Weights were calculated as the inverse of predicted values from a logistic regression of the following variables on retention until the end point: treatment group, baseline maternal characteristics (age, gravidity, gestational age, educational level, marital status, employment, body mass index, mid-upper arm circumference, frequency of meat consumption, and use of antimalarial agents), household characteristics (house ownership, roof and floor materials, and per capita food expenditure), adherence to the regimen, and pairwise interactions among these. The effect of iron supplementation was then estimated using weighted log-binomial models with empirical SEs.[21] Data analysis was performed from November 1, 2013, through November 15, 2014. All *P* values were 2-sided. SAS statistical software, version 9.3 (SAS Institute Inc), was used for all analyses.

Results

No significant baseline differences were found between women in the iron and placebo groups (**Table 1**). The mean gestational age at randomization was 18.2 weeks (*P* = .96). The mean (median [SD]) time between randomization and delivery was 148 (148 [36]) days (*P* = .33), and the mean (median) time between randomization and the 6-week postpartum visit was 305

Figure. Study Enrollment, Randomization, and Pregnancy Outcomes



HIV indicates human immunodeficiency virus.

(237 [177]) days (P = .82). The mean (median [SD]) adherence (number of tablets absent from returned regimen bottles divided by the number of days the participant had the bottle) was not different between the groups (iron: 76% [84% (25%)]; placebo: 78% [85% (23%)]; P = .67).

The overall prevalence of placental malaria was 6.6%; the risk of placental malaria was not different between the groups (relative risk [RR], 1.03; 95% CI, 0.65-1.65), and there was no increased risk for microscopic (RR, 1.14; 95% CI, 0.49-2.65) or submicroscopic (RR, 1.12; 95% CI, 0.65-1.92) placental malaria (**Table 2**). The use of marginal structural models to adjust for potential selection bias did not materially change the findings (RR, 1.09; 95% CI, 0.68-1.75; P = .71). All infections detected by PCR and microscopy were *P falciparum*. Maternal peripheral malaria was low, with only 12 women ever being positive during pregnancy.

Risk of maternal death was not significantly affected by iron supplementation (RR, 0.67; 95% CI, 0.11-3.98) (Table 2). Although there were fewer maternal hospitalizations in the iron group compared with the placebo group (RR, 0.57; 95% CI, 0.32-1.02), no single type of hospitalization was significantly associated with iron supplementation. Prevalence of nausea, vomiting, or diarrhea reported at monthly visits did not differ across the 2 arms of the study (P > .99). In addition, no difference was found in ever reporting temporary discontinuation of the regimen because of perceived adverse effects (P = .58, Table 2).

Mean hemoglobin (P = .98) and ferritin (P > .99) levels at randomization did not differ between the groups (**Table 3**). The mean (SD) hemoglobin level at delivery was significantly higher in the iron group (11.8 [2.0] g/dL) than in the placebo group (10.9 [1.9] g/dL, P < .001). Iron supplementation significantly decreased the risk of anemia at delivery by 40% (RR, 0.60; 95% CI, 0.51-0.71) but not severe anemia (RR, 0.68; 95% CI, 0.41-1.14). The serum ferritin level at delivery was significantly higher in the iron group (P < .001). Iron supplementation significantly reduced the risk of maternal iron deficiency at delivery by 52% (RR, 0.48; 95% CI, 0.32-0.70) and the risk of iron

deficiency anemia by 66% (RR, 0.34; 95% CI, 0.19-0.62). After adjusting for inflammation, iron supplementation significantly reduced the risk of maternal ID at delivery by 37% and the risk of iron deficiency anemia (IDA) by 59% (Table 3).

Among live births, iron supplementation did not affect birth weight (P = .89) or risk of LBW (RR, 0.91; 95% CI, 0.62-1.34). Iron supplementation did not significantly affect the risks of fetal loss, early child mortality, or preterm births (**Table 4**).

Discussion

In this randomized, double-blind, placebo-controlled clinical trial, no evidence of harm was identified from iron supplementation of pregnant women who were iron replete and nonanemic at initiation of antenatal care in a malaria-endemic area. Iron supplementation was not significantly associated with placental malaria or birth weight, whereas iron significantly reduced risks of anemia and ID compared with placebo.

The safety of iron supplementation in malaria-endemic settings has been questioned because iron may increase malaria-associated morbidity and mortality. A large randomized trial in Pemba, Tanzania, identified an excess of all-cause and malaria-related serious adverse events in children who received iron, resulting in a premature halt of the study.[6] Both human host and malaria parasite require iron as an essential nutrient. In the presence of infection, the host reallocates iron stores away from invading pathogens by reducing the availability of serum iron. Malaria disrupts host iron distribution and use through mechanisms that involve the erythrocyte (hemolysis and erythropoiesis), iron metabolism (ferritin recycling by the macrophage and dietary iron absorption), and anemia.[24,25]

Previous studies[6,10,11,26] have raised concern regarding iron supplements for individuals who are iron replete with expected benefits among those with ID. In a trial from Ghana, iron fortification among children reduced the incidence of malaria in the iron group compared with the no-iron group; in subgroup analyses, iron was protective for children with IDA at baseline but not for nonanemic, iron-replete individuals.[26] Similarly, iron supplementation in the Pemba trial was apparently beneficial among children with IDA at baseline but had no effect in the nonanemic, iron-replete group.[6] The plausibility of risk has been extended to pregnancy; however, no trial has evaluated this risk among baseline iron-replete, nonanemic women. In observational studies,[10,11] ID was associated with increased risk of placental malaria among iron-replete women.

In our trial, we noted no evidence of increased risk of malaria, maternal hospitalizations, or other serious adverse events associated with iron supplementation. A systematic review[27] revealed that risk associated with iron supplementation in children was noted in trials in which malaria interventions were not available, such as the Pemba trial. Although malaria remains endemic in Tanzania, infection prevalence in Dar es Salaam for children younger than 5 years was 3.6% as of 2012.[28] Malaria prevalence and mortality have steadily decreased since reaching a peak in 2003, with the decrease in Tanzania among the greatest in Africa at 7% to 8%.[29] Such reductions are likely the result of improved preventive measures.[30] Use of ITNs among pregnant women in Tanzania has markedly increased in the last decade from 16% in 2004 to 75% in 2012.[28] The uptake of intermittent preventive treatment during pregnancy has somewhat improved but remains suboptimal in Dar es Salaam (2 doses, 48.3%).[28] Several randomized clinical trials suggest that the downstream effects of preventing malaria in preg-

Table 1. Participant Characteristics at Baseline[a]

Characteristic	Iron Group (n = 750)	Placebo Group (n = 750)
Age, mean (SD), y	23.7 (4.1)	24.1 (4.2)
Primigravida	451 (60.1)	415 (55.3)
Gestational age at randomization, mean (SD), wk	18.2 (4.3)	18.2 (4.4)
Educational level, y		
0-4	18 (2.5)	14 (1.9)
5-7	404 (55.0)	389 (53.6)
8-11	211 (28.8)	202 (27.8)
≥12	101 (13.8)	121 (16.7)
Marital status		
Married or cohabitating	595 (79.7)	600 (80.5)
Other	152 (20.4)	145 (19.5)
Employment status		
Skilled	140 (18.7)	149 (19.9)
Unskilled or informal	226 (30.1)	226 (30.1)
Housewife or unemployed	362 (48.3)	348 (46.4)
Other	22 (2.9)	27 (3.6)
Housing type		
Own	139 (18.6)	143 (19.1)
Rent	534 (71.5)	534 (71.3)
Other	74 (9.9)	72 (9.6)
Roof		
Metal	730 (97.7)	734 (98.0)
Other	17 (2.3)	15 (2.0)
Floor		
Concrete	701 (93.5)	704 (93.9)
Tile	43 (5.7)	41 (5.5)
Dirt or wood	6 (0.8)	5 (0.7)
Malaria interventions		
Uses spray, coils, or ITN nightly	668 (90.6)	686 (92.7)
Has ITN	663 (88.5)	666 (88.9)
Uses ITN	628 (86.2)	638 (87.2)
BMI at enrollment, mean (SD)	24.5 (4.4)	24.6 (4.7)
BMI category at enrollment		
<18.5	32 (4.3)	36 (4.8)
18.5-24.9	434 (58.3)	415 (55.6)
≥25	278 (37.4)	295 (39.5)
Baseline MUAC, mean (SD), mm	26.5 (3.6)	26.5 (3.6)

Abbreviations: BMI, body mass index (calculated as the weight in kilograms divided by height in meters squared); ITN, insecticide-treated net; MUAC, mid-upper arm circumference.

[a] Data are presented as number (percentage) of study participants unless otherwise indicated. No significant differences were found between the iron and placebo groups (P > .05 in all cases).

Table 2. Effect of Iron Supplementation vs Placebo on Maternal Outcomes[a]

Outcome	Iron Group		Placebo Group		Relative Risk (95% CI)	*P* Value
	Finding	No. of Participants or Events	Finding	No. of Participants or Events		
Placental malaria[b]						
Microscopic	11 (2.3)	471	10 (2.1)	487	1.14 (0.49-2.65)	.77
Submicroscopic	26 (5.3)	486	24 (4.8)	501	1.12 (0.65-1.92)	.69
Any placental malaria	33 (6.7)	493	33 (6.5)	510	1.03 (0.65-1.65)	.89
Placental weight, mean (SD), g	458 (140)	443	456 (144)	467	NA	.97
Maternal death	2 (0.3)	750	3 (0.4)	750	0.67 (0.11-3.98)	>.99
Maternal hospitalizations	20 (0.4)	4380	36 (0.8)	4474	0.57 (0.32-1.02)	.06
Malaria	2 (0)	4380	7 (0.2)	4474	0.29 (0.06-1.40)	.12
Abdominal pain	3 (0.1)	4380	4 (0.1)	4474	0.80 (0.17-3.40)	.73
Vomiting	1 (0)	4380	1 (0)	4474	1.02 (0.06-16.30)	.99
Other	14 (0.3)	4380	24 (0.5)	4474	0.60 (0.31-1.15)	.12
Potential adverse effects ever reported[c]	227 (30.3)	750	226 (30.1)	750	1.00 (0.86-1.17)	>.99
Ever temporarily discontinued regimen because of perceived adverse effects	25 (3.3)	750	30 (4.0)	750	0.83 (0.49-1.40)	.58

Abbreviation: NA, not applicable.

[a] Data are presented as number (percentage) of participants unless otherwise indicated.

[b] Microscopic infections were detected by placental histopathologic analysis. Submicroscopic infections were detected by quantitative polymerase chain reaction on maternal placental tissue. Five participants (4 in the iron group, 1 in the placebo group) had both microscopic and submicroscopic infections.

[c] Nausea, vomiting, or diarrhea reported at monthly visit.

Table 3. Effect of Iron Supplementation vs Placebo on Maternal Hematologic and Iron Status[a]

Outcome	Iron Group		Placebo Group		Relative Risk (95% CI)	*P* Value
	Finding	No. of Participants or Events	Finding	No. of Participants or Events		
Hemoglobin, mean (SD), g/dL						
Randomization	11.7 (1.4)	738	11.7 (1.3)	741	NA	.98
Delivery	11.8 (2.0)	481	10.9 (1.9)	501	NA	<.001
Mean change in hemoglobin	0.1 (2.2)	473	−0.7 (2.0)	495	NA	<.001
Anemia at delivery[b]	144 (29.9)	481	248 (49.5)	501	0.60 (0.51-0.71)	<.001
Severe maternal anemia at delivery[c]	23 (4.8)	481	35 (7.0)	501	0.68 (0.41-1.14)	.14
Ferritin, µg/L						
Randomization	40.9 (33.3)	741	40.7 (31.4)	742	NA	>.99
Delivery	92.5 (171.1)	483	54.1 (90.3)	509	NA	<.001
Mean change in ferritin	41.3 (295.6)	476	11.3 (92.1)	503	NA	<.001
Iron deficiency						
At delivery[d]	33 (6.8)	483	73 (14.3)	509	0.48 (0.32-0.70)	.001
At delivery (CRP adjusted)[e]	227 (47.3)	480	379 (75.3)	503	0.63 (0.56-0.70)	<.001
Anemia at delivery[b,d]	14 (3.0)	464	43 (8.8)	491	0.34 (0.19-0.62)	<.001
Anemia at delivery (CRP adjusted)[c,e]	74 (16.1)	461	191 (39.4)	485	0.41 (0.32-0.52)	<.001

Abbreviations: CRP, C-reactive protein; NA, not applicable.

SI conversion factors: To convert hemoglobin to grams per liter, multiply by 10; ferritin to picomoles per liter, multiply by 2.247.

[a] Data are presented as number (percentage) of participants unless otherwise indicated.

[b] Hemoglobin level less than 11 g/dL.

[c] Hemoglobin level less than 8.5 g/dL.

[d] Serum ferritin level less than 12 µg/L.

[e] Serum ferritin level less than 70 µg/L and CRP level greater than 8.2 µg/mL or serum ferritin level less than 30 µg/L and CRP level of less than or equal to 8.2 µg/mL.

nancy include significant reductions in LBW and neonatal mortality and provide support for programmatic adoption of intermittent preventive treatment during pregnancy and ITNs for reducing malaria in pregnancy.[31] Nevertheless, in the absence of malaria interventions, the risk of placental infection during pregnancy in Dar es Salaam is 40% to 50% in primigravidae and secundigravidae women.[32] In our study, use of malaria interventions at home had wide coverage (>90%), and all women enrolled in our study were given intermittent preventive treatment with sulfadoxine and pyrimethamine. This high coverage of malaria prevention, coupled with the nonanemic, iron-replete baseline status of our participants, is

Table 4. Effect of Iron Supplementation vs Placebo on Perinatal Outcomes[a]

Outcome	Iron Group		Placebo Group		Relative Risk (95% CI)	*P* Value
	Finding	No. of Participants or Events	Finding	No. of Participants or Events		
Fetal loss[b]	56 (7.7)	731	45 (6.1)	738	1.26 (0.86-1.92)	.24
Infant mortality at 6 wk[c]	20 (3.0)	675	16 (2.3)	692	1.28 (0.67-2.45)	.45
Birth weight, mean (SD), g	3155 (545)	672	3137 (519)	691		.89
Birth weight group, g						
<2500	45 (6.7)	672	51 (7.4)	691	0.91 (0.62-1.34)	.62
<2000	15 (2.2)	672	14 (2.0)	691	1.10 (0.54-2.26)	.79
Gestational age at delivery, mean (SD), wk	39.3 (2.5)	665	39.2 (2.7)	685		.37
Preterm birth, wk[b]						
<37	100 (15.0)	665	113 (16.5)	685	0.91 (0.71-1.17)	.46
<34	21 (3.2)	665	27 (3.9)	685	0.80 (0.46-1.40)	.44
Low birth weight and preterm birth[d,e]	27 (4.1)	665	21 (3.1)	685	1.32 (0.76-2.32)	.32
Low birth weight and term birth[d,e]	18 (2.7)	665	30 (4.4)	685	0.62 (0.35-1.10)	.10
Small for gestational age (Alexander et al standard)[d,f]	121 (18.0)	672	125 (18.1)	691	1.00 (0.79-1.25)	.97
Small for gestational age (INTERGROWTH-21st standard)[g]	101 (15.8)	641	105 (16.2)	648	0.97 (0.76-1.25)	.83

Abbreviation: INTERGROWTH-21st, International Fetal and Newborn Growth Consortium for the 21st Century.

[a] Data are presented as number (percentage) of participants unless otherwise indicated.

[b] Stillbirth and miscarriage.

[c] Live birth only.

[d] Restricted to births at 21 to 44 weeks of gestation.

[e] Low birth weight was defined as less than 2500 g.

[f] Small for gestational age was defined as birth weight below the 10th percentile for gestational age using the standard of Alexander et al.[18]

[g] Small for gestational age was defined as birth weight below the 10th percentile for gestational age using the INTERGROWTH-21st standard,[19] restricted to births at 30 to 42 weeks of gestation.

likely to be an important factor to explain the difference in findings between our trial and the Pemba trial.

The low overall risk of malaria in the present trial (resulting from successful malaria control) reduced the power to address a primary aim of the trial: the effect of iron on placental malaria risk. The question of what degree of malaria control is required to offset the risks of supplementation identified in studies with poor or no malaria control remains; however, this question may be difficult to directly answer because it would be hard to justify conducting placebo-controlled studies of iron supplementation without proper measures of malaria control. A 2011 Cochrane review of iron supplementation trials in children supports our findings: the risk of malaria was higher with iron (RR, 1.13; 95% CI, 1.01-1.26) when no malaria surveillance and control were present, but no effect of iron supplementation was noted otherwise.[27] The loss to follow-up that resulted in reduced sample sizes could also affect our results by reducing the power to detect differences between the iron and placebo arms or by creating biases in our estimates of these differences. However, our analyses that used marginal structural models to adjust for potential selection bias because of loss to follow-up did not materially change the findings for placental malaria.

Another important consideration in prenatal supplementation is the potential benefits on hematologic status. We found that women receiving iron supplementation had improved hematologic and iron status from baseline to delivery. Our RR estimates for maternal anemia, ID, and IDA at delivery are consistent with published results, including those presented in a recent comprehensive meta-analysis.[33] Measures associated with ID, including hemoglobin and serum ferritin concentrations, decrease during pregnancy, even in women with adequate iron stores. The physiologic requirements for iron in pregnancy are substantial and unlikely to be met even under an optimal diet,[34] indicating that even women who are iron replete early in pregnancy require supplementation to maintain nonanemic, iron-replete status.

Among the secondary outcomes evaluated in this study, iron supplementation was not associated with LBW, preterm birth, small for gestational age deliveries, or other adverse perinatal outcomes. Two meta-analyses[35,36] identified a decreased risk of LBW among women receiving iron supplementation but did not identify risks for our other secondary birth outcomes, confirming the results of an updated Cochrane review. Although these meta-analyses provide support for iron supplementation in the overall reduction of LBW, the benefit in nonanemic, iron-replete women is less known. Among studies[37-45] of women who are nonanemic at initiation of supplementation, only one study[40] found a significantly protective effect for iron on LBW, underscoring the multifactorial origin of poor fetal growth.

Conclusions

Our trial has indicated the lack of harm from prenatal iron supplementation in iron-replete, nonanemic women when good malaria control is present. The observed benefits associated with the improved iron and hematologic status pro-

vide support for continuing universal prenatal supplementation. Because anemia during pregnancy is associated with adverse outcomes for both mother and child, we recommend continuation of iron supplementation for all pregnant women as standard of care in this and similar environments with adequate concomitant malaria control.

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