Original Investigation

Effect of Daily Antenatal Iron Supplementation on *Plasmodium* Infection in Kenyan Women
A Randomized Clinical Trial

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IMPORTANCE Anemia affects most pregnant African women and is predominantly due to iron deficiency, but antenatal iron supplementation has uncertain health benefits and can increase the malaria burden.

OBJECTIVE To measure the effect of antenatal iron supplementation on maternal *Plasmodium* infection risk, maternal iron status, and neonatal outcomes.

DESIGN, SETTING, AND PARTICIPANTS Randomized placebo-controlled trial conducted October 2011 through April 2013 in a malaria endemic area among 470 rural Kenyan women aged 15 to 45 years with singleton pregnancies, gestational age of 13 to 23 weeks, and hemoglobin concentration of 9 g/dL or greater. All women received 5.7 mg iron/day through flour fortification during intervention, and usual intermittent preventive treatment against malaria was given.

INTERVENTIONS Supervised daily supplementation with 60 mg of elemental iron (as ferrous fumarate, n = 237 women) or placebo (n = 233) from randomization until 1 month postpartum.

MAIN OUTCOMES AND MEASURES Primary outcome was maternal *Plasmodium* infection at birth. Predefined secondary outcomes were birth weight and gestational age at delivery, intrauterine growth, and maternal and infant iron status at 1 month after birth.

RESULTS Among the 470 participating women, 40 women (22 iron, 18 placebo) were lost to follow-up or excluded at birth; 12 mothers were lost to follow-up postpartum (5 iron, 7 placebo). At baseline, 190 of 318 women (59.7%) were iron-deficient. In intention-to-treat analysis, comparison of women who received iron vs placebo, respectively, yielded the following results at birth: *Plasmodium* infection risk: 50.9% vs 52.1% (crude difference, −1.2%, 95% CI, −11.8% to 9.5%; $P = .83$); birth weight: 3202 g vs 3053 g (crude difference, 150 g, 95% CI, 56 to 244; $P = .002$); birth-weight-for-gestational-age z score: 0.52 vs 0.31 (crude difference, 0.21, 95% CI, −0.11 to 0.52; $P = .20$); and at 1 month after birth: maternal hemoglobin concentration: 12.89 g/dL vs 11.99 g/dL (crude difference, 0.90 g/dL, 95% CI, 0.61 to 1.19; $P < .001$); geometric mean maternal plasma ferritin concentration: 32.1 µg/L vs 14.4 µg/L (crude difference, 123.4%, 95% CI, 85.5% to 169.1%; $P < .001$); geometric mean neonatal plasma ferritin concentration: 163.0 µg/L vs 138.7 µg/L (crude difference, 17.5%, 95% CI, 2.4% to 34.8%; $P = .02$). Serious adverse events were reported for 9 and 12 women who received iron and placebo, respectively. There was no evidence that intervention effects on *Plasmodium* infection risk were modified by intermittent preventive treatment use.

CONCLUSIONS AND RELEVANCE Among rural Kenyan women with singleton pregnancies, administration of daily iron supplementation, compared with administration of placebo, resulted in no significant differences in overall maternal *Plasmodium* infection risk. Iron supplementation led to increased birth weight.

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Anemia in pregnancy is a moderate or severe health problem in more than 80% of countries worldwide, but particularly in Africa, where it affects 57% of pregnant women.[1] Iron deficiency is the most common cause and may be even more widespread, but global or regional estimates are lacking.

Although universal antenatal iron supplementation has been recommended since 1959,[2] delivery and adherence have been notably poor in low- and middle-income countries.[3,4] It is well established that antenatal iron supplementation leads to increased maternal hemoglobin concentrations and a reduced risk of anemia at term,[5] but the functional significance of this hematologic response is uncertain.[6] Iron deficiency in pregnancy has been associated with severe anemia and maternal death, but causal evidence from randomized trials is inconclusive.[5] A meta-analysis of randomized trials of iron supplementation during pregnancy found no evidence of benefits for maternal and neonatal health outcomes.[5]

Added to these uncertainties, a trial among children[7] reinforced earlier concerns that iron supplementation can increase rates of infectious diseases, including malaria.[8] Antenatal supplementation nonetheless continues to be recommended,[9] despite reports that iron deficiency is associated with protection against *Plasmodium* infection in placental blood.[10] In a previous randomized trial, antenatal iron supplementation did not increase susceptibility to *Plasmodium* infection,[11] but its design had limitations in allocation concealment, blinding, and data completeness.[5] In highly endemic areas, *P falciparum* infections in pregnancy are usually asymptomatic, but they increase the risk of adverse birth outcomes (reduced birth weight, intrauterine growth retardation, preterm delivery, increased neonatal mortality) and adverse maternal outcomes (severe anemia and death).[12]

We aimed to measure the effect of daily iron supplementation during pregnancy on maternal *Plasmodium* infection risk. We conducted preplanned analysis of effect modification by baseline iron status, gravidity, age, and HIV infection. We also assessed effects of iron supplementation on gestational age at delivery, newborn size, and maternal and neonatal iron status at 1 month postpartum.

Methods

The study was a double-blind, randomized trial comparing daily supplementation with iron vs placebo, with 2 parallel groups of pregnant women receiving daily supplementation with and without iron. (Additional details are provided in the trial protocol in Supplement 1, the statistical analysis plan in Supplement 2, and the eMethods in Supplement 3.) The study received ethical clearance in Kenya (Kenyatta National Hospital/University of Nairobi) and England (London School of Hygiene and Tropical Medicine). All women in the trial provided written informed consent.

The study was conducted (October 2011-April 2013) in Nyanza Province, Kenya, where malaria is highly endemic and transmission is perennial. As per national and international guidelines,[13-15] pregnant women should receive daily iron supplementation and intermittent preventive treatment (IPT) for malaria with sulfadoxine-pyrimethamine. In 2008 to 2009, 31% of women in Nyanza Province reported not having taken any iron supplements during their last pregnancy, while 54% took them for less than 60 days.[16]

Sample

Local women aged 15 to 45 years who were married or cohabitating were included in a community surveillance program and invited for pregnancy screening when having missed their menstrual period for 10 weeks. Those who were not in stable relationships were invited for pregnancy testing every 12 weeks. At screening, we collected stool, and we administered a urine pregnancy test, a medical examination including obstetric ultrasonography, and preventive antihelminth chemotherapy with praziquantel and albendazole (**Figure 1**).

At 14 to 21 days after the initial visit but before randomization, we collected venous blood and measured hemoglobin concentration (HemoCue301) and zinc protoporphyrin (ZPP; Aviv206d). Erythrocytes and plasma were stored in liquid nitrogen and dry ice until analysis.

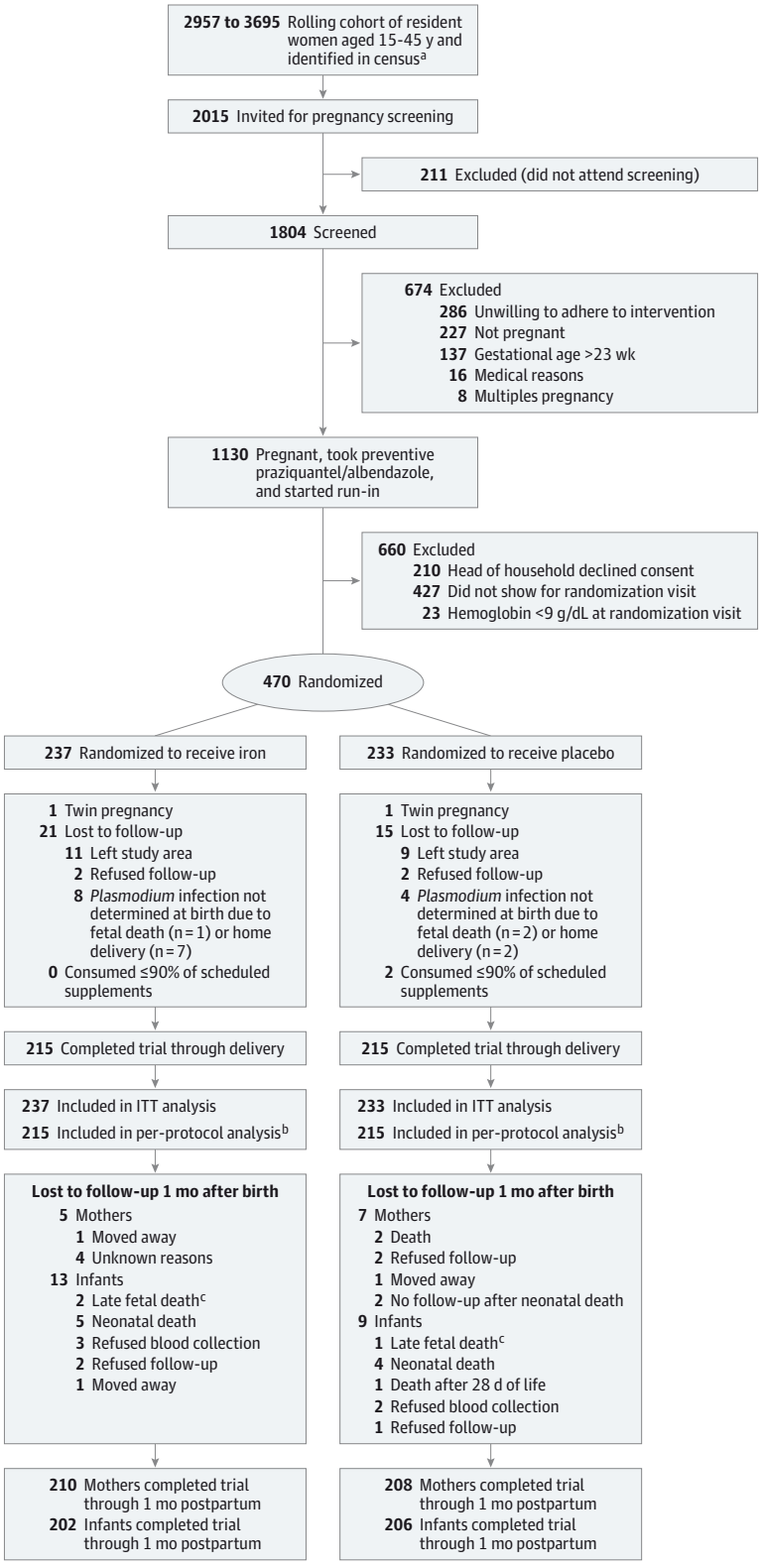
Recruitment continued until the target number of women was reached. We calculated that we would need 225 pregnant women per intervention group (450 women total) to give 92% probability of excluding no effect, assuming a *Plasmodium* infection risk of 50% in the placebo group, a relative risk increase due to iron of 35%, 5% drop-out of the iron group, and no "drop-in" of women crossing over from the placebo group to the iron group. To enroll this number of women, we anticipated an 18-month recruitment period.

Women were included when they were aged 15 to 45 years, written informed consent had been obtained, they were likely to be available for study until 1 month postpartum, and they were planning to deliver in the predesignated health facility. Women were excluded if they had obvious intellectual disabilities or a metabolic disorder (eg, diabetes); they had a medical history of sickle cell anemia or epilepsy or an obstetric history suggestive of eclampsia or preeclampsia; they were carrying multiples; gestational age at the second visit was less than 13 weeks or more than 23 weeks; no venous blood was collected; or hemoglobin concentration was less than 9 g/dL (which has previously been associated with adverse events such as prematurity, low birth weight, and fetal death).[17,18]

Randomization and Blinding

Supplements were given as capsules that were identical in appearance except for shell color. The code linking each color to supplement type was kept in sealed envelopes. One of us (H.V.) not involved in the field work used tables with random numbers to produce sequentially numbered, sealed, opaque envelopes containing the code, using simple randomization with a 1:1 allocation ratio. Eligible women were allocated in order of enrollment to the color indicated in the next available envelope. Participants and field staff were blinded to intervention until data analysis.

Figure 1. Participant Flow in Trial of Iron Supplementation During Pregnancy



[a] During the study, women entered the cohort as they immigrated into the study area or attained the minimum eligible age (15 years) and left the cohort as they emigrated or attained the maximum eligible age (45 years).

[b] Sample sizes <215 are due to missing data, which varied by outcome. In the intention-to-treat (ITT) analysis, missing values were replaced by multiple imputation.

[c] Maternal samples were collected at delivery so that primary outcome could be established.

Interventions

Supplements contained iron (60 mg of elemental iron as ferrous fumarate, which has similar iron bioavailability as ferrous sulfate) or placebo; they contained no other micronutrients. Research assistants administered supplements and daily observed that women swallowed their supplements.

From screening until end of intervention, local mill operators added fortificant iron (target dose: 20 mg/kg flour) to grain routinely brought for milling by homestead members of participating women. Based on weighed intake studies, we estimate that fortification supplied on average 5.7 mg of elemental iron as NaFeEDTA (ferric sodium ethylenediaminetetraacetate) daily in pregnant women.

Follow-up

Women were referred to regular health services for routine antenatal and medical care during intervention, including IPT for malaria and (as required) antiretroviral therapy, but they were instructed not to take micronutrient supplements other than those supplied by our field staff. They were asked to ensure that all diagnoses, treatments, and drugs administered were recorded in antenatal health booklets and to alert the field team as soon as they went into labor. An obstetric nurse assisted in delivery; complicated cases were brought to a tertiary facility. Birth weight was measured (within 10 g) immediately after delivery or, for those born at home, on presentation in the research clinic.

We collected maternal venous blood, maternal placental blood,[19] cord blood, and placental biopsies within 1 hour postpartum. For home deliveries, samples were collected within 2 hours postpartum. All women received therapy with artemether-lumefantrine, praziquantel, and albendazole immediately postpartum.

Supplementation with iron or placebo continued for 1 month postpartum. We then collected maternal venous blood and neonatal capillary blood. We also extracted information from antenatal health booklets about IPT use, possession of mosquito nets, and micronutrient supplements supplied during antenatal visits.

Laboratory Measurements

We used dipstick tests (Access Bio) to detect histidine-rich protein-2 (HRP2) and lactate dehydrogenase (pLDH) specific to either *P falciparum* or to nonfalciparum human *Plasmodium* species. Whereas HRP2-based tests detect current or recent *P falciparum* infection, pLDH-based tests only indicate current infection.[20] Placental biopsies were examined histologically[21] to detect *Plasmodium* infection. We used real-time polymerase chain reaction (PCR) to detect *P falciparum*–specific DNA in erythrocytes and DNA in stool of intestinal helminths associated with blood loss (*Schistosoma* spp, *Trichuris trichiura*, hookworm).

We assessed plasma iron markers (concentrations of ferritin, soluble transferrin receptor, transferrin) and plasma inflammation markers (concentrations of C-reactive protein [CRP] and α_1-acid glycoprotein) on a Beckman Coulter UniCel DxC 880i analyzer and HIV infection by point-of-care antibody tests.

Outcomes

The primary outcome was defined as past or present maternal *Plasmodium* infection assessed at parturition, regardless of species, as indicated by 1 or more positive results for the presence of pLDH or HRP2 in plasma or by placental histopathology or *P falciparum* DNA in maternal erythrocytes from venous or placental blood by PCR test. Secondary (exploratory) outcomes were patent *Plasmodium* infection (similarly defined as primary outcome but restricted to ≥1 positive result from dipstick tests or placental histopathology); presence of *P falciparum* DNA (as primary outcome, but restricted to ≥1 positive result for PCR tests); current or recent *Plasmodium* infection (as primary outcome, but restricted to ≥1 positive result for dipstick tests or PCR tests); birth weight; gestational age at delivery; intrauterine growth as indicated by birthweight-for-gestational-age *z* score; and maternal and neonatal iron status at 1 month postpartum.

Statistical Analysis

We used SPSS version 21 (IBM) and a predefined plan (see the statistical analysis plan in Supplement 2). We estimated effects when possible; *P* values, where reported, are 2-sided. Birth-weight-for-gestational-age *z* scores were derived with Kenyan children as a reference[22] and a coefficient of variation of 12.8% (estimated from the present study).

Anemia was defined as a hemoglobin concentration less than 11 g/dL. A participant had an iron deficiency if ferritin concentration was less than 15 µg/L and was "iron-replete" if ferritin concentration was 15 µg/L or greater without inflammation. Iron status was considered uncertain if ferritin concentration was 15 µg/L or greater with inflammation. Inflammation was defined as concentrations of CRP of greater than 10 mg/L or α_1-acid glycoprotein greater than 1 g/L.

The preplanned primary analysis was per protocol. It included all individuals with singleton pregnancies who complied with intervention (ie, consumed >90% of scheduled supplements) and for whom outcomes were available. We also conducted intention-to-treat analysis with multiple imputations to replace missing values. Results reported are by intention-to-treat analysis unless indicated otherwise. In the analysis of birth weight, infants born at referral facilities and those born at home whose weight was measured more than 24 hours postpartum were included in intention-to-treat analysis but excluded from per-protocol analysis.

For binary outcomes, effects are reported as the absolute difference in proportions except for the risk of low birth weight, which we also report as a relative effect to facilitate extrapolation of results to different settings. Continuous outcomes were log-transformed as needed to normalize distributions; effects and corresponding confidence interval limits were exponentiated and expressed as proportional differences in geometric means. We used multiple linear regression and multiple logistic regression to compare effect estimates with and without adjustment for baseline factors (gravidity, maternal age, HIV infection, *Plasmodium* infection status, hemoglobin concentration, iron deficiency, and gestational age). For complete accounting, we report the adjusted effect for the primary outcome, with the odds ratio re-expressed as a risk difference; for secondary outcomes (birth weight, gestational age at delivery, and maternal and infant hemoglobin concentration at 30 days after birth), adjusted results are not reported because adjustment did not markedly affect the magnitude of the intervention effects.

We anticipated that iron absorption and thus effects of administered iron would depend on baseline iron status and that baseline proxy markers of immunity against malaria (gravidity, age, HIV infection) might determine the ability to suppress a possible increase in parasitemia resulting from administered iron. We used stratified and direct multivariate analyses to assess effect modification by these baseline factors, with *Plasmodium* infection, birth weight, and maternal hemoglobin concentration at 1 month after birth as outcomes. We also assessed to what extent the use of IPT influenced the magnitude of intervention effects.

Results

Of 2015 women invited for screening, 470 (23%) were randomized (237 to the iron group and 233 to the placebo group) and were included in the intention-to-treat analysis (Figure 1). Per-protocol analysis included 430 or fewer cases, depending on the volume of missing data for each outcome. For the primary outcome, 363 women (77%) were available for analysis with missing data from 55 in the iron group and 52 in the placebo group. Adherence in the iron and placebo groups was 100% and 99.1%, respectively. The groups were similar regarding the percentage who received iron supplements supplied by external sources (9.3% vs 9.9%) and who possessed insecticide-treated bed nets (15.2% vs 15.9%). Only on 1 occasion (at baseline) did we find a participant to be infected by a *Plasmodium* species other than *P falciparum*.

We found no evidence that iron supplementation caused serious adverse events (Figure 1 and eTable 2 in Supplement 3). Serious adverse events were reported for 9 and 12 women who received iron and placebo, respectively. There were 2 maternal deaths, which both occurred in the placebo group; one woman was reported to have died due to postpartum hemorrhage and another at 2 weeks postpartum due to pneumonia and cardiac arrest. There were 7 fetal or neonatal deaths in each group; in addition, 1 child died in each group after 28 days of life.

Four hundred fifteen women (88.3%) delivered newborns at the research clinic, 38 (8.1%) in referral hospitals, and 17 (3.6%) at home. Placenta was missing or poorly preserved for 42 women (8.9%) due to delivery at home or in referral hospitals; in addition, placental biopsies were unavailable for 85 women first enrolled (18.1%) due to incorrect preservation of samples. Two mothers refused consent for neonatal blood collection. Plasma sample volumes from 2 infants in the control group were insufficient for all biochemical tests.

Baseline

Intervention groups were similar except for mild imbalances in marital status (married or living together: 86.1% vs 79.0% in groups receiving iron or placebo) and gravidity (secundigravida: 24.1% vs 15.0% in groups receiving iron or placebo) (**Table 1**). Of women without inflammation, 59.7% (190/318) were iron-deficient. Infection by *Schistosoma* spp and *T trichiura* was relatively common, whereas *Necator americanus* was rarely found. *Ancylostoma* DNA was absent in all stool samples.

Table 1. Baseline Characteristics of Study Participants by Intervention Group

Characteristics	Iron	Placebo
No. of participants	237	233
Height, mean (SD), cm	162.4 (5.7)	162.4 (6.7)
Weight, mean (SD), kg	58.2 (7.6)	57.5 (7.5)
Body mass index, mean (SD)[a]	22.1 (2.7)	21.8 (2.6)
Marital status, No. (%)		
Married or living together	204 (86.1)	184 (79.0)
Divorced or separated	8 (3.4)	10 (4.3)
Never married	25 (10.5)	39 (16.7)
Age, median (IQR), y	24.0 (20.0-28.5)	24.0 (20.0-29.0)
Gestational age, median (IQR), wk[b]	17.6 (15.7-19.6)	17.4 (15.6-19.8)
Gravidity, No. (%)		
Primigravida	37 (15.6)	48 (20.6)
Secundigravida	57 (24.1)	35 (15.0)
Multigravida	143 (60.3)	150 (64.4)
Plasmodium infection, No. (%)		
Any *plasmodium* spp by any dipstick or PCR[c]	89 (37.6)	86 (36.9)
P falciparum by HRP2- or pLDH-based dipstick or PCR	88 (37.1)	86 (36.9)
Current or recent *P falciparum* infection by either HRP2- or pLDH-based dipstick	49 (20.6)	46 (19.8)
P falciparum by PCR	81 (34.2)	82 (35.2)
HIV infection, No. (%)[d]	48 (20.3)	51 (22.1)
Plasma CRP concentration, median (IQR), mg/L	4.2 (2.1-10.1)	4.3 (2.1-11.0)
Plasma AGP concentration, mean (SD), g/L	0.8 (0.3)	0.8 (0.3)
Inflammation, No. (%)[e]		
Plasma CRP concentration ≥10 mg/L	61 (25.7)	65 (27.9)
Plasma AGP concentration ≥1.0 g/L	43 (18.1)	42 (18.0)
Plasma CRP concentration ≥10 mg/L or AGP ≥1.0 g/L	76 (32.1)	76 (32.6)
Hemoglobin concentration, mean (SD), g/dL	11.39 (1.09)	11.25 (1.19)
Anemia, hemoglobin concentration <11.0 g/dL, No. (%)	82 (34.6)	93 (39.9)
Plasma ferritin concentration, median (IQR), µg/L	13.9 (8.2-29.2)	13.8 (8.3-28.5)
Plasma sTfR concentration, median (IQR), mg/L	1.9 (1.4-2.5)	2.0 (1.6-2.7)
Plasma transferrin concentration, mean (SD), g/L	3.1 (0.5)	3.1 (0.6)
Iron deficiency, plasma ferritin concentration <15 µg/L		
All women, No. (%)	126 (53.2)	122 (52.4)
Those with CRP concentration <10 mg/L, No./total No. (%)	101/176 (57.4)	96/168 (57.1)
Those with AGP concentration <1.0 g/L, No./total No. (%)	115/194 (59.3)	106/191 (55.5)
Those with concentrations of CRP <10 mg/L or AGP <1.0 g/L, No./total No. (%)	97/161 (60.2)	93/157 (59.2)
ZPP-heme ratio, median (IQR), µmol/mol		
Whole blood	89.0 (67.8-119.3)	89.5 (67.3-126.3)
Erythrocyte	37.5 (19.8-63.3)	35.5 (19.8-72.3)

(continued)

Table 1. Baseline Characteristics of Study Participants by Intervention Group (continued)

Characteristics	Iron	Placebo
Intestinal helminth infections[f]		
Schistosoma spp, No. (%)		
Absent (Ct = 45)	165 (69.7)	149 (63.7)
Low (35 < Ct < 45)	10 (4.4)	10 (4.2)
Moderate (30 ≤ Ct ≤ 35)	22 (9.4)	19 (8.0)
High (Ct < 30)	39 (16.6)	56 (24.1)
Trichuris trichiura, No. (%)		
Absent (Ct = 45)	185 (78.1)	198 (84.8)
Low (35 < Ct < 45)	8 (1.0)	8 (3.4)
Moderate (30 ≤ Ct ≤ 35)	12 (4.9)	13 (5.5)
High (Ct < 30)	32 (13.5)	15 (6.2)
Necator americanus, No. (%)		
Absent (Ct = 45)	211 (88.9)	209 (89.8)
Low (35 < Ct < 45)	9 (3.7)	9 (3.9)
Moderate (30 ≤ Ct ≤ 35)	10 (4.2)	10 (4.2)
High (Ct < 30)	8 (3.2)	5 (2.0)

Abbreviations: AGP, α_1-acid glycoprotein; CRP, C-reactive protein; Ct, cycle threshhold; HRP2, *P falciparum*–specific histidine-rich protein-2; IQR, interquartile range; pLDH, *P falciparum*-specific lactate dehydrogenase; PCR, polymerase chain reaction; sTfR, soluble transferrin receptor; ZPP, zinc protoporphyrin.

[a] Calculated as weight in kilograms divided by height in meters squared.

[b] All women except 1 were in the second trimester of pregnancy.

[c] Only 1 participant (iron group) had infection by a *Plasmodium* species other than *P falciparum*.

[d] HIV status of 2 participants was not determined.

[e] Only 1 participant (iron group) had current fever defined as axillary temperature ≥37.5°C.

[f] Missing values occurred because stool samples could not be collected for some women. Cycle threshold values are inversely proportional to the amount of target DNA in the sample. Ct = 45: no detectable levels of target nucleic acid; 35 < Ct < 45: weak reactions indicative of marginal amounts of target nucleic acid; 30 ≤ Ct ≤ 35: positive reactions indicative of moderate amounts of target nucleic acid, compatible with low parasite load that is unlikely to be detectable by microscopy; Ct < 30: strong positive reactions indicative of abundant target nucleic acid compatible with a parasite load that is probably detectable by microscopy.

Maternal Outcomes at Delivery

Forty women (22 in the iron group and 18 in the placebo group) were lost to follow-up or excluded at birth. There was no evident effect of iron supplementation on *Plasmodium* infection risk (50.9% vs 52.1% for iron vs placebo groups, respectively; difference, −1.2%, 95% CI, −11.8% to 9.5%; P = .83) (**Table 2**). This effect was not influenced by baseline imbalances (crude and adjusted risk difference, −1.2% vs −0.5%). Subgroup analysis showed no evidence that the effect of iron on *Plasmodium* infection risk depended on any of the baseline factors investigated (**Figure 2**) or on IPT use (**Figure 3**).

Iron supplementation led to improved maternal iron status as indicated by hemoglobin concentrations, prevalence of anemia, and ZPP-heme ratios (Table 2).

Neonatal Outcomes at Delivery

Iron supplementation resulted in increased birth weight by 150 g (95% CI, 56 to 244) (Table 2) and reduced the risk of low birth weight by 58% (95% CI, 22% to 87%). The absolute risk reduction was 6.0% (95% CI, 0.8% to 11.1%) (Table 2). Thus, on average, 16.8 women (95% CI, 9.0 to 61.3) needed to receive supplementation to prevent 1 case of low birth weight. The effect of iron on birth weight was larger in women with iron deficiency at baseline than in those who were initially iron-replete (234 g vs 39 g; difference, 195 g; 95% CI, −3 to 393; P = .05) (Figure 2). There was no evidence that the effect of iron on birth weight was influenced by IPT use (Figure 3).

Iron supplementation led to an increased gestational age at delivery by 3.4 days and neonatal length by 0.9 cm; it reduced the risk of premature birth by 7% (Table 2). We found no effect of iron on neonate iron markers (hemoglobin concentration, ZPP-heme ratios).

Maternal Outcomes at 1 Month After Birth

Twelve mothers were lost to follow-up postpartum (5 in the iron group and 7 in the placebo group). Iron supplementation improved maternal iron status. Hemoglobin concentration and geometric mean plasma ferritin concentration increased by 0.90 g/dL and 123%, respectively; geometric mean plasma transferrin receptor concentration was reduced by 29% (**Table 3**). Gains in iron status were greater in women with poor iron status at baseline. Iron supplementation increased hemoglobin concentration by 1.52 g/dL and 0.44 g/dL in women with and without initial anemia, respectively. Corresponding values were 1.29 g/dL and 0.60 g/dL for women who were initially iron-deficient and -replete, respectively (Figure 2).

Neonatal Outcomes at 1 Month Postpartum

Twenty-two infants were lost to follow-up (13 in the iron group and 9 in the placebo group). Iron supplementation increased geometric mean plasma ferritin concentration by 18%. There was no evidence that it affected hemoglobin and plasma transferrin receptor concentrations.

Per-Protocol Analysis

Results of the per-protocol analysis were similar to those obtained by intention-to-treat analysis (eTables 3 and 4, eFigures 1 and 2 in Supplement 3). There was no evident effect of iron on *Plasmodium* infection, however defined (eTables 4 and 5 in Supplement 3). Iron supplementation led to a decrease in the risk of low birth weight by 64% (95% CI, 12%-86%). Among women who were initially iron deficient, iron supplementation increased birth weight by 263 g (95% CI, 136-362). There was some evidence that IPT use modified the effect of iron on maternal hemoglobin concentration (P = .04 for interaction), but there was no monotonic trend in effect by IPT dose.

Discussion

Overall, we found no effect of daily iron supplementation during pregnancy on risk of maternal *Plasmodium* infection. Iron supplementation resulted in an increased birth weight (by 150 g), gestational duration, and neonatal length; enhanced maternal and infant iron stores at 1 month after birth; and a decreased risk of low birth weight (by 58%) and prematurity. The

Table 2. Effect of Iron on Selected Maternal and Neonatal Outcomes at Delivery[a]

Indicator	No. of Missing Outcome Data (% of Randomized Persons)		Iron (n = 237)	Placebo (n = 233)	Effect (95% CI)	*P* Value
	Iron	Placebo				
Maternal outcomes						
Plasmodium infection, %						
Any evidence of infection (primary outcome)[b]	55 (23.2)	52 (22.3)	50.9	52.1	−1.2 (−11.8 to 9.5)	.83
Patent infection[c]	57 (24.1)	59 (25.3)	47.3	45.6	1.7 (−8.5 to 11.9)	.74
Presence of *P falciparum* DNA[d]	22 (9.3)	21 (9.0)	25.1	26.9	−1.8 (−11.1 to 7.4)	.69
Current or recent infection[e]	56 (23.6)	52 (22.3)	38.6	40.0	−1.4 (−12.8 to 10.0)	.81
Hemoglobin concentration, g/dL	22 (9.3)	19 (8.2)	12.08	11.15	0.93 (0.59 to 1.26)	<.001
Anemia (hemoglobin concentration <11.0 g/dL), %	22 (9.3)	19 (8.2)	22.0	50.4	−28.4 (−36.9 to −19.8)	<.001
Hemoglobin concentration >13.0 g/dL, %	22 (9.3)	19 (8.2)	31.3	17.3	14.0 (6.0 to 22.0)	<.001
ZPP-heme ratio, µmol/mol						
Whole blood	21 (8.9)	16 (6.9)	108.7	158.3	−31.3% (−37.9% to −24.0%)[f]	<.001
Erythrocyte	23 (9.7)	16 (6.9)	29.9	66.9	-55.3% (-62.5% to -46.7%)[f]	<.001
Neonatal outcomes						
Birth weight, g[g]	28 (11.8)	23 (9.9)	3202	3053	150 (56 to 244)	.002
Low birth weight (<2500 g), %	28 (11.8)	23 (9.9)	4.3	10.3	−6.0 (−11.1 to −0.8)	.02
Gestational age at delivery, d	1 (0.4)	1 (0.4)	274.4	271.0	3.4 (0.8 to 5.9)	.009
Premature birth (<37 wk gestation), %	1 (0.4)	1 (0.4)	9.1	16.2	−7.1 (−13.2 to −1.1)	.02
Birth-weight-for-gestational-age *z* score	27 (11.4)	25 (10.7)	0.52	0.31	0.21 (−0.11 to 0.52)	.20
Length, cm	40 (16.9)	36 (15.5)	50.6	49.7	0.9 (−0.1 to 1.8)	.07
Head circumference, cm	40 (16.9)	35 (15.0)	34.9	34.6	0.3 (−0.2 to 0.8)	.28
Hemoglobin concentration in cord blood, g/dL	31 (13.1)	24 (10.3)	15.43	15.11	0.32 (−0.11 to 0.75)	.14
ZPP-heme ratio, µmol/mol						
Cord blood	31 (13.1)	25 (10.7)	138.6	139.7	−0.8% (−7.8% to 6.6%)[f]	.82
Cord erythrocyte	31 (13.1)	25 (10.7)	55.3	55.8	−0.9% (−11.9% to 11.5%)[f]	.88

Abbreviations: HRP2, *P falciparum*–specific histidine-rich protein-2; pLDH, *P falciparum*-specific lactate dehydrogenase; PCR, polymerase chain reaction; ZPP, zinc protoporphyrin.

[a] Values indicate group prevalence or group mean, and effects are indicated as absolute difference unless indicated otherwise. See Methods for definitions of indicators.

[b] At least 1 positive result for dipstick tests (HRP2 or pLDH for any human *Plasmodium* species) in maternal venous or placental blood, or by *P falciparum*–specific PCR tests in maternal erythrocytes from venous or placental blood, or presence of parasites or pigment in placental biopsies by histopathology.

[c] At least 1 positive result for dipstick tests (HRP2 or pLDH for any human *Plasmodium* species) in maternal venous or placental blood or presence of parasites or pigment in placental biopsies by histopathology.

[d] Presence of *P falciparum*–specific DNA in maternal erythrocytes from venous or placental blood by PCR test.

[e] At least 1 positive result for dipstick tests (HRP2 or pLDH for any human *Plasmodium* species) in maternal venous or placental blood, or presence of parasites in maternal erythrocytes or pigment in erythrocytes/monocytes in intervillous space by placental histopathology.

[f] Relative differences in geometric means, with placebo as the reference group.

[g] Including neonates born in hospital or for whom weight was measured after 24 hours postpartum.

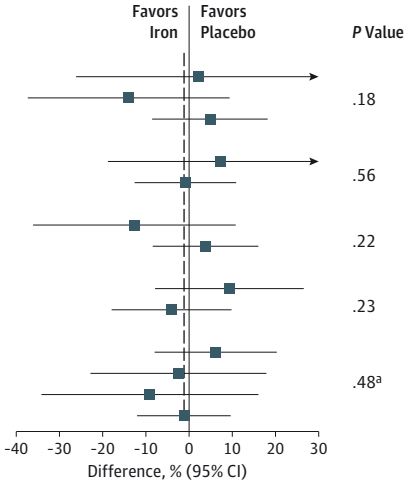
effect on birth weight was influenced by initial maternal iron status. Correction of maternal iron deficiency led to an increase in birth weight by 239 g.

Supplementation with iron-containing micronutrients can increase malaria rates in young children with iron deficiency,[23] perhaps through transiently increased counts of young erythrocytes that are more susceptible to invasion and propagation by *P falciparum* merozoites than mature erythrocytes.[24] Although we found no evidence in the present study that iron increased *Plasmodium* infection risk in women who were iron-deficient or anemic at baseline, we could not exclude such an effect (see upper limit of 95% CIs

Figure 2. Effect of Iron Supplementation on Selected Outcomes by Subgroup

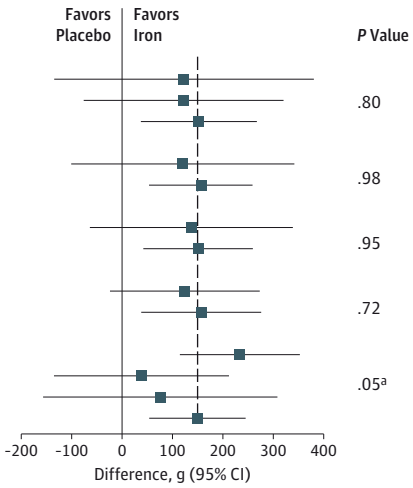
A *Plasmodium* infection assessed at delivery

	Iron Group		Placebo Group		
	No. of Participants/ Total No.	Risk, %	No. of Participants/ Total No.	Risk, %	Difference, % (95% CI)
Gravida status					
Primigravid	27/37	73	34/48	71	2 (-26 to 31)
Secundigravid	30/57	52	23/35	66	-14 (-37 to 9)
Multigravid	67/143	47	63/150	42	5 (-8 to 18)
Maternal age					
<20 y	34/48	71	31/49	64	7 (-19 to 33)
≥20 y	89/189	47	89/184	48	-1 (-12 to 11)
HIV infection					
Yes	15/48	32	23/51	44	-13 (-36 to 11)
No	109/189	57	97/182	54	4 (-8 to 16)
Anemia					
Yes	49/82	60	47/93	50	9 (-8 to 26)
No	75/155	48	73/140	52	-4 (-18 to 10)
Iron status					
Deficient	62/126	50	53/122	43	6 (-8 to 20)
Replete	33/64	51	34/64	54	-2 (-23 to 18)
Uncertain	29/47	61	33/47	70	-9 (-34 to 16)
Overall	121/237	51	121/233	52	-1 (-12 to 10)



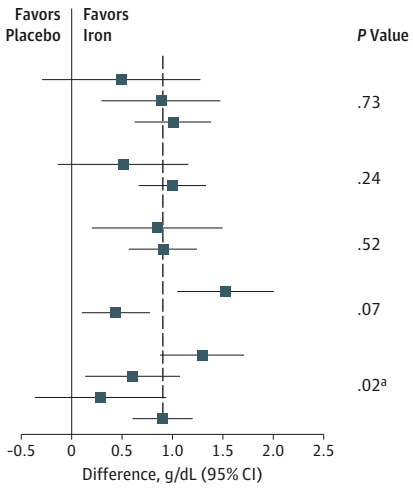
B Birth weight

	Iron Group		Placebo Group		
	No. of Participants	Birth Weight Mean (SE), g	No. of Participants	Birth Weight Mean (SE), g	Difference, g (95% CI)
Gravida status					
Primigravid	37	3051 (95)	48	2928 (81)	123 (-134 to 380)
Secundigravid	57	3223 (65)	35	3101 (75)	122 (-75 to 319)
Multigravid	143	3233 (42)	150	3081 (41)	152 (38 to 266)
Maternal age					
<20 y	48	3095 (77)	49	2974 (79)	121 (-100 to 341)
≥20 y	189	3230 (37)	184	3073 (36)	156 (54 to 258)
HIV infection					
Yes	48	3127 (74)	51	2989 (71)	138 (-63 to 338)
No	189	3222 (38)	182	3071 (38)	151 (43 to 259)
Anemia					
Yes	82	3134 (54)	93	3009 (54)	125 (-23 to 272)
No	155	3238 (42)	140	3081 (41)	157 (38 to 276)
Iron status					
Deficient	126	3288 (44)	122	3055 (42)	234 (116 to 351)
Replete	64	3168 (59)	64	3129 (67)	39 (-134 to 211)
Uncertain	47	3019 (82)	47	2943 (81)	76 (-155 to 307)
Overall	237	3202 (34)	233	3053 (33)	150 (56 to 244)



C Maternal hemoglobin concentration at 1 mo after birth

	Iron Group		Placebo Group		
	No. of Participants	Hemoglobin Mean (SE), g/dL	No. of Participants	Hemoglobin Mean (SE), g/dL	Difference, g/dL (95% CI)
Gravida status					
Primigravid	37	12.38 (0.30)	48	11.89 (0.27)	0.49 (-0.29 to 1.27)
Secundigravid	57	12.90 (0.17)	35	12.01 (0.25)	0.88 (0.30 to 1.47)
Multigravid	143	13.01 (0.13)	150	12.01 (0.14)	1.00 (0.63 to 1.38)
Maternal age					
<20 y	48	12.41 (0.23)	49	11.89 (0.24)	0.51 (-0.13 to 1.16)
≥20 y	189	13.01 (0.11)	184	12.01 (0.13)	1.00 (0.67 to 1.33)
HIV infection					
Yes	48	12.59 (0.22)	51	11.74 (0.24)	0.85 (0.20 to 1.50)
No	189	12.96 (0.11)	182	12.06 (0.13)	0.91 (0.57 to 1.24)
Anemia					
Yes	82	12.52 (0.18)	93	11.00 (0.17)	1.52 (1.05 to 2.00)
No	155	13.08 (0.12)	140	12.64 (0.12)	0.44 (0.10 to 0.77)
Iron status					
Deficient	126	12.88 (0.13)	122	11.58 (0.16)	1.29 (0.88 to 1.71)
Replete	64	13.23 (0.18)	64	12.62 (0.16)	0.60 (0.14 to 1.07)
Uncertain	47	12.45 (0.22)	47	12.17 (0.26)	0.29 (-0.36 to 0.93)
Overall	237	12.89 (0.10)	233	11.99 (0.11)	0.90 (0.61 to 1.19)



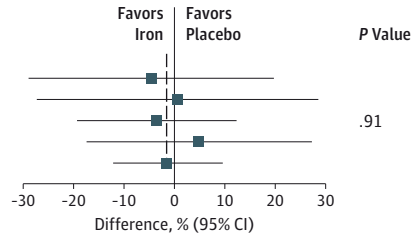
Analyses were by intention-to-treat, with multiple imputations to replace missing values. Where pooling of results from multiple imputations led to noninteger counts, we rounded those values. *P* values indicate the 2-sided probability that group effects are as different as observed or more extreme when assuming that they are identical. Dashed lines indicate overall effect; solid lines, no effect.

[a] Analysis restricted to those who were iron-deficient or iron-replete.

Figure 3. Effect of Iron Supplementation on Selected Outcomes by Use of Intermittent Preventive Treatment

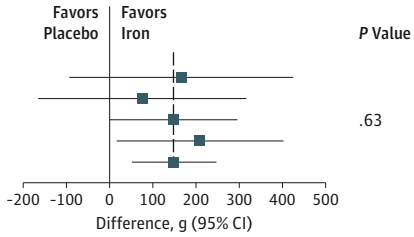
A *Plasmodium* infection assessed at delivery

	Iron Group		Placebo Group			
	No. of Participants/ Total No.	Risk, %	No. of Participants/ Total No.	Risk, %	Difference, % (95% CI)	*P* Value
IPT use						
None	20/48	41.0	20/43	45.4	-4.3 (-28.5 to 19.8)	
Single dose	23/47	48.5	19/39	47.7	0.8 (-26.8 to 28.5)	
2 Doses	49/91	54.2	52/90	57.5	-3.3 (-19.0 to 12.4)	.91
3 Doses	29/51	56.6	31/60	51.5	5.0 (-17.1 to 27.2)	
Overall	121/237	50.9	121/233	52.1	-1.2 (-11.9 to 9.5)	



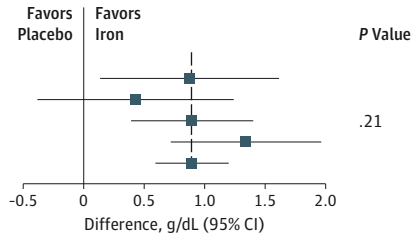
B Birth weight

	Iron Group		Placebo Group			
	No. of Participants	Birth Weight Mean (SE), g	No. of Participants	Birth Weight Mean (SE), g	Difference, g (95% CI)	*P* Value
IPT use						
None	48	3130 (91)	43	2962 (84)	167 (-91 to 426)	
Single dose	47	3176 (83)	39	3101 (92)	76 (-164 to 316)	
2 Doses	91	3221 (51)	90	3073 (57)	149 (0 to 297)	.63
3 Doses	51	3264 (76)	60	3054 (64)	210 (19 to 401)	
Overall	237	3202 (34)	233	3053 (33)	150 (56 to 244)	



C Maternal hemoglobin concentration at 1 mo after birth

	Iron Group		Placebo Group			
	No. of Participants	Hemoglobin Mean (SE), g/dL	No. of Participants	Hemoglobin Mean (SE), g/dL	Difference, g/dL (95% CI)	*P* Value
IPT use						
None	48	12.72 (0.24)	43	11.84 (0.30)	0.88 (0.15 to 1.61)	
Single dose	47	12.67 (0.26)	39	12.24 (0.32)	0.43 (-0.37 to 12.4)	
2 Doses	91	12.83 (0.17)	90	11.93 (0.20)	0.90 (0.40 to 1.40)	.21
3 Doses	51	13.36 (0.23)	60	12.01 (0.21)	1.34 (0.73 to 1.96)	
Overall	237	12.89 (0.10)	233	11.99 (0.11)	0.90 (0.61 to 1.19)	



Analyses were by intention-to-treat, with multiple imputations to replace missing values. Where pooling of results from multiple imputations led to noninteger counts, we rounded those values. *P* values indicate the 2-sided probability that group effects are as different as observed or more extreme when assuming that they are identical. Dashed lines indicate overall effect; solid lines, no effect; IPT, intermittent preventive treatment.

in Figure 2). Should this raise concerns? *P falciparum* infections in pregnancy notably cause increased risks of low birth weight and severe anemia.[12] In our study, however, women who were initially iron-deficient benefited most in terms of birth weight, indicating that any potential decrease in birth weight due to the effect of iron supplements on *Plasmodium* infection was outweighed by beneficial effects of iron on birth weight through other mechanisms.

Depending on local conditions, approximately one-quarter of severe anemia cases among pregnant women are attributable to malaria,[12] suggesting that most cases are due to iron deficiency. Our data show that women who were initially iron-deficient or anemic benefited particularly in terms of hemoglobin concentrations (Figure 2), thus decreasing their risk of severe anemia. Women with increased hemoglobin concentrations due to iron supplementation may also be less prone to develop severe anemia during or following episodes of malarial illness.

Per-protocol analysis suggested that IPT use may have modified the effect of iron on hemoglobin concentration (eFigure 2 in Supplement 3), but the absence of a clear trend with dose indicates that this finding may be spurious. Interpretation of these data is further complicated because IPT use was not a baseline factor and may have acted as a mediating factor.

One limitation of our study concerns the missing data. Multiple imputation yields valid effect estimates when data are missing at random,[25] which seems a reasonable assumption given the reasons for missingness of most primary outcome data (exclusion of twin pregnancies, women leaving the study area, absent placental samples because of delivery at home or in tertiary facilities, or incorrect preservation of samples).

Another limitation of our study concerns the lack of detailed, reliable morbidity data during follow-up. To avoid ethical dilemmas, we did not collect blood samples during intervention, and we referred women for antenatal care after enrollment to regular health services, where they received standard service. *Plasmodium* infection was determined in samples collected at delivery, which may not have captured all infections or symptomatic episodes during intervention. *Plasmodium* pigment persists in the placenta, however, and placental histopathology probably captures most infections in the second half of pregnancy, even though it may have low sensitivity in capturing earlier infections.[21,26]

Table 3. Effect of Iron on Selected Maternal and Neonatal Outcomes at 1 Month After Birth[a]

Indicator	No. of Missing Outcome Data (% of Randomized Persons)		Iron (n = 237)	Placebo (n = 233)	Effect (95% CI)	*P* Value
	Iron	Placebo				
Maternal outcomes						
Hemoglobin concentration, g/dL	22 (9.3)	19 (8.2)	12.89	11.99	0.90 (0.61 to 1.19)	<.001
Anemia (hemoglobin concentration <12.0 g/dL), %	22 (9.3)	19 (8.2)	43.2	65.7	−22.4 (−31.4 to −13.5)	<.001
Plasma ferritin concentration, µg/L	23 (9.7)	21 (9.0)	32.1	14.4	123.4% (85.5% to 169.1%)[b]	<.001
Iron deficiency (plasma ferritin concentration <15 µg/L)						
All persons, %	23 (9.7)	21 (9.0)	19.8	56.0	−36.2 (−44.9 to −27.5)	<.001
Those without inflammation, %[c]	23 (9.7)	21 (9.0)	21.1	59.5	−38.5 (−49.7 to −27.3)	<.001
Plasma transferrin concentration, g/L	24 (10.1)	21 (9.0)	2.67	3.07	−0.40 (−0.49 to −0.30)	<.001
Plasma transferrin receptor concentration, mg/L	23 (9.7)	21 (9.0)	1.81	2.53	−28.6% (−35.5% to −21.0%)[b]	<.001
ZPP-heme ratio, µmol/mol						
Whole blood	22 (9.3)	19 (8.2)	100.4	152.0	−33.9% (−40.3% to −26.8%)[b]	<.001
Erythrocyte	24 (10.1)	19 (8.2)	31.9	74.5	−52.7% (−64.0% to −49.1%)[b]	<.001
Neonatal outcomes						
Hemoglobin concentration, g/dL	33 (13.9)	30 (12.9)	13.45	13.32	0.13 (−0.52 to 0.78)	.69
Plasma ferritin concentration, µg/L	34 (14.3)	31 (13.3)	163.0	138.7	17.5% (2.4% to 34.8%)[b]	.02
Plasma transferrin receptor concentration, mg/L	35 (14.8)	31 (13.3)	1.21	1.27	−4.4% (−11.3% to 2.9%)[b]	.23

Abbreviation: ZPP, zinc protoporphyrin.

[a] Values indicate group prevalence or group mean, and effects are absolute differences unless indicated otherwise. See Methods for definitions of indicators.

[b] Relative differences in geometric means, with placebo as the reference group.

[c] Concentrations of C-reactive protein <10 mg/L and α1-acid glycoprotein <1.0 g/L.

The use of placebo in a population that included women with anemia and iron deficiency was justified by our concern that risks inherent to iron supplementation outweighed possible benefits; however, it also provided a unique opportunity to assess benefits of antenatal iron supplementation. Recent meta-analyses, published after we started our trial, found no[27] or only small effects (31 g[28] and 41 g[29]) of antenatal iron supplementation on birth weight. In these meta-analyses, however, subgroup analysis by initial iron status could not be investigated, because women with anemia or iron deficiency were excluded from the studies reviewed, women (also in the placebo group) received iron as rescue therapy during intervention, or initial anemia status and iron status had not been specified.[27] Consistent with the findings reported in these meta-analyses,[27-29] our data showed no evident effect of iron on birth weight among iron-replete women.

The beneficial effect of maternal iron supplementation on birth weight found in our study may also be explained in part by a superior adherence compared with previous studies.[27-29] Whereas we observed daily whether women swallowed supplemental capsules, supervision was unclear in other studies, or contact with participants was limited to repeated visits or telephone calls. Although several studies used capsules or coated tablets,[30,31] iron was mostly given as tablets with ferrous salts, which produce a strong, unpleasant taste that may have discouraged adherence. Attrition was high in many studies, although it is unclear whether this occurred selectively in the iron groups. Adherence in most studies was not reported or poor, or supplement use was assessed by tablet counts or self-reports, which tend to overestimate adherence.[32,33]

The gain in birth weight was due at least in part to a longer gestational duration, with perhaps some contribution by improved fetal growth. In magnitude, this effect (150 g) is comparable or exceeds effects of interventions to prevent malaria in pregnancy, namely, IPT (79-135 g, depending on frequency of administration) and insecticide-treated nets (55 g),[34] and is particularly important because low birth weight is associated with neonatal and postneonatal mortality, morbidity, inhibited growth, cognitive development, and potentially chronic diseases later in life.[35] This comparison should not detract from the benefits of these malaria control interventions, but is rather made to indicate the importance that should be given to increased coverage of iron supplementation.

In accordance with our finding on birth weight, the hemoglobin response to iron was larger in women who were initially iron-deficient as compared with those who were iron-replete. A differential benefit of iron by anemia status was

apparent for hemoglobin concentration but not birth weight (Figure 2), consistent with initial anemia being due only partly to iron deficiency and partly to other causes.

Our finding of increased plasma ferritin concentrations at 1 month postpartum adds to growing evidence,[36] so far confirmed only in a single trial,[37] that antenatal iron supplementation improves neonatal iron stores, thus delaying the age at which iron deficiency is likely to develop during infancy.

The baseline characteristics of our study population were typical for pregnant women in many rural settings in low- and middle-income countries. There was no evidence that gains in birth weight depended on gravidity, maternal age, HIV infection, anemia, and IPT use, suggesting benefits from iron for all subgroups thus defined, including primigravidae and those who did not receive IPT. Thus, our results may apply to pregnant women in other low- and middle-income countries, although the effect on birth weight can vary depending on the prevalence of iron deficiency. Our results cannot be extrapolated to daily antenatal supplementation with 120 mg, for which safety data are lacking, or to children, for whom there is substantial evidence that iron supplementation can increase malaria rates.[38]

In low- and middle-income countries, it is generally impractical to screen for iron status, and most countries have policies for universal iron supplementation for pregnant women. Based on our results, we believe that the benefits of universal supplementation outweigh possible risks.

Conclusions

Among rural Kenyan women with singleton pregnancies, administration of daily iron supplementation, compared with administration of placebo, resulted in no significant differences in overall maternal *Plasmodium* infection risk. Iron supplementation led to increased birth weight.

ARTICLE INFORMATION

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Acquisition, analysis, or interpretation of data: M. Mwangi, Roth, Smit, Trijsburg, Demir, Wielders, Mens, Verweij, Prentice, Savelkoul, Andang'o, Verhoef.
Drafting of the manuscript: M. Mwangi, Verhoef.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: M. Mwangi, Verhoef.
Obtained funding: Brouwer, Verhoef.
Administrative, technical, or material support: M. Mwangi, Roth, Smit, Trijsburg, A. Mwangi, Demir, Wielders, Mens, Verweij, Brouwer, Savelkoul, Andang'o, Verhoef.
Study supervision: Andang'o, Verhoef.

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Correction: This article was corrected online October 1, 2015, to add 2 individuals to the Additional Contributions section.

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