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Iron supplementation during pregnancy, anemia, and birth weight: a randomized controlled trial[1–3]

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ABSTRACT

Background: The need for prophylactic iron during pregnancy is uncertain.

Objective: We tested the hypothesis that administration of a daily iron supplement from enrollment to 28 wk of gestation to initially iron-replete, nonanemic pregnant women would reduce the prevalence of anemia at 28 wk and increase birth weight.

Design: Between June 1995 and September 1998, 513 low-income pregnant women in Cleveland were enrolled in the study before 20 wk of gestation. Of these, 275 had a hemoglobin concentration ≥ 110 g/L and a ferritin concentration ≥ 20 μg/L and were randomly assigned to receive a monthly supply of capsules containing either 30 mg Fe as ferrous sulfate or placebo until 28 wk of gestation. At 28 and 38 wk of gestation, women with a ferritin concentration of 12 to < 20 μg/L or < 12 μg/L received 30 and 60 mg Fe/d, respectively, regardless of initial assignment. Almost all the women received some supplemental iron during pregnancy. We obtained infant birth weight and gestational age at delivery for 117 and 96 of the 146 and 129 women randomly assigned to receive iron and placebo, respectively.

Results: Compared with placebo, iron supplementation from enrollment to 28 wk of gestation did not significantly affect the overall prevalence of anemia or the incidence of preterm births but led to a significantly higher mean (± SD) birth weight (206 ± 565 g; $P = 0.010$), a significantly lower incidence of low-birth-weight infants (4% compared with 17%; $P = 0.003$), and a significantly lower incidence of preterm low-birth-weight infants (3% compared with 10%; $P = 0.017$).

Conclusion: Prenatal prophylactic iron supplementation deserves further examination as a measure to improve birth weight and potentially reduce health care costs. *Am J Clin Nutr* 2003;78:773–81.

KEY WORDS Iron deficiency, anemia, iron supplementation, pregnancy, low birth weight, small-for-gestational age infants, preterm delivery

INTRODUCTION

For women who are initially iron replete and not anemic, the need for supplemental iron during pregnancy is uncertain. Present evidence is insufficient for the US Preventive Services Task Force to either recommend or not recommend routine use of iron supplements in pregnant women with a hemoglobin concentration ≥ 100 g/L (1, 2). Specifically, evidence about beneficial effects of iron supplementation during pregnancy on functional outcomes is inconclusive. Moreover, the theoretical possibility of adverse effects, such as oxidative damage, with administration of iron supplements during pregnancy has been raised (3).

In 1993, the Institute of Medicine (IOM) proposed a complex program of selective iron supplementation during pregnancy, which was based on screening pregnant women in their first and second trimesters by using hemoglobin and ferritin concentrations (**Table 1**) (4). In particular, the IOM recommended that iron supplements not be given to women who are nonanemic (hemoglobin concentration ≥ 110 g/L) and iron replete (ferritin concentration > 20 μg/L). Women with anemia or decreased iron stores would receive different doses of iron depending on their hemoglobin or ferritin concentrations. In the third trimester, all women would receive iron supplements, but the dose would differ by hemoglobin concentration. Recognizing the lack of available evidence, the IOM recommended that the guidelines be evaluated before implementation (4). The Centers for Disease Control and Prevention and the American College of Obstetricians and Gynecologists recommend a daily iron supplement (30 mg) as prophylaxis for iron deficiency during pregnancy (5, 6). In the present study, we tested the hypothesis that administration of a daily iron supplement from enrollment to 28 wk of gestation to initially iron-replete, nonanemic pregnant women would reduce the prevalence of anemia at 28 wk of gestation. During the study, we added birth weight and gestational age as outcomes because of a need for more information on the functional consequences of iron supplementation during pregnancy (1, 2).

SUBJECTS AND METHODS

Study population

Eligible participants were legally competent, nonimprisoned pregnant women at < 20 wk of gestation. All were enrolled in the Cuyahoga County, MetroHealth Medical Center, Supplemental

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TABLE 1
Summary of Institute of Medicine guidelines for iron supplementation during pregnancy[1]

Hemoglobin	Ferritin	Recommended management
	μg/L	
First trimester		
<90 g/L	Any	Obtain medical evaluation
90–109 g/L	>30	Obtain medical evaluation
90–109 g/L	12–20	30 mg supplemental Fe/d
≥110 g/L	≤20	30 mg supplemental Fe/d
90–109 g/L	<12	60–120 mg supplemental Fe/d
≥110 g/L	>20	No iron
Second trimester		
<90 g/L	Any	Obtain medical evaluation
≥105 g/L	≤20	30 mg supplemental Fe/d
90–104 g/L	<12	60–120 mg supplemental Fe/d
≥105 g/L	>20	No iron
Third trimester		
<90 g/L	Any	Obtain medical evaluation
≥110 g/L	Any	30 mg supplemental Fe/d
90–109 g/L	Any	60–120 mg supplemental Fe/d

[1] From reference 4.

Nutrition Program for Women, Infants, and Children (WIC) in Cleveland between June 1995 and September 1998; were registered patients at the MetroHealth Medical Center; and gave written informed consent to participate in the study. The study protocol was approved by the institutional review boards of the MetroHealth Medical Center and the Centers for Disease Control and Prevention.

Enrollment

The purpose and design of the study was explained to each eligible participant in writing (fact sheet written by GMB) and verbally by a WIC dietitian (LI) trained in the informed consent procedure, with emphasis on the requirement for random allocation to receive no supplemental iron or standard iron supplementation. Among the WIC enrollees, 513 agreed to participate in the study. No compensation was given for participation. Initial venous blood samples (5 mL) were drawn, and each volunteer was given an appointment for her first study visit within 10 d of enrollment. The protocol required participants to visit the WIC clinic monthly and to have blood drawn at 28 and 38 wk of gestation. At their monthly visits to the WIC clinic, the participants returned their supplement bottles with any unused capsules to the WIC dietitian (LI), who asked the participants about any side effects that they had experienced.

Iron and hematologic measurements

Iron and hematologic studies, including complete blood count, ferritin, and erythrocyte protoporphyrin, were conducted on the blood samples at enrollment, 28 wk of gestation, and 38 wk of gestation. Measurement of erythrocyte protoporphyrin, however, was added after the start of the study, and thus data are not available for all the subjects. After collection in the WIC clinic, the blood samples were sent immediately by a pneumatic tube carrier transport system to the Hematology Research Laboratory, which is housed in an adjacent section of the building. The samples were immediately removed from the transport system and refrigerated at 4 °C until analysis (whole blood or red cell studies) or separation of the blood components (serum, plasma) for storage at −70 °C. Complete blood counts and zinc erythrocyte protoporphyrin measurements were carried out within 4 h of receipt. Serum iron studies and ferritin measurements were carried out in the order of receipt in batches of ≈100 samples per assay after the first thawing of previously frozen specimens.

Hemoglobin concentrations and mean corpuscular volumes were measured with an electronic counter (Coulter Electronics, Hialeah, FL). Plasma ferritin concentrations were measured by using enzyme-linked immunosorbent assay (Ramco Laboratories, Inc, Houston). Zinc erythrocyte protoporphyrin was measured fluorimetrically (ZnP Model 4000 Hematofluorimeter; Environmental Sciences Associates, Inc, Bedford, MA). An internal quality control system ensured that appropriate controls were run with each assay and that the calibration of spectrophotometers, the hematofluorimeter, balances, pipettes, and other equipment was periodicly verified.

Randomization

The women were randomly allocated to treatment groups on the basis of random numbers generated by the study data manager using a computerized algorithm (STATVIEW II; Abacus Concepts, Inc, Berkeley, California). Of the 513 women who gave initial blood samples, 275 had a hemoglobin concentration ≥110 g/L and a ferritin concentration ≥20 μg/L (**Figure 1**). These women were randomly allocated to receive by mouth each day soft gelatin capsules containing either 30 mg Fe as ferrous sulfate (n = 146) or placebo (n = 129) until 28 wk of gestation. Of the 238 women not included in this report, 95 had a ferritin concentration of 12 to <20 μg/L and were randomly assigned to receive daily capsules containing 30 or 60 mg Fe, 126 had a ferritin concentration <12 μg/L and were prescribed 60 mg Fe/d, 15 were referred for medical evaluation (because they were anemic with a serum ferritin concentration >20 μg/L), and 2 were missing data for ferritin or hemoglobin concentration.

Intervention

The capsules containing placebo were formulated to be indistinguishable from those containing iron, and at the first visit, each subject was given a bottle containing a 31-d supply of either the placebo or the iron capsules. Additional study capsules were dispensed at subsequent monthly clinic visits until the time of the second assessment at 28 wk of gestation. Repeated attempts were made by telephone and mail to reschedule appointments for study participants who did not return to the WIC clinic. A label on each bottle included a number linking the bottle to the type of capsule. The data manager (who did not have contact with patients) assigned participants to their groups and held the link. WIC personnel, laboratory analysts, and patients were not aware of the type of capsules.

Reassessments at 28 and 38 wk of gestation

At 28 wk of gestation, venous blood samples were drawn, and iron and hematologic studies were conducted. The women were assigned to the prescriptions listed in **Table 2** according to their hemoglobin and serum ferritin concentrations, and capsules were dispensed in a bottle containing a 31-d supply at the monthly clinic visit. This procedure was repeated at 38 wk of gestation.

Program records

Maternal prepregnancy weight, smoking status at entry into WIC, race or ethnic designation, years of maternal education, number of previous live births, maternal weight at delivery, birth weight, birth length, and date of delivery were obtained

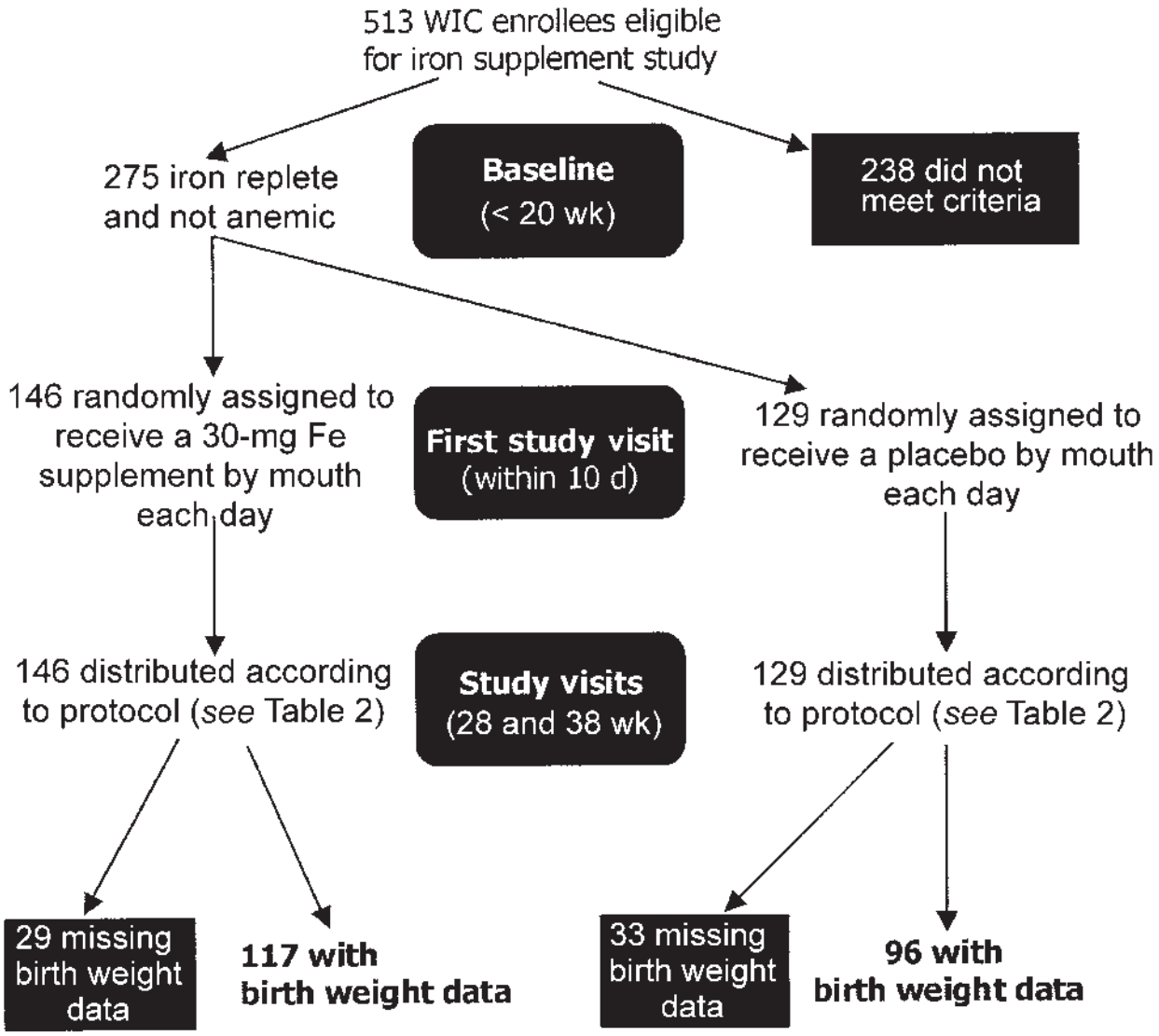

FIGURE 1. Flow of participants in the randomized controlled trial from the baseline examination at < 20 wk of gestation to the end of pregnancy. Of the 238 initially eligible women who were excluded, 236 were either anemic or had a ferritin concentration < 20 μg/L, and 2 were missing data on ferritin or hemoglobin concentrations. WIC, Supplemental Nutrition Program for Women, Infants, and Children.

from the WIC program records. Gestational age at delivery was calculated as the number of weeks from the reported last menstrual period to the delivery date. Weight gain during pregnancy was calculated as the reported weight at delivery minus the prepregnancy weight.

Main outcomes

The primary outcome was the prevalence of anemia (hemoglobin concentration < 110 g/L) at the 28-wk visit. We also examined the distributions of birth weight, birth length, and gestational age and the proportion of infants who had a low birth weight (< 2500 g), were born preterm (< 37 wk of gestation), were born at term but had a low birth weight (≥ 37 wk of gestation and < 2500 g), and were small-for gestational age (7). We compared means and SDs between term births because this approach provides another way to compare fetal growth between groups (8). We also examined the proportion of infants who were small and preterm based on *1*) a birth weight < 2500 g and a gestation of < 37 wk, and *2*) their "residual birth weight" (8).

Subsidiary outcomes

Subsidiary outcomes included average maternal weight gain, iron status at 28 wk of gestation, adherence to iron supplementation, and side effects. Iron-status measurements included mean cell volume and hemoglobin, ferritin, and erythrocyte protopophyrin concentrations. For this study, "iron deficiency anemia" was defined as a hemoglobin concentration < 110 g/L and a ferritin concentration < 12 μg/L, and "absent iron stores" was defined as a ferritin concentration < 12 μg/L.

Adherence to the supplementation regimen was calculated on the basis of the number of capsules remaining in the bottle at each return visit:

TABLE 2
Study protocol at 28 wk of gestation according to initial treatment category[1]

Measurement at 28 wk			Treatment at 28 wk	
Hemoglobin	Ferritin	Descriptor	Initial iron supplement group (n = 146)	Initial placebo group (n = 129)
	$\mu g/L$			
>110 g/L	>20	Not anemic, iron replete	Continued with 30-mg Fe supplements (n = 18)	Continued with placebo (n = 15)
>110 g/L	12–20	Not anemic, iron depleted	Continued with 30-mg Fe supplements (n = 22)	Assigned 30-mg Fe supplements (n = 10)
>90 g/L	<12	Absent iron stores	Increased to 60-mg Fe supplements (n = 62)	Assigned 60-mg Fe supplements (n = 56)
90–109 g/L	>20	Anemic, ferritin >20 μg/L	Excluded and referred for medical evaluation (n = 5)	Excluded and referred for medical evaluation (n = 4)
90–109 g/L	12–20	Anemic, iron depleted	Continued with 30-mg Fe supplements (n = 3)	Assigned 30-mg Fe supplements (n = 1)
<90 g/L	Any	Severely anemic	Excluded and referred for medical evaluation (n = 0)	Excluded and referred for medical evaluation (n = 0)
No measurement	No measurement	Lost to follow-up of iron measures	Unknown (n = 36)	Unknown (n = 43)

[1] Iron-status measures were reassessed at 38 wk of gestation among 144 women who had not given birth and who returned for assessment. Treatment was then reassigned for the 144 women on the basis of the iron measures at 38 wk of gestation according to the same protocol as for 28 wk of gestation.

$$\text{Adherence (\%)} = [(31 - \text{number of pills left in the bottle})/\text{number of days between dispensing date and return date}] \times 100 \quad (1)$$

We also calculated the proportion of women who took >50% of the capsules received. Total iron taken was defined as the sum of the number of capsules taken times the amount of iron per capsule.

Side effects were determined at each visit by the WIC dietitian (LI), who asked the subjects, "Did you have any complaints or difficulty with taking the capsules?" The women who responded positively to this question at ≥1 visit were recorded as having experienced side effects. Some women who returned for monthly visits did not bring their bottles but answered questions about side effects, and thus the proportion of women with data on side effects was larger than that with data on adherence.

Sample size and power

Sample size calculations for the study were originally based on a relative difference of 50% between the 2 groups in the prevalence of anemia during the third trimester, ie, 30% compared with 15%. Although the prevalence of anemia at 28 wk of gestation among women in the study population who were not exposed to prenatal iron supplements was unknown, the prevalence of anemia during the third trimester among low-income women enrolled in WIC was ≈30% before this study. On the basis of 2 independent samples with a two-sided significance level of 0.05 and a β-error specification of 0.20, 120 subjects per group were needed (9). On the assumption of an overall rate of loss to follow-up of 10%, 133 subjects per group were required (266 nonanemic, iron-replete women). Actual loss to follow up (Table 2) affected the power of the study. With 86 women in the placebo group who had hemoglobin data at 28 wk of gestation, the statistical power to find a 50% difference between the 2 groups in anemia risk at 28 wk of gestation at a two-tailed probability of <0.05 was ≈40% (10).

We also hypothesized that the 2 study groups would differ in their distributions of birth weight and gestational age. With 96 women in the placebo group (n = 192 in the study population), and if the true difference between the 2 treatments was 0.407 times the SD, the probability of detecting a difference between the 2 groups at a two-sided significance level of 5% was 80% (10). Given that the SDs for mean birth weight and gestational age at delivery in the study sample were 573 g and 2.2 wk, respectively, the study had ≥80% power at a two-sided significance level of 5% to detect differences in mean birth weight and mean gestational age of 233 g and 0.9 wk, respectively. The minimum detectable difference may actually have been a bit smaller because the group that was provided with iron supplements from enrollment to 28 wk of gestation actually included 117 women. Because infants born to mothers who smoke during pregnancy weigh, on average, 150–300 g less than do those born to mothers who do not smoke (11), we thought that the difference in birth weight described above (ie, 233 g) was clinically significant.

Statistical analyses

We analyzed the outcomes on the basis of the randomization of the study participants. Because of losses to follow-up, we did not have data on third-trimester iron status, infant birth weight, gestational age at delivery, or adherence for some of the women. We compared the distributions of sociodemographic, health, and behavioral characteristics and of initial iron-status measures between the women with missing data and those with complete data. We also compared the reasons for missing data by randomization group. Among the women with third-trimester iron-status measures, we compared average iron-status measures between the 2 study groups and calculated the absolute difference between the 2 groups in the proportion of women with third-trimester anemia, low iron stores, and iron deficiency anemia. Among women with data on infant birth weight, we compared distributions of birth weight, gestational age, birth length, and maternal weight gain during pregnancy between the 2 study groups. We also calculated the absolute difference between the 2 groups in the risks of low birth weight, preterm delivery, small-for-gestational age, preterm delivery with low birth weight, and term delivery with low birth weight.

We used t tests to compare continuous outcomes between the iron and placebo groups. Because the distributions of ferritin

TABLE 3
Baseline characteristics of the study subjects by treatment category

Characteristic	Iron supplement group (n = 146)	Placebo group (n = 129)
Age (y)	24.3 ± 5.3[1]	24.5 ± 5.1
Race or ethnicity (n)		
White	82	73
Black	35	34
Hispanic	23	22
Other	5	0
No. of previous live births (n)[2]		
0	62	59
1	39	38
≥2	45	31
Prepregnancy weight (kg)	72.5 ± 20.3	77.9 ± 24.3[3]
Cigarette smoking (%)[4]	39.6	35.9
Gestational age at study entry (wk)	10.9 ± 3.5	10.6 ± 4.1
Hemoglobin (g/L)	129 ± 9	127 ± 10
Ferritin (μg/L)	44.7 (30.0, 59.7)[5]	49.4 (34.1, 76.7)[6]
Mean corpuscular cell volume (fL)	89.1 ± 4.7	89.2 ± 5.0
Erythrocyte protoporphyrin (μg/dL)[7]	53.7 ± 14.0	55.6 ± 16.5

[1] $\bar{x}$ ± SD.
[2] In the placebo group, one woman was missing data on the number of previous live births.
[3,6] Significantly different from the iron supplement group (t test): [3] P = 0.049, [6] P = 0.0168.
[4] n = 134 in the iron supplement group, and n = 117 in the placebo group.
[5] Antilog of the mean; 25th and 75th antilog percentiles in parentheses.
[7] n = 108 in the iron supplement group, and n = 100 in the placebo group.

concentrations were skewed, we log-transformed ferritin concentrations before comparing the means with a t test. The results were then retransformed into antilogarithms to recover the original units and were expressed as geometric means and percentiles in the tables. We used chi-square tests and Fisher exact tests, where appropriate, to compare differences in categorical outcomes (12). To adjust for factors that differed by randomization group, we calculated mean differences in continuous outcomes by using multiple linear regression, and we adjusted odds ratios for dichotomous outcomes by using multiple logistic regression. We used SAS (version 8; SAS Institute Inc, Cary, NC) for all analyses (13).

To determine whether missing data biased our results, we compared our complete case analysis (n = 213) with that obtained by using a 3-step multiple imputation process (n = 275) (14, 15). We created 40 imputed datasets, 10 for each of 4 theoretical scenarios: *1*) the women who were missing birth-weight data took the prescribed supplement or placebo, *2*) regardless of initial treatment group assignment, the women who were missing birth-weight data did not take iron supplements, *3*) regardless of initial treatment group assignment, the women who were missing birth-weight data took iron supplements, and *4*) the women who were missing birth-weight data switched treatments (ie, the women assigned to receive iron supplements did not take them, and the women assigned to receive placebos took iron supplements instead). With multiple imputation, data are assumed to be missing at random (ie, the lack of data depends on the observed variables only, including supplemental iron on the basis of 1 of the 4 theoretical scenarios), and we felt that our data did not violate this assumption. Variables used to impute missing data included the following: theoretical supplementation after assignment (on the basis of 1 of the 4 scenarios presented above), gestational age at study entry, natural log of initial ferritin concentration, initial hemoglobin concentration, maternal age at last menstrual period, ethnic group, parity, smoking status at entry into WIC, prepregnancy weight (in kg), and adherence to the supplementation regimen. We used the PROC MI and PROC MIANALYZE procedures in the SAS software with a Markov chain Monte Carlo Approach to account for the complicated nature of the missing data (13–15).

RESULTS

Among the women who were enrolled in the study, those who were randomly assigned to the placebo group had, on average, significantly higher prepregnancy weight and iron stores than did those who were randomly assigned to the supplement group (**Table 3**). There were no other significant differences between the groups in demographic characteristics, behavioral characteristics, or initial iron status (Table 3).

Some of the women were missing data on the primary study outcomes because they did not return to the clinic (n = 49), developed other medical conditions (eg, miscarriage) that prevented them from participating (n = 11), or declined participation after random assignment (n = 35). Seventy-nine women (28.7%) were missing data on third-trimester iron status, 62 (22.5%) were missing data on infant birth weight, and 43 (15.6%) were missing data on both (Figure 1 and Table 2). Compared with the women with complete data, the women without data on either of these 2 outcomes entered the study, on average, 1.5 wk earlier in pregnancy (P = 0.001) and were more likely to be black and non-Hispanic (36.2% compared with 19.4%; P = 0.0025). Initial iron status and other maternal characteristics did not differ significantly between these groups. The proportion of women with missing data and the reasons for missing data did not differ significantly between the 2 treatment groups (P = 0.198, chi-square test).

At 28 wk of gestation, 196 women returned for iron measures (Table 2). For most of these women, the dose of supplemental iron was increased at 28 wk of gestation. Among 110 women who were initially assigned to receive 30 mg Fe/d and who returned for iron measures at 28 wk of gestation, 62 (56%) were without iron stores and were prescribed 60 mg Fe/d, 25 (23%) had depleted iron stores and continued to receive 30 mg Fe/d, 18 (16%) were iron replete and continued to receive 30 mg Fe/d (Table 2), and 5 (5%) were referred for medical evaluation. Among 86 women who were initially assigned to receive placebo and who returned for iron measures at 28 wk of gestation, 56 (65%) were without iron stores and were prescribed 60 mg Fe/d, 11 (13%) were iron depleted and were prescribed 30 mg Fe/d, 15 (17%) were iron replete and continued to receive placebo, and 5 (6%) were referred for medical evaluation. None of the women had a hemoglobin concentration < 90 g/L at 28 wk of gestation. At 38 wk of gestation, 144 women returned for iron measures. Only one woman treated with placebo throughout her pregnancy remained nonanemic and iron replete (data not shown).

Seventy-three women were missing adherence data. Compared with the women with adherence data, those without adherence data entered the study, on average, 1.2 wk earlier in pregnancy (P = 0.019). Otherwise, maternal characteristics did not differ significantly between these 2 groups. Adherence to the supplementation regimen between the initial visit and 28 wk of gestation did not differ significantly between the 2 treatment

TABLE 4
Adherence to supplementation regimen and reported side effects between enrollment and 28 wk of gestation

	Iron supplement group (n = 112)	Placebo group (n = 90)
Total capsules consumed (no.)	67 ± 42[1]	71 ± 39
Adherence (%)	63.4 ± 20.6	65.2 ± 21.7
Women taking ≥50% of capsules (%)	78.6 ± 41.2	80.0 ± 40.2
Iron dose (g)	2.0 ± 1.3	0[2]
Side effects reported at ≥1 visit (%)[3]	24.6 ± 43.2	18.5 ± 39.0

[1] $\bar{x}$ ± SD.
[2] Significantly different from the iron supplement group, $P < 0.0001$ (t test).
[3] n = 126 women randomly assigned to receive supplements from enrollment to 28 wk of gestation, and n = 108 women randomly assigned to receive placebo from enrollment to 28 wk of gestation.

groups (**Table 4**). The women who were randomly assigned to receive iron took a mean of 2 g Fe between the initial study visit and 28 wk of gestation. By delivery, the women who were initially assigned to receive iron had taken a mean (± SD) of 2.9 ± 1.8 g Fe, and the women who were initially assigned to receive placebo had taken a mean of 0.8 ± 1.1 g Fe ($P < 0.0001$).

At 28 wk of gestation, neither the proportions of women with anemia, low iron stores, or iron deficiency anemia nor mean iron-status measures differed significantly between the iron and placebo groups (**Table 5**). The associations of iron supplementation with absent iron stores and iron deficiency anemia were stronger after adjustment for prepregnancy weight and log of initial ferritin concentration: the prevalence of absent iron stores and of iron deficiency anemia was 14.3 percentage points ($P = 0.031$) and 10 percentage points ($P = 0.062$) lower, respectively, among the women assigned to receive iron supplements than among those assigned to receive placebo.

Compared with the birth-weight distribution among the women assigned to receive placebo from enrollment to 28 wk of gestation, the birth weight distribution among those assigned to receive iron supplements was shifted to the right (**Figure 2**). The distribution of gestational age at birth was also shifted to the right among the women assigned to receive iron from enrollment to 28 wk of gestation, with the curves for the 2 treatment groups meeting at 36 wk of gestation (**Figure 3**). Ten infants born to women in the iron supplement group were delivered at 36 wk of gestation, whereas only 2 infants were born to women in the placebo group at 36 wk of gestation. Compared with the infants born to the women in the placebo group, the infants born to the women in the iron supplement group were significantly heavier (by a mean of 206 g) and significantly less likely to have a low birth weight (**Table 6**). Most of the difference between the 2 treatment groups in the incidence of low birth weight was accounted for by the difference in the incidence of small preterm infants. When the incidence of small preterm infants was estimated by analysis of residual birth-weight distributions (data not shown), small preterm infants were born to 1.6% of the women in the iron supplement group and to 12.7% of the women in the placebo group ($P = 0.004$). Although the difference between the 2 treatment groups in the proportion of small-for-gestational age infants was significant, the difference in the proportion of term infants with low birth weight was not. Among the women who gave birth at term (≥ 37 wk of gestation), the mean (± SD) birth weight of the infants born to the women who received iron supplements from enrollment to 28 wk of gestation was 118 ± 460 g higher than that of the infants born to the women who received placebo ($P = 0.0836$). These data also suggest that most of the difference between the 2 treatment groups in the proportion of small-for-gestational age infants occurred preterm. The overall difference in preterm births between the 2 groups was not significant, primarily because most of the births at 36 wk of gestation occurred in the iron supplement group (Figure 3). Adjustment for self-reported prepregnancy weight and the log of initial ferritin concentration did not significantly change the associations of iron supplementation with infant birth outcomes. After adjustment for the log of initial ferritin concentration and prepregnancy weight, the odds ratio for low birth weight among the infants born to the women in the iron supplement group was 0.24 (95% CI: 0.08, 0.68) relative to that of the infants born to the women in the placebo group. The corresponding odds ratios

TABLE 5
Third-trimester iron status among initially iron-replete, nonanemic pregnant women by treatment category[1]

Characteristic	Iron supplement group (n = 110)	Placebo group (n = 86)	Difference	P[2]
Hemoglobin (g/L)	117 ± 9[3]	116 ± 10	1 ± 9	0.499
Ferritin (μg/L)	7.4 (3.7, 20.5)[4]	7.4 (4.5, 17.1)	0	0.985
Mean cell volume (fL)	90.8 ± 4.6	90.3 ± 4.3	0.5 ± 5.2	0.443
Erythrocyte protoporphyrin (μg/dL)[5]	59.3 ± 17.0	62.9 ± 16.0	−3.6 ± 16.6	0.140
Anemia (%)[6]	19.8	26.7	−6.9	0.251
Absent iron stores(%)[7]	56.4	65.1	−8.7	0.214
Iron deficiency anemia (%)[8]	12.7	20.9	−8.2	0.123

[1] At the initial study visit (<20 wk of gestation), women were considered nonanemic if they had a hemoglobin concentration ≥110 g/L and were considered iron replete if they had a ferritin concentration >20 μg/L.
[2] t test for mean (±SD) values, and chi-square test for percentage values.
[3] $\bar{x}$ ± SD.
[4] Antilog of the mean; 25th and 75th antilog percentiles in parentheses.
[5] n = 107 in the iron supplement group, and n = 84 in the placebo group.
[6] Defined as a hemoglobin concentration <110 g/L.
[7] Defined as a serum ferritin concentration <12 μg/L.
[8] Defined as a hemoglobin concentration <110 g/L and a serum ferritin concentration <12 μg/L.

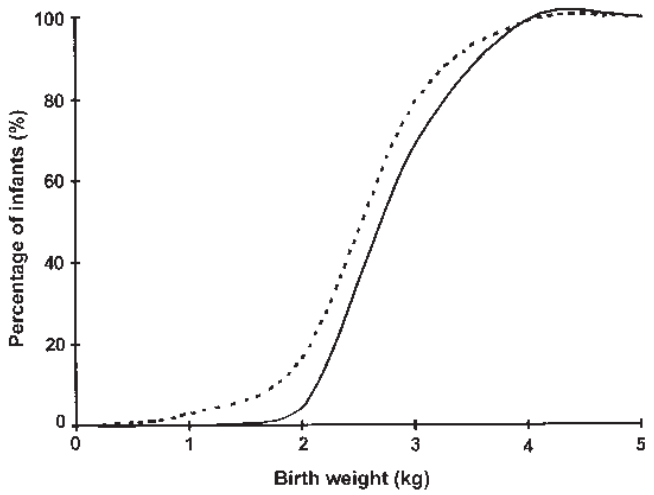

FIGURE 2. Cumulative distribution of infant birth weight among women randomly assigned to receive either iron capsules (—, $n = 110$) or placebo (- - -, $n = 96$) from the initial visit to 28 wk of gestation.

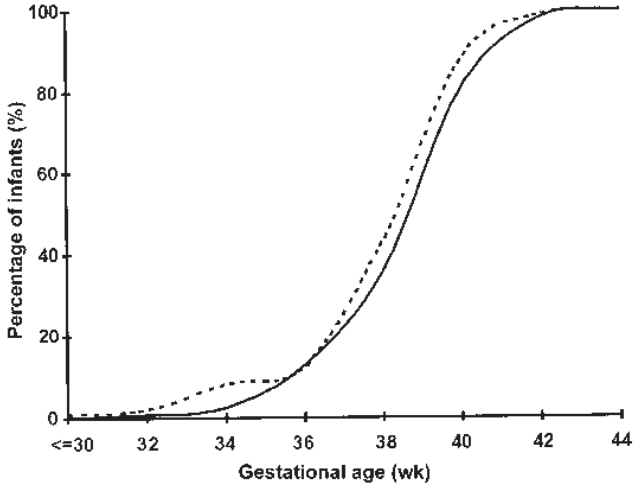

FIGURE 3. Cumulative distribution of infant gestational age at birth among women randomly assigned to receive either iron capsules (—, $n = 110$) or placebo (- - -, $n = 96$) from the initial visit to 28 wk of gestation.

adjusted for log of initial ferritin concentration and prepregnancy weight from the multiple imputation analyses of the 275 women and infants were 0.25–0.34.

DISCUSSION

Compared with placebo, daily 30-mg Fe supplements given from enrollment to 28 wk of gestation to initially iron-replete, nonanemic pregnant women did not lead to a significantly lower prevalence of anemia during the third trimester. The results of our study provide evidence, however, that, compared with placebo, daily iron supplements given from enrollment to 28 wk of gestation to initially iron-replete, nonanemic pregnant women lead to a significantly lower incidence of infants with low birth weight and to significantly higher mean birth weight, principally because of a lower proportion of small preterm infants. Although the lower incidence of infants with low birth weight is significant, the design of our study may underestimate the effect of iron. Importantly, *1*) a low dose of iron (30 mg, once daily) was given as the supplement, and *2*) for most of the women, placebo treatment was limited to the period between enrollment (mean of 11 wk of gestation) and 28 wk of gestation. At 28 wk, evidence of depleted or absent iron stores was found in 78% of the women who were originally assigned to receive placebo; these women were then prescribed supplemental iron for the remainder of their pregnancies.

A recent comprehensive review concluded that virtually all published intervention studies had major flaws that preclude definitive conclusions about the effect of iron supplementation on birth outcomes (16). In our study, the randomization, the use of the placebo control until 28 wk of gestation, and double blinding eliminated many factors (eg, internal biases and confounding) other than iron that could have potentially explained the higher observed birth weight in the iron supplement group. Nonetheless, our study also has limitations.

Despite randomization, the women who were assigned to the placebo group had significantly higher prepregnancy weight and initial iron stores than did the women who were assigned to the iron supplement group, and these differences could contribute to an underestimate of the effect of iron supplementation. Although adjustment for these characteristics made no difference with respect to the main outcomes, we found larger reductions among the iron supplement group in both the prevalence of absent iron stores and iron deficiency anemia in the third trimester after adjustment for prepregnancy weight and initial iron stores.

We did not find any differences between the 2 treatment groups in the proportion of women lost to follow-up or in the reasons for the loss to follow-up, but the women who were lost to follow-up entered the study earlier in gestation than did those who were not lost to follow-up and were more likely to be black than were those who were not lost to follow-up. We used these factors as well as others to conduct a sensitivity analysis based on low-birth-weight data multiply imputed for women with missing data by using 4 assumptions for actual iron supplementation (14, 15) ($n = 275$) and found that the reductions in low birth weight in the iron supplement group were substantial regardless of the assumption.

Although self-reported birth-weight data have previously been shown to be accurate (17), the reporting of the last menstrual period has not been evaluated among women enrolled in WIC. It is possible that errors in reporting may have affected our estimates of gestational age and attenuated the differences in the incidence of preterm delivery. We found that most of the births at 36 wk ($n = 12$) occurred among the women who received iron supplements ($n = 10$), and all of the infants resulting from these births weighed ≥ 2500 g. When we defined small preterm infants by using residual birth weight (8), we found larger reductions in the incidence of small preterm infants in the iron supplement group than when we defined small preterm infants as infants who were born at < 37 wk of gestation and who had a birth weight < 2500 g. Because the analysis of residual birth weight does not include gestational age, it may be more accurate.

In our study, the rates of low birth weight (9.9%) and preterm delivery (12.7%) were higher than those in the US population during the study years of 1995–1998 (ie, 7.3–7.6% and 11.0–11.6%, respectively) (18). Although the women were enrolled in our study before 20 wk of gestation, MetroHealth Medical Center acts as the referral center for women with high-risk pregnancies, and a substantial proportion of the women in our population were

TABLE 6
Birth outcomes for initially iron-replete, nonanemic pregnant women by treatment category[1]

Characteristic	Iron supplement group (n = 117)	Placebo group (n = 96)	Difference	P[2]
Birth weight (g)	3277 ± 501[3]	3072 ± 635	206 ± 565	0.010
Gestational age at delivery (wk)	38.9 ± 1.9	38.3 ± 2.5	0.6 ± 2.2	0.049
Low birth weight (%)[4]	4.3	16.7	−12.4	0.003
Preterm delivery (%)[5]	12.8	12.5	0.3	0.944
Preterm delivery with low birth weight (%)	2.6	10.4	−7.8	0.017
Term delivery with low birth weight (%)	1.7	6.3	−4.5	0.083
Small-for-gestational age (%)[6]	6.8	17.7	−10.9	0.014
Weight gain during pregnancy (kg)	15.3 ± 7.7	13.3 ± 11.3	2.0 ± 9.5	0.144
Birth length (cm)[7]	49.7 ± 2.9	49.3 ± 3.7	0.4 ± 3.3	0.464

[1] At the initial study visit (<20 wk of gestation), women were considered nonanemic if they had a hemoglobin concentration ≥110 g/L and were considered iron replete if they had a ferritin concentration >20 μg/L.
[2] t test for mean (±SD) values, and chi-square test for percentage values.
[3] $\bar{x}$ ± SD.
[4] Defined as a birth weight <2500 g.
[5] Defined as a date of delivery <37 wk from the date of the last menstrual period.
[6] Defined as <10th percentile of weight at birth for gestational age (7).
[7] n = 106 in the iron supplement group, and n = 90 in the placebo group.

smokers (36%), thus increasing the risk of adverse outcomes. We did not have data on pregnancy complications (eg, hypertension) in the study population. Even with randomization, it is possible that the rates of pregnancy complications were higher in the placebo group than in the iron supplement group and that these differences accounted for the difference in the proportion of small preterm infants. However, the rates of smoking and gestational weight gain were not significantly different between the 2 treatment groups, suggesting that these 2 factors at least did not account for the difference in the proportion of small preterm infants.

Little is known about the pathophysiologic means whereby iron supplementation may affect the regulation of gestation and fetal growth. Iron deficiency and iron deficiency anemia may lead to changes in factors such as norepinepherine concentration (19, 20), cortisol and corticotropin-releasing hormone concentrations (21), and indexes of oxidative stress that may adversely affect gestation, fetal growth, or both (21). In the future, researchers should consider measuring these factors.

In our study, iron supplementation had a strong effect on birth weight, but the effect on anemia during the third trimester was not significant. Most of the cases of anemia appeared to be due to iron deficiency in the iron supplement group (12.7/19.8 × 100 = 64%) and in the placebo group (20.9/26.7 × 100 = 78%), but the incidence of iron deficiency anemia in the iron supplement group was also not significantly lower than that in the placebo group. Adjustment of the results for initial ferritin concentration and prepregnancy weight suggested that, compared with placebo, iron supplementation from enrollment to 28 wk of gestation led to a significantly lower proportion of women with low iron stores at 28 wk; however, even with iron supplementation, most of the women developed low iron stores. How can iron supplementation have little to no effect on iron status at 28 wk of gestation but increase infant birth weight?

It is possible that iron supplements may be preferentially transferred to the placenta and fetus, thus contributing to higher birth weight rather than to higher maternal iron stores. However, the mechanisms for the preferential transfer of iron and for higher birth weight as a result of increased iron to the fetus are unknown. Maternal iron deficiency in rats results in compensatory changes in the iron transport mechanisms of the placenta, which in turn minimizes the level of iron deficiency in the fetus (22), but how this applies to humans is uncertain. In the present study, 30 mg Fe in the form of supplements may have been enough to meet fetal requirements but may not have been enough to maintain maternal stores.

Other mechanisms may have little to do with iron deficiency. Iron supplementation may improve the appetite of the mother (23), thus increasing energy consumption and resulting in increased intrauterine growth. Our study did not include measures of energy consumption. Another explanation is that iron supplementation may lead to increased plasma volume expansion, and thus in the placebo group in our study, plasma volume may have failed to expand adequately. Although this seems like a plausible explanation, one study indicated that iron supplements resulted in increased red blood cell volume, but not plasma volume, during pregnancy (24).

The 1993 IOM recommendations to prevent iron deficiency anemia (4) are complex and based on the assumption that measurements of hemoglobin and of ferritin (which is costly to measure) provide reliable means of identifying women who would benefit from iron supplementation during the first and second trimester of pregnancy. In our study, most of the initially iron-replete, nonanemic women had decreased or absent iron stores by 28 wk of gestation. Indeed, only one woman treated with placebo throughout her pregnancy remained nonanemic and without iron depletion. Most importantly, in contrast to the US Preventive Services Task Force (1, 2) and IOM recommendations (4), our study provides evidence that infants may benefit substantially from maternal iron supplementation beginning early in gestation. Iron supplementation of initially nonanemic pregnant women without iron depletion from the first trimester to 28 wk of gestation may have important benefits by preventing small preterm births. Because small preterm births are a major determinant of perinatal morbidity and mortality (8), iron supplementation could reduce health care costs. Furthermore, accumulating evidence suggests that in adult life, infants with low birth weight may be at greater risk of various chronic disorders, including type 2 diabetes, hypertension, and coronary artery disease (25, 26). Iron supplementation during pregnancy deserves further examination as a measure to improve birth outcomes and reduce health care costs.

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GMB, LI, IP, and RY designed the randomized controlled trial. GMB, MEC, and IP designed the birth-weight study. LI enrolled the participants, dispensed the study drugs, performed pill counts, and asked subjects about their side effects. MEC led the data analysis. All investigators contributed to data interpretation and the writing of the manuscript. None of the authors had any conflicts of interest.

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