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BRIEF REPORT

Weekly Iron as a Safe Alternative to Daily Supplementation for Nonanemic Pregnant Women

Esther Casanueva,[a] Fernando E. Viteri,[b] Mónica Mares-Galindo,[a] Carlos Meza-Camacho,[a] Alvar Loría,[c] Lourdes Schnaas,[a] and Roxana Valdés-Ramos[d]

[a]*Instituto Nacional de Perinatología Isidro Espinosa de los Reyes (INPer), México, D.F., Mexico*
[b]*University of California, Berkeley and Children's Hospital, Oakland Research Institute, Oakland, California*
[c]*Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México, D.F., Mexico*
[d]*Universidad Autónoma del Estado de México, Mexico*

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Background. We undertook this study to compare the effectiveness and safety of antenatal daily and weekly supplementation with iron, folic acid, and vitamin B_{12} in healthy, pregnant women who were not anemic at gestational week 20.

Methods. Women with singleton pregnancies and blood hemoglobin (Hb) >115 g/L at gestational week 20 (equivalent to 105 g/L at sea level) were randomly assigned to two groups, one consuming one tablet containing 60 mg iron, 200 μg folic acid and 1 μg vitamin B_{12} daily (DS, $n = 56$); the other consuming two tablets once weekly (WS, $n = 60$). Blood Hb and serum ferritin concentrations were measured every 4 weeks from weeks 20 to 36, and pregnancy outcomes were evaluated.

Results. Mild anemia and hypoferritinemia throughout pregnancy occurred less frequently in DS than WS. None of the 116 women had Hb concentrations <103 g/L at any evaluation point. In contrast, hemoconcentration (Hb >145 g/L) from gestational week 28 onwards occurred in 11% in DS and 2% in WS. We observed *ex post facto* that hemoconcentration at gestational week 28 was associated with a significantly higher relative risk of low birth weight (RR 6.23, 95% CI 1.46–26.57) and premature delivery (RR 7.78, 95% CI 1.45–24.74).

Conclusions. In women who were nonanemic at gestational week 20, both schemes (DS and WS) prevented the occurrence of Hb levels <100 g/L. DS women had a higher incidence of hemoconcentration. Hemoconcentration was associated with increased risk of low birth weight and premature delivery.

Key Words: Pregnancy, Anemia, Iron, Folate, Vitamin B_{12}, Daily/weekly supplementation, Mexico, Randomized controlled trial.

Introduction

Iron requirements increase during pregnancy (1,2), and iron deficiency and anemia are common in pregnant women throughout the world (3). Iron-deficiency anemia occurring early in pregnancy is associated with low birth weight and premature delivery, and severe anemia near term imposes a maternal health risk (4,5). In an effort to reduce and prevent these risks, iron supplementation programs are being used in many countries. However, the problem persists because of a variety of factors, the most important being inadequate knowledge of anemia and iron deficiency, poor supply of supplements and poor adherence to supplementation regimens (6–11).

Recently, preventive weekly supplementation, rather than daily supplementation, has been proposed because the turnover of the small intestinal mucosa takes place every 5–6 days in humans (12–14). Intestinal cells subjected to high luminal doses of iron reduce iron absorption possibly by several mechanisms that are the object of active research and that may involve a combination of mechanisms: crypt cell pre-programming (15), rapid response by mature

Address reprint requests to: Esther Casanueva, PhD, Instituto Nacional de Perinatología Isidro Espinosa de los Reyes, Montes Urales 800, México, D.F. CP 11000 Mexico; E-mail: casanuev@servidor.unam.mx

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cells (16), mucosal block (17) or intracellular iron trafficking (18). Given that new cells may be programmed to absorb iron according to the extent of body iron reserves (16), this approach could fine-tune iron absorption, avoid iron-induced oxidative damage, and avoid daily flooding of the intestinal lumen and mucosa with iron and the resulting negative consequences (19–21).

Several epidemiological studies carried out among different risk groups have shown that weekly and daily supplementation schemes can be similarly efficacious in preventing anemia and iron deficiency as well as improving adherence, reducing side effects and correcting mild-to-moderate iron-deficiency anemia (19,22–24). To our knowledge, no longitudinal comparison of daily and weekly prenatal iron supplementation in women who are nonanemic at 20 weeks of pregnancy has been conducted.

The objective of this study was to compare the effectiveness* and safety† of antenatal daily and weekly supplementation with iron, folic acid, and vitamin B_{12} in healthy pregnant women who were not anemic at gestational week 20.

Subjects and Methods

The study was performed at the Instituto Nacional de Perinatología Isidro Espinosa de los Reyes (INPerIER) in Mexico City, located at an altitude of 7350 feet above sea level (2240 m). INPerIER is a teaching and research center that includes a hospital specializing in the care of women of childbearing age. Its patient population consists mainly of women from the middle and low–middle socioeconomic classes. Women admitted to the study were recruited during their first visit for prenatal care. Inclusion requirements were as follows: first hospital consultation at gestational week ≤20, absence of disease or addictions (including tobacco), no nutritional supplements taken before gestational week 20, single-fetus pregnancy, and blood Hb >115 g/L (equivalent to 105 g/L at sea level) at gestational week 20.

Subjects signed an informed consent form and the study was approved by the Committees for the Protection of Human Subjects at INPer and at the University of California at Berkeley, CA, according to the to the principles of the Declaration of Helsinki. If the pregnant woman was <18 years of age, consent of one parent or legal guardian was also required.

The women were seen every 4 weeks from week 20 to week 36 of pregnancy except for those who had premature delivery (two at week 34 and five at week 35), in which instances examination and blood sampling at labor were designated as 36-week data.

Supplementation Groups

One hundred and twenty pregnant women who fulfilled the inclusion criteria were recruited and were randomly assigned to one of two groups by drawing lots without replacement; there was a 50% probability of being placed in either group. The groups received either daily supplementation (DS) or weekly supplementation (WS) at no cost. Supplement tablets were identical in content and were to be ingested from the 20th week of pregnancy until delivery. The tablets contained 60 mg of elemental iron (ferrous sulfate), 200 μg of folic acid and 1 μg of vitamin B_{12}.

The 60 women in the DS group were supplied monthly with 30–31 tablets and were instructed to ingest one tablet daily. The women in the WS group were supplied monthly with 8–10 tablets and were instructed to consume two tablets once each week. If the scheduled day was missed, they were instructed to take the tablets the next day and not to wait until the following week before ingesting them. Both groups were told that the tablets were to be ingested with water at least 1 h after a meal and preferably before going to bed at night to decrease gastrointestinal side effects.

Adherence to Supplementation

Adherence to the regimen was evaluated using information from supplementation diaries completed by the participants. Adherence was calculated as the lowest percentage of tablets ingested (according to either the diary or the residual tablet counts) divided by the total number of tablets that should have been ingested. The correlation coefficient between these two estimates of adherence (i.e., diary vs. tablet count) was 0.81.

Side Effects

A list of symptoms related to iron intake was included in the diaries and were required to be flagged by participants. A score for each side effect was calculated as the total number of days women had experienced the side effect divided by the total number of days from week 20 to week 36 divided by the number of women in the supplementation group. For the WS group, a second score was calculated using only the side effects reported during 24 h following the ingestion of the two tablets.

Dietetic Iron Consumption

Each subject completed a food frequency questionnaire previously validated in Mexican women and considered the habitual intake of the previous year (25).

The study was not totally blinded because the nutritionist had to know the identity of the treatment group to dispense the tablets and to evaluate adherence to supplementation.

*Effectiveness: The ability of the supplementation scheme to prevent gestational anemia and hemoglobin levels associated with perinatal risk during pregnancy.

†Safety: The ability of the supplementation scheme to avoid high hemoglobin concentrations and other undesirable side effects.

However, other personnel, attending physicians, and researchers were blinded. A placebo control group was not included because Mexican law demands that all pregnant women receive iron and folate supplementation (26). Current practice in Mexico is to start supplementation at or after gestational week 20.

Laboratory Methods

At every maternal visit, 10 mL of venous blood was drawn into two metal-free BD Vacutainer (BD, Franklin Lakes, NJ) tubes. One contained EDTA and the other contained no anticoagulant so that serum could be obtained. A complete blood count was done using an automatic cell counter (Coulter T890, version 3D, $<2\%$ coefficient of variation, Beckman, Gouda, The Netherlands), and serum was used to measure ferritin concentrations (ELISA kit, $<5\%$ coefficient of variation, Opus, Dade Behringer, Newark, DE). All serum samples were stored frozen at -70°C. Ferritin measurements for each participant were performed in the same run to avoid interassay error.

Cutoff Levels for Anemia, Hemoconcentration, and Hypo- and Hyperferritinemia in Pregnant Women

For comparison with other studies, we adopted the altitude-adjusted Hb cutoffs for gestational anemia proposed by U.S. Centers for Disease Control (CDC) (Hb of 115, 115, 117, 120, and 124 g/L for the 20th, 24th, 28th, 32nd, and 36th weeks of pregnancy) (27). Gestational hemoconcentration was diagnosed when Hb level was >145 g/L. The 145 g/L Hb cut-off level used to designate a state of hemoconcentration was based on results of studies by Scanlon et al. (28), Murphy et al. (29) and Steer et al. (30). These studies, involving 605,334 women, showed a clearly increased risk for low birth weight and/or premature delivery at Hb values >132 g/L during the first and/or second trimesters, >135.5 g/L during the third trimester, or 135 g/L at any time during pregnancy (mean + two Z-scores at gestational weeks 24–30). The sea level value of 135 g Hb/L was adjusted for the altitude of Mexico City by the addition of 10 g/L (27,31). Cutoffs for hypoferritinemia and hyperferritinemia during pregnancy were serum ferritin concentrations <10 μg/L and >30 μg/L, respectively (32).

Infant Characteristics at Birth

Gestational age at delivery was estimated from the date of the last menstrual period and from the newborn's characteristics, using a standard method (33). At birth, the newborn's weight was measured on an electronic scale sensitive to 0.1 g (Tanita 1582, Mexico City) with an error for repeated measurements of <1%.

Statistical Analysis

Sample size of each group was estimated to be sufficient to distinguish a 10% intersubject difference in Hb concentration according to our previous experience of intrasubject and intersubject variability of Hb concentrations during pregnancy (34). We used the formula for bioequivalence studies (35), considering a 10% difference of anemia probability between groups at gestational week 36. The *post facto* power was estimated to be 98%.

Ferritin concentration values were converted to logarithms to correct for the skewed distribution. Differences between groups were compared using Student's *t*-test. Statistical differences in proportions and in nonparametric variables were analyzed using Fisher's exact test and the differences in distributions were evaluated using the Kolmogorov–Smirnov formula. Relative risk (RR) and 95% confidence intervals were established using standard methods.

Results

Four pregnant women in the DS group did not complete the study for reasons unrelated to supplementation (Figure 1). These women did not differ from the others who completed the study.

Table 1 shows general characteristics at gestational week 20 for the women who completed the study and their gestational weight gain. They were mainly young (17% were <16 years old) and fairly well educated by Mexican standards (mean years of schooling = 11). Gestational weight gain was adequate and similar for both groups. Food habits included small amounts of heme-iron every day and the mean of the iron intake was not different between groups (DS 14.25 ± 4.25; WS 14.37 ± 5.36 mg/day; $p = 0.90$).

Table 2 shows the concentrations of Hb from weeks 20 to 36 and the presence of hemoconcentration and of anemia according to the CDC cutoff values adjusted for altitude (27). Hb concentration did not differ significantly between the DS and WS groups at weeks 20 and 24, but it was significantly higher in the DS group from gestational weeks 28–36. These higher Hb levels of the DS group resulted in a significantly lower frequency of anemia in weeks 32 and 36 and in a significantly higher prevalence of hemoconcentration in weeks 28–36 (Figure 2). None of the women studied had an Hb concentration of <103 g/L (93 g/L at sea level) during pregnancy (Figure 2). No undesirable pregnancy outcomes were associated with low Hb concentrations in either group.

Table 3 presents the geometric means and ranges of serum ferritin concentrations and the prevalence of hypoferritinemia and hyperferritinemia. Ferritin concentration decreased significantly as pregnancy progressed in both groups. The WS group exhibited a greater decrease in ferritin levels than the DS group and between-group

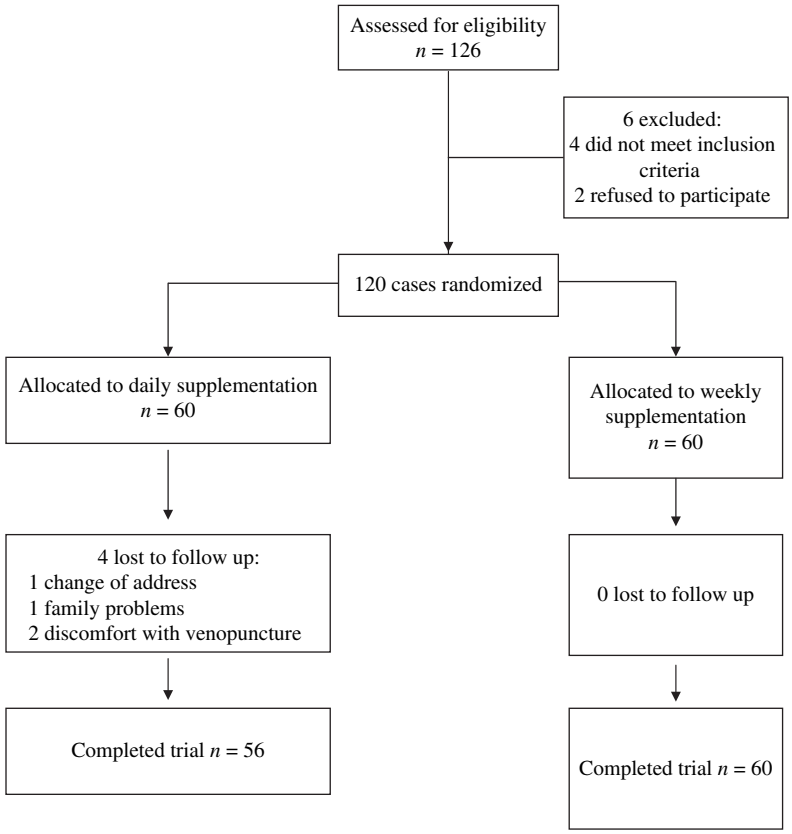

Figure 1. Flow chart describing the progress of the sample of pregnant women during the trial.

differences were significant for weeks 24–36. The variability in ferritin levels remained practically unchanged throughout pregnancy in both groups (standard deviation ranged from 2.0 to 2.7 μg/L).

Hypoferritinemia was present in more than a third of all cases at week 20 despite their nonanemic status at that time. At weeks 32 and 36, hypoferritinemia was observed in half of the DS group and in two thirds of the WS group. Hypoferritinemia was more prevalent than anemia at every time point.

Hyperferritinemia was less than 15% in both groups at all time points, with the exception of the WS group at week 20 (24%). This was the only time point at which the WS group had a higher prevalence of hyperferritinemia than the DS group. Ferritin levels in the WS women were lower than in the DS women during the remainder of pregnancy.

Adherence to supplementation did not differ significantly between groups, although it was slightly higher in the WS group, i.e., the 50th percentile of adherence was 93% (WS group) vs. 90% (DS group).

The incidence of days with side effects attributable to iron ingestion shown in Table 4 was significantly higher in the DS group than in the WS group with the exception of the metallic aftertaste, which was marginally significant ($p = 0.057$). However, when data of the WS group were restricted to side effects experienced during the 24 h after the double dose of iron, the incidence of days with side effects was higher for WS than for DS, with the exception of constipation, which was not reported by any of the women in the WS group.

Table 1. Characteristics at recruitment of the women in the two supplementation groups

	Supplementation	
Indicator	Daily $n = 56$	Weekly $n = 60$
Age, years	23.5 ± 6.1 (12–35)	24.3 ± 6.4 (14–35)
Height, cm	155.6 ± 6.3 (141–168)	155.9 ± 5.6 (146–172)
Education, years	11.0 ± 3.1 (5–16)	10.8 ± 2.7 (6–18)
Gestations	2.0 ± 1.3 (1–6)	2.1 ± 1.3 (1–6)
Pregestational BMI	23.3 ± 3.7 (17–33.3)	22.7 ± 3.3 (18–30.0)
% weight for height at week 20[a]	107 ± 15 (92–144)	106 ± 13 (93–132)
Weight gain (kg) (weeks 20–36)	11.9 ± 5.5 (3–31)	11.6 ± 4.3 (5–23.0)

Values are mean ± SD (range).
[a]Weight for height at gestational week 20 (real weight/normal weight for height at gestational week 20) × 100, according to Arroyo et al. (61).

Table 2. Blood hemoglobin levels and prevalence of anemia and hemoconcentration by supplementation groups

	Hemoglobin g/L Mean ± SD (range)		Anemia %		Hemoconcentration %	
Gestational week	Daily $n = 56$	Weekly $n = 60$	Daily $n = 56$	Weekly $n = 60$	Daily $n = 56$	Weekly $n = 60$
20	128.7 ± 8.5 (116–157)	131.2 ± 9.7 (116–158)	0 -	0 -	2 ($n = 1$)	7 ($n = 4$)
24	127.3 ± 9.6 (103–157)	126.6 ± 8.9 (113–154)	5 ($n = 3$)	7 ($n = 4$)	2 ($n = 1$)	3 ($n = 2$)
28	130.5 ± 10.8 (114–167)	125.0 ± 9.1* (104–151)	9 ($n = 5$)	17 ($n = 10$)	11 ($n = 6$)	2* ($n = 1$)
32	134.5 ± 10.3 (114–1639)	124.5 ± 11.4* (103–161)	9 ($n = 5$)	25* ($n = 5$)	13 ($n = 7$)	5* ($n = 3$)
36	135.8 ± 10.0 (109–161)	126.3 ± 10.3* (106–160)	9 ($n = 5$)	43** ($n = 26$)	18 ($n = 10$)	7* ($n = 4$)

Differences evaluated by Student's t-test for hemoglobin and by χ^2 test for anemia and hemoconcentration.
*$p < 0.05$; **$p < 0.01$.

None of the women had any complications requiring medical treatment. The average gestational age at delivery was 39 weeks (SD = 1 week) and the birth weight was 3.10 kg (SD = 0.44 kg). Six percent ($n = 7$) of all babies were of low birth weight (≤2500 g) and 7.8% ($n = 9$) were born prematurely before gestational week 37 (weeks 34–36). The frequency of low birth weight and premature delivery did not differ significantly between groups and was not associated with anemia or with serum ferritin levels at any point during gestation. However, the relative risk for low birth weight was 6.2 times higher among mothers with hemoconcentration at gestational week 28 (7 subjects) than among mothers with a lower Hb concentration at that time point, i.e., the prevalence of low birth weight at week 28 was 0.286 (2/7) in women with Hb >145 g/L vs. 0.046 (5/109) in the remainder (RR 6.23, 95% CI = 1.46–26.57). Similarly, the relative risk for premature delivery was 7.8 times higher among mothers with hemoconcentration at week 28, i.e., 0.429 (3/7) in hemoconcentrated women vs. 0.055 (6/109) in the others (RR 7.78, 95% CI = 2.45–24.74).

Discussion

In the women who were nonanemic at gestational week 20, weekly supplementation was less effective than daily supplementation in preventing Hb levels considered anemic according to the CDC recommended cutoff points corrected for altitude (27). The absence of a placebo control group [Mexican law does not allow such a group (26)] only allows the comparison of effects between DS and WS groups. However, a historical comparison in a study of

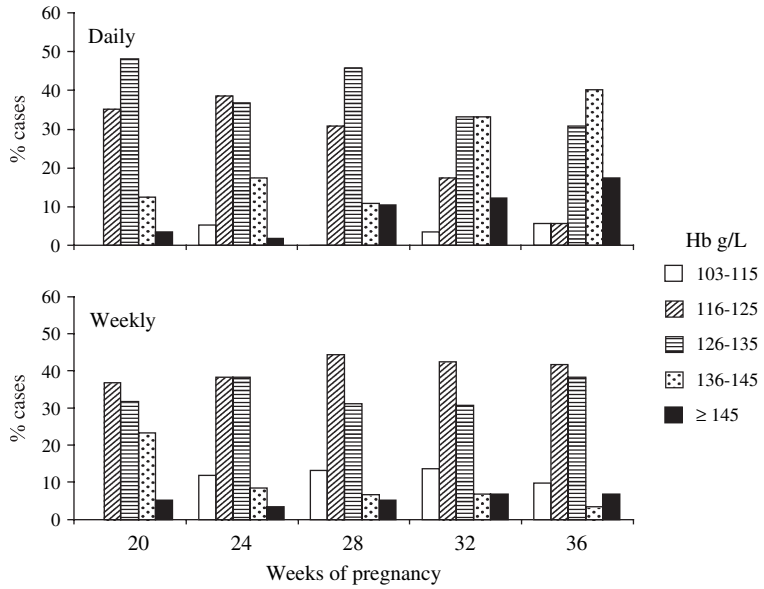

Figure 2. Evolution of Hb according to gestational age and type of supplementation.

Table 3. Geometric mean (±SD) of serum ferritin levels and prevalence of hypoferritinemia and hyperferritinemia in the two supplemented groups

	Ferritin μg/L		Hypoferritinemia		Hyperferritinemia	
Gestational week	Daily $n = 56$	Weekly $n = 60$	Daily $n = 56$	Weekly $n = 60$	Daily $n = 56$	Weekly $n = 60$
20	13.1 ± 2.4 (1–70)	12.8 ± 2.7 (1–106)	36 ($n = 20$)	40 ($n = 24$)	14 ($n = 8$)	23 ($n = 14$)
24	11.8 ± 2.0 (1–51)	8.5 ± 2.4* (1–37)	34 ($n = 19$)	55** ($n = 33$)	7 ($n = 4$)	3 ($n = 2$)
28	9.6 ± 2.5 (1–109)	6.4 ± 2.2** (1–26)	38 ($n = 21$)	63** ($n = 38$)	4 ($n = 2$)	0 -
32	10.1 ± 2.1 (2–81)	6.2 ± 2.3** (1–43)	50 ($n = 28$)	65 ($n = 39$)	5 ($n = 3$)	2 ($n = 1$)
36	11.9 ± 2.1 (2–87)	7.3 ± 2.6* (1–77)	46 ($n = 26$)	68** ($n = 41$)	3 ($n = 7$)	7 ($n = 4$)

Between-group differences evaluated by Student's *t*-test and by Fisher exact test for hypo- and hyperferritinemia.
*$p < 0.05$; **$p < 0.01$.

pregnant women entering prenatal care at INPerIER at gestational weeks 20, 28 and 36 without having received prior iron containing supplements provides some pertinent data, even though they are not a perfect negative control (36). Based on hemoglobin values for the 25th, 50th and 75th percentiles at those gestational weeks, anemia prevalence based on the CDC cutoff points was near 25, 48 and 70%, respectively. Moreover, the range of hemoglobin values was 98–137, 92–142 and 95–144 g/L. Evidently, there were women with hemoglobin concentration <100 g/L, a level that carries maternal and perinatal risk in every group, although there were none in either DS or WS group. These data are suggestive of some benefit derived from DS and WS.

Several studies show that anemia occurring during pregnancy increases the risk of low birth weight and/or premature delivery (4,28,37,38). The sea level Hb concentrations at which these negative effects begin to occur during pregnancy vary among studies from 90 g/L (28,30,39) to 100 g/L (28,30,37).

Importantly, in our study none of the women considered anemic had Hb levels <103 g/L from gestational week 20 onwards, and none of the women considered anemic had gestational and/or perinatal complications such as premature delivery or low birth weight. Lack of perinatal complications could be because anemia was mild: >85% of anemic cases in the WS group and 50% in the DS anemic group had Hb concentrations >115 g/L (Figure 2).

Further analysis of our results revealed that the incidence of Hb levels >145 g/L (equivalent to 135 g/L at sea level) was significantly higher from gestational week 28 onwards among the DS group than among the WS group in spite of the relatively low number of cases. Furthermore, we found *ex post facto* that hemoconcentration was associated with both premature delivery and low birth weight. This finding is consistent with those of other epidemiological and clinical studies (28–37) in showing that women at the higher segments of the Hb distribution have a higher risk of low birth weight and premature delivery. This suggests there may be a causal relationship between daily supplementation of 60

Table 4. Frequency of side effects in the two supplemented groups

	% of all days				% at day after iron intake[c]		
Side effect	DS[a] $n = 56$	WS[b] $n = 60$	Ratio WS/DS	p*	WS[c] $n = 60$	Ratio[d] WS/DS	p*
Nausea	10.1 (0–28)[e]	3.3 (1–5)	0.3	0.003	25.0 (7–50)	2.5	0.007
Heartburn	8.5 (0–31)	3.3 (1–7)	0.4	0.031	31.3 (5–61)	3.7	0.007
Metallic aftertaste	5.5 (0–28)	1.7 (0.7)	0.3	0.057	16.7 (0–63)	3.1	0.053
Constipation	3.7 (0–21)	0 (0–1)	0.0	0.001	0.0 (0–11)	0.0	0.062

[a]Percentage of all days = % of days with side effects/all days from weeks 20 to 36 for DS group (daily supplementation).
[b]Percentage of all days = % of days with side effects/all days from weeks 20 to 36 for WS group (weekly supplementation).
[c]Percentage at day after supplement ingestion = % of days with side effects at day after ingestion/all days after weekly iron intake (weekly supplementation).
[d]Median of [c]/median of[a].
[e]Median (range).
*p value with Kolmogorov-Smirnov test for two samples.

mg iron and hemoconcentration by the end of the second trimester, possibly due to excessive iron intake beyond that needed to prevent anemia. The WS group took the supplement intermittently, and the total amount of iron ingested was 28% of that ingested by the DS group. The intake of iron by the DS group thus had a significant association with an increase in perinatal risk of premature delivery and low birth weight. Pritchard and Hunt (40) showed that the daily ingestion of 104 mg of iron as ferrous gluconate for an average of 11 weeks (a total of 8 g of iron), as well as the IM administration of 1000 mg of iron ''late in the second trimester of pregnancy'' resulted in Hb concentrations >130 g/L in about 40% of women. In the placebo group, only 8% had Hb levels >130 g/L. These figures agree with those obtained among normal Northern European pregnant women supplemented with between 100 and 200 mg of iron daily, and from which data the CDC derived the norms and cutoff points for anemia in pregnancy: the prevalence of Hb >135 g/L was about 39% in the iron-treated group and about 8.5% in the nonsupplemented groups (41–44). In our study, the incidence of Hb >145 g/L in the DS group was 18%. This prevalence is close to half that observed in all the previous studies, in which daily iron doses were significantly greater. Importantly, in the WS group the incidence of Hb >145 g/L was only 7%.

The apparent association between the amount of ingested supplemental iron and the development of undesirably high Hb concentrations during gestation has received little attention. The Newcastle research group and others (45–48) have considered possible iron-induced hemoconcentration among women receiving daily iron supplements. A meta-analysis using the Cochrane method of iron supplementation during pregnancy indicates that the risk of hemoconcentration increases if daily iron intake is >60 mg/day in comparison to ≤60 mg/day, and that weekly supplementation with 120 mg of iron does not appear to carry such risk (49).

This raises the question of how much supplemental iron is safe. This question has been posed from different angles, including possible damage due to oxidative stress (50–53) and hemoconcentration (45–46). Milman et al. indicated that doses of iron >40 mg/day did not confer further benefits. Their results show that, as iron doses increase, the number of women with Hb >135 g/L and with high ferritin levels at term increases significantly. This is significantly associated with lower birth weight and weight-for-length ratio (53).

We strongly hypothesize that excess iron at gestational week 28 can lead to hemoconcentration due to a specific pharmacological effect that forcibly elevates hemoglobin production. Normal pregnancies involve rapid increments in the production of erythropoietin and Hb before the last trimester of gestation and decline in hepcidin production by mid-pregnancy (54). It is important to note that hepcidin is a cysteine-rich peptide exhibiting antimicrobial activity. It has been isolated from human blood and urine and is synthesized predominantly in the liver as a precursor. It is secreted as a 25-amino acid peptide form and is evolutionarily conserved. Its name comes from hepatic bactericidal protein. Low levels are found in the intestine, stomach, colon, lungs, and heart. It belongs to a class of antimicrobial peptides, some of which are involved in inflammation, hepcidin being a type II acute phase protein. There is one gene in humans whose functional loss results in raised serum iron, decreased reticuloendothelial iron stores, and increased intestinal iron absorption. Anemia, hypoxia and iron deficiency result in reduced hepcidin expression and iron overload increases its expression resulting in lowered serum iron, increased reticuloendothelial iron stores, and decreased intestinal iron absorption. Hepcidin is a potent internal signal in the conservation of iron homeostasis (55–57).

Several retrospective studies show that higher rates of premature delivery and low birth weight occur when Hb levels during pregnancy are 130 g/L at sea level (140 g/L at Mexico City's altitude) (28–30). A prospective study in China disclosed an association between high Hb values between the fifth and eighth gestational month and premature delivery (37). Our prospective study showed, *ex post facto*, a significantly higher risk of low birth weight and premature delivery when maternal hemoconcentration (>Hb 145 g/L) occurred at gestational week 28. Importantly, in spite of the relatively small sample size of our study compared with large epidemiological studies, it was large enough to yield significant differences in relative risks of these undesirable perinatal events.

The frequency of hypoferritinemia during gestation was consistently higher than that of anemia. However, the value of serum ferritin as an indicator of iron depletion in pregnancy is dubious because it decreases with advancing gestation despite iron supplementation and can be elevated by common inflammatory conditions (e.g., vaginosis and chorioamnionitis). The timing of blood sampling after ingestion of iron is also critical because ferritin concentrations increase for a few days after ingestion of iron doses typical of supplements or therapy. Hyperferritinemia has been associated with infection and with increased risk of premature delivery and low birth weight (58). We observed some instances of hyperferritinemia during pregnancy in this study, but they were not associated with any undesirable effects. Hyperferritinemia has also been reported to occur after apparently excessive iron supplementation (59–60).

In this study, adherence to the supplementation programs was remarkably high. We attribute this to the provision of appropriate information initially and to monthly reinforcement during routine antenatal visits. It was made clear to the women that they should not wait for a week to take the next supplement if they neglected to take the supplement on the selected day, and that they should take it as soon as they became aware of the omission.

Our main conclusions are as follows: 1) Weekly supplementation for women who were nonanemic at mid-gestation

was not as effective as daily supplementation in preventing mild anemia in late pregnancy. However, both supplementation schemes appeared to be equally effective in preventing Hb concentration below the cutoff point associated with perinatal risk. 2) Daily supplementation was associated with an 18% incidence of hemoconcentration, which began at gestational week 28 and persisted during the third trimester of pregnancy. Weekly supplementation was associated with a 7% incidence of hemoconcentration. 3) An *ex post facto* observation was that hemoconcentration at week 28 was associated with increased risk of premature delivery and low birth weight.

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