

Changes in iron status during pregnancy in Peruvian women receiving prenatal iron and folic acid supplements with or without zinc¹⁻⁴

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ABSTRACT

Background: Iron deficiency anemia is the most prevalent nutrient deficiency during pregnancy, yet there are few data on the effect of prenatal iron supplementation in women in developing countries.

Objective: Our objective was to describe the effect of iron supplementation on hematologic changes during pregnancy, and the effect on those changes of adding zinc to the supplements.

Design: Pregnant women were enrolled in a randomized, double-masked study conducted at a hospital in a shantytown in Lima, Peru. Women were supplemented daily from 10–24 wk gestation to 4 wk postpartum with 60 mg Fe and 250 µg folic acid with or without 15 mg Zn. Hemoglobin and ferritin concentrations were measured in 645 and 613 women, respectively, at enrollment, at 28–30 and 37–38 wk gestation, and in the cord blood of 545 neonates.

Results: No differences in iron status were detected by supplement type, but hematologic changes were related to initial hemoglobin status. Women with anemia (hemoglobin <110 g/L) showed steady increases in hemoglobin concentration throughout pregnancy whereas women with relatively higher initial hemoglobin concentrations had declining values during mid pregnancy, then rising values by 37–38 wk gestation. Women with an initial hemoglobin concentration >95 g/L showed increases in serum ferritin by the end of the pregnancy. Despite supplementation, women with poorer hematologic status; who were younger, single, and multiparous; and who consumed fewer supplements were more likely to have anemia at the end of pregnancy.

Conclusions: These hematologic changes are congruent with the effects of iron supplementation reported in placebo-controlled trials and the addition of zinc did not significantly affect them. *Am J Clin Nutr* 2000;71:956–61.

KEY WORDS Maternal iron supplementation, pregnancy, anemia, zinc supplementation, Peru, hemoglobin, ferritin, developing countries

INTRODUCTION

Iron deficiency anemia is recognized as the most prevalent nutritional problem in the world (1). Pregnant women are at particularly high risk of iron deficiency anemia, and in developing countries prevalences range from 35% to 75% (2). In

Peru, 35% of women of childbearing age and 50% of pregnant women have anemia (3, 4).

Iron needs during pregnancy are very high; it is estimated that during pregnancy ≈850 mg absorbed Fe is needed to meet the requirements for the fetus, placenta, and maternal red cell expansion (5). Such demands are 3–4 times the iron requirements of nonpregnant women and cannot be met by diet alone, particularly in developing countries where the diets are usually low in bioavailable iron. Because iron deficiency and anemia have been associated with poor pregnancy outcomes (6, 7), iron supplements are prescribed daily for pregnant women in many countries during the second and third trimesters of pregnancy (8).

Despite widespread policies advocating iron supplementation during pregnancy, few effective prenatal iron supplementation programs have been identified (9). Reasons given for the low effectiveness of these programs include 1) low compliance because of inadequate patient motivation, 2) low motivation of health personnel, 3) poor access to health services, 4) adverse effects, and 5) inadequate supplies of supplement tablets.

In 1995, we began a controlled trial to investigate the effect of prenatal zinc supplementation on maternal and infant health in a maternity hospital of the Peruvian Ministry of Health. In this study, nearly 1300 women were randomly assigned at entry to receive daily prenatal iron and folic acid supplements with or without zinc. With this approach, we investigated the benefits of prenatal supplementation administered through a regular Ministry of Health program. In this article, we describe the effect of the prenatal iron supplementation program on the iron status of participating mothers and their infants. Because of the known

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²Presented in part at Experimental Biology '97 in New Orleans, 9 April 1997 [FASEB J 1997;11:A443 (abstr)].

³Supported by DAN-5116-A-00-8051-00 and HRN-A-00-97-00015-00, cooperative agreements between the US Agency for International Development/Office of Human Nutrition and The Johns Hopkins University.

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Received February 26, 1999.

Accepted for publication September 22, 1999.

competition between iron and zinc (10), we also examined whether adding zinc to these supplements affected iron measures in the mothers and newborns.

SUBJECTS AND METHODS

The study was carried out between 1995 and 1997 in Villa El Salvador, a periurban shantytown in the southern part of metropolitan Lima, Peru. Pregnant women receiving prenatal care between 10 and 24 wk gestation in the study hospital were invited to participate in a randomized, double-masked study of maternal zinc supplementation. Eligible women had low-risk, singleton pregnancies; were eligible for vaginal delivery; and had been living in Lima or other coastal regions of Peru for ≥ 6 mo before the pregnancy. Signed, informed consent was obtained from each woman at enrollment. The protocol was approved by the Ethical Committee at the Instituto de Investigación Nutricional (IIN) and the Committee for Human Research at The Johns Hopkins School of Hygiene and Public Health.

At enrollment, subjects were randomly assigned within parity (nulliparous or multiparous) and gestational age (< 17 wk of gestation) strata into 2 groups. One group received daily supplements of 60 mg Fe (as ferrous sulfate) and 250 μg folic acid; the other group received the same amounts of iron and folic acid along with 15 mg Zn (as zinc sulfate). All of the supplements had the same brick color and shape and were specially produced in coded blister packs for the study by a local pharmaceutical company (Instituto Quimioterápico, SA, Lima, Peru). Both the health personnel and the investigators were blinded to the coding scheme until analyses of the data were completed.

Women received prenatal supplements from 10–24 wk of pregnancy to 4 wk postpartum. The tablets were given out monthly during prenatal visits with the recommendation to take one tablet every day, between meals, with lemonade, water, or a juice rich in ascorbic acid. The number of pills given to each woman was monitored at the hospital. In addition, fieldworkers visited the women in their homes biweekly to inquire about their health and supplement consumption. The fieldworkers were trained to respond to minor concerns regarding adverse effects and to motivate women who were less compliant. On average, women took 85% of the assigned number of tablets, and there was no difference in compliance by type of prenatal supplement. A more complete analysis of patterns of compliance with supplementation will be described in a subsequent article.

Information was collected on maternal sociodemographic characteristics and clinical history at enrollment. Duration of pregnancy was calculated on the basis of the woman's reported date of last menses as well as by clinical indications of pregnancy duration at enrollment. Maternal anthropometric measurements (weight, height, body circumferences, and skinfold thicknesses) were taken at enrollment, 28–30 wk, and 37–38 wk gestation. Blood samples were taken from the women at each of the 3 time points and from the umbilical cord at delivery to determine hemoglobin, serum ferritin, and serum zinc concentrations. A report of the influence of zinc supplementation on maternal and neonatal zinc status was published elsewhere (11). Clinical follow-up of the pregnancy was done monthly or more frequently as necessary by the hospital staff and by the project obstetrician. Infants were weighed at birth by hospital personnel and crown-heel length and various circumferences and skinfold thicknesses were measured on day 1 by study personnel.

Blood samples were collected between 0800 and 1000 from the cubital veins of subjects who were in a fasting state. Hemoglobin concentration was measured by the cyanomethemoglobin method (12) at the hospital under IIN laboratory staff supervision. The blood was centrifuged at the hospital at room temperature for 5 min at $2264\text{--}2430 \times g$ within 30 min of collection and the serum was frozen at -20°C . The serum samples were transported on ice to the IIN laboratory 45 min away. Serum ferritin was analyzed at the IIN laboratory in duplicate by enzyme-linked immunosorbent assay by using human antiferritin and antiferritin peroxidase antibodies (DAKO, Santa Barbara, CA); ferritin standards were obtained from Diagnostic Products Corporation (Los Angeles). Women were considered to have anemia if their hemoglobin concentration was < 110 g/L and to have iron deficiency if their serum ferritin concentration was < 12 $\mu\text{g/L}$ (13, 14).

A total of 1295 pregnant women initially enrolled in the study. From this group, 279 women dropped out of the study: 92 declined to participate shortly after enrolling, 89 moved to another community, 58 left the study later for other reasons, 30 miscarried, 7 developed pregnancy complications, and 3 had twins. We obtained all 3 hemoglobin values for 645 of the 1016 women who remained in the study; that is, 63% of the women completed the study. Serum ferritin concentrations were available for 613 of these women and iron status was determined in the cord blood of 545 neonates.

Baseline characteristics of the supplementation groups were compared by means of *t* tests or chi-square analyses. Hemoglobin concentrations were normally distributed, but for the analyses, serum ferritin concentrations were normalized by a natural logarithmic transformation. For presentation, the values for the adjusted medians ± 1 SDs of serum ferritin were transformed to their original units ($\mu\text{g/L}$). Repeated-measures analysis of variance (ANOVA) was used to test whether the changes in maternal iron status differed by supplement type, whereas *t* tests were used to compare iron measurements in the newborns. These same techniques were used to examine changes in iron status in stratified analyses in which women were categorized into 5 groups on the basis of their initial hemoglobin concentration: 1) < 95 g/L, 2) 95–109 g/L, 3) 110–119 g/L, 4) 120–129 g/L, and 5) ≥ 130 g/L. Finally, logistic regression techniques were used to identify factors influencing the likelihood of having anemia at 37–38 wk gestation despite iron supplementation. From the regression model, odds ratios (ORs) and 95% CIs were calculated to describe the effect of each factor on risk of anemia at 37–38 wk gestation. For continuous variables, we calculated the effect on risk of anemia per change in a common unit (eg, per 1 wk or per 10 g/L), or in units commonly reported in the literature (per 20 tablets). During analyses, statistical significance was defined as $P < 0.05$. Data were analyzed by using SPSS for WINDOWS, Release 7.5 (SPSS Inc, Chicago).

RESULTS

Selected characteristics of the 645 participants with complete serial hemoglobin measurements are presented in **Table 1**. There were no significant differences between the 2 treatment groups at enrollment in duration of pregnancy, parity, weight, height, body mass index (BMI; in kg/m^2), education, or various indicators of socioeconomic status. However, women consuming supplements containing zinc were significantly younger than those consuming supplements containing iron and folic acid only.

TABLE 1

Selected characteristics of Peruvian women at entry into prenatal care and the study, 10–24 wk gestation

Characteristic	Supplement type	
	Iron + folate + zinc (<i>n</i> = 325)	Iron + folate (<i>n</i> = 320)
Age (y)	24.2 ± 5.3 ¹	25.3 ± 5.5 ²
Gestational age (wk)	16.0 ± 4.7	15.7 ± 4.5
Height (cm)	151.6 ± 5.3	151.3 ± 5.9
Weight (kg)	55.0 ± 8.0	55.2 ± 8.6
BMI (kg/m ²)	24.0 ± 3.2	24.2 ± 3.4
Married or in consensual union (%)	84.9	86.6
Parity (%)		
0	48.9	45.8
1	28.5	24.3
2–3	17.8	19.4
≥4	4.8	10.5
In-home services (%)		
Potable water	60.0	57.4
Sewage	66.2	62.7
Electricity	75.2	81.8
Housing material (%)		
Cardboard	22.8	26.0
Wood	16.0	16.9
Brick	60.9	56.1

¹ $\bar{x} \pm SD$.

²Significantly different from the other supplement type, *P* < 0.05.

Changes in iron-status indicators throughout pregnancy are presented in **Table 2** by type of prenatal supplement. Overall, hemoglobin and ferritin concentrations declined from initial values at 10–24 wk gestation and then rose to approximately baseline values by 37–38 wk gestation. As expected, hemoglobin and serum ferritin concentrations in the neonates were considerably higher than maternal values. As shown, there were no significant differences by prenatal supplement type. Furthermore, there were no differences observed by supplement type when the changes were compared with stratification by initial maternal hemoglobin concentration (data not shown). Because the addition of zinc to the supplements did not affect the hematologic changes during pregnancy, the data from both supplement groups were pooled for further analyses.

Changes in hemoglobin and serum ferritin concentrations during pregnancy varied according to initial hemoglobin status (**Table 3**). Increasing hemoglobin concentrations during pregnancy were observed in women with initial hemoglobin concentrations <110 g/L, whereas in women with higher initial hemoglobin concentrations, hemoglobin concentrations declined at 28–30 wk and then remained constant or rose at 37–38 wk gestation. Serum ferritin concentration increased throughout pregnancy only in women with initial hemoglobin concentrations <95 g/L. In women with hemoglobin concentrations >95 g/L, serum ferritin values declined at 28–30 wk and then rose at 37–38 wk gestation. No differences in cord blood hemoglobin or serum ferritin concentrations were observed according to initial maternal hemoglobin concentration; however, a positive correlation was observed between neonatal serum ferritin concentration and that of the mother at 37–38 wk gestation (*r* = 0.20, *P* < 0.05).

At enrollment, 33% of the women had anemia (hemoglobin concentration < 110 g/L). The prevalence of anemia was reduced

only slightly to 31% by 37–38 wk gestation. There was no difference in the proportion of anemic subjects by prenatal supplement type at any of the 3 time points during pregnancy. Among women with hemoglobin concentrations indicative of anemia at enrollment, 52% had iron deficiency; at 37–38 wk gestation, only 48% still had anemia and 33% had iron deficiency. Among women with hemoglobin concentrations >110 g/L at enrollment, the prevalence of anemia by the end of pregnancy rose to 23%, whereas the prevalence of iron deficiency rose from 24% to 33%.

A multiple logistic model was developed to identify risk factors for anemia at the end of pregnancy. The adjusted OR and 95% CI for each risk factor are presented in **Table 4**. As expected, women entering pregnancy with poorer initial hematologic status were more likely to have anemia at the end of pregnancy. Risk of anemia decreased with the number of supplements consumed. After adjustment for supplement consumption, duration of supplementation was associated with increased risk, indicating that women who were less compliant were also more likely to have anemia. Apart from these factors, anemia at the end of pregnancy was more likely in younger women and in those not married or in a consensual union. Finally, the likelihood of anemia increased substantially with each additional previous pregnancy.

DISCUSSION

In the present study, women were randomly assigned to receive daily prenatal iron and folic acid supplements with or without 15 mg Zn. As would be expected because of the large ratio of iron to zinc in the preparation (10), the addition of zinc did not affect hematologic status in either the mothers or the newborns. Furthermore, no differences were observed in the percentage absorption or red cell incorporation by supplement type (15). Inclusion of zinc in the supplements did, however, improve the zinc status of both the mothers and their infants (11). Thus, we showed that it is possible to improve maternal iron and zinc status during pregnancy with a prenatal supplement that combines these nutrients.

This study also provides one of the first descriptions of hematologic changes during pregnancy in a large sample of women in

TABLE 2

Iron status in Peruvian women during pregnancy and in neonates by type of maternal prenatal supplement¹

	Supplement type	
	Iron + folate + zinc (<i>n</i> = 325)	Iron + folate (<i>n</i> = 320)
Hemoglobin (g/L)		
10–24 wk	116 ± 12 ²	115 ± 14
28–30 wk	110 ± 13	110 ± 13
37–38 wk	114 ± 13	115 ± 13
Cord vein ³	175 ± 23	177 ± 24
Serum ferritin (μg/L)		
10–24 wk	21.8 (8.9, 53.3) ⁴	19.7 (7.7, 50.0)
28–30 wk	13.9 (7.2, 27.1)	14.1 (7.0, 28.7)
37–38 wk	17.6 (8.1, 38.5)	17.8 (8.2, 38.9)
Cord vein ³	167.3 (94.0, 297.7)	167.0 (92.3, 302.4)

¹There were no significant differences between supplement groups.

² $\bar{x} \pm SD$.

³Cord values were available for 275 and 270 neonates born to mothers who did or did not receive supplemental zinc, respectively.

⁴Median; –1 SD and +1 SD in parentheses.

TABLE 3

Iron status during pregnancy of Peruvian women who consumed iron and folic acid supplements (with or without zinc) and of their neonates, stratified by initial hemoglobin concentration¹

Variable	Initial hemoglobin (g/L)				
	<95 (n = 42)	95–109 (n = 168)	110–119 (n = 189)	120–129 (n = 169)	≥130 (n = 77)
Enrollment gestation (wk, completed)	16.9 ± 5.0 ²	18.0 ± 4.4	16.1 ± 4.3	14.5 ± 4.4	13.2 ± 3.5
Hemoglobin (g/L)					
10–24 wk	88.4 ± 5.5 ^a	103.5 ± 4.2 ^a	115.0 ± 2.8 ^b	124.2 ± 2.9 ^c	136.0 ± 5.3 ^b
28–30 wk	97.9 ± 15.2 ^b	106.7 ± 12.1 ^b	110.8 ± 12.1 ^a	112.3 ± 11.3 ^a	118.6 ± 11.4 ^a
37–38 wk	105.5 ± 14.8 ^c	110.9 ± 13.2 ^b	115.4 ± 12.2 ^b	117.4 ± 11.5 ^b	120.5 ± 13.3 ^a
Cord vein	178.7 ± 21.7	176.4 ± 23.5	176.6 ± 23.0	174.4 ± 22.5	177.6 ± 19.8
Serum ferritin (µg/L)					
10–24 wk	8.5 (3.4, 21.5) ^{a,3}	15.5 (6.3, 38.1) ^b	21.0 (12.1, 51.3) ^c	26.4 (12.2, 57.9) ^c	33.3 (15.3, 72.2) ^c
28–30 wk	12.3 (5.3, 28.4) ^b	12.7 (6.6, 24.6) ^a	14.4 (7.4, 27.9) ^a	14.4 (7.6, 27.3) ^a	16.4 (7.5, 35.9) ^a
37–38 wk	14.3 (6.8, 29.8) ^b	16.8 (7.3, 38.5) ^c	17.8 (8.2, 38.7) ^b	18.5 (8.9, 38.2) ^b	19.6 (8.7, 44.1) ^b
Cord vein	151.2 (64.5, 354.3)	170.4 (104.0, 279.2)	166.3 (95.6, 289.2)	169.7 (90.1, 319.6)	168.8 (94.1, 302.7)

¹ Values within strata with different superscript letters are significantly different, *P* < 0.05.

² \bar{x} ± SD.

³ Median; -1 SD and +1 SD in parentheses.

a developing country who were provided 60 mg Fe/d from 10–24 wk gestation to 4 wk postpartum through the prenatal care delivery system. This amount and duration of supplementation is consistent with the new guidelines proposed by the International Consultative Group on Nutritional Anemias in 1998 (16). As shown, the benefits to maternal iron status from iron supplementation depend on maternal iron status early in pregnancy. With supplementation, hemoglobin concentrations increased by 7.4–17.1 g/L from ≈17–18 wk gestation to 37–38 wk gestation in women entering pregnancy with hemoglobin concentrations <110 g/L. Accompanying these changes were increases in serum ferritin concentrations by the end of pregnancy. Hemoglobin and serum ferritin concentrations declined at 28–30 wk in women entering pregnancy with hemoglobin concentrations ≥110 g/L, but rose again by 37–38 wk gestation. Differences in plasma volume expansion between women may confound the results and the results are subject to regression to the mean (17). We did not assess plasma volume expansion; however, because of the low within-subject variability inherent in venous hemoglobin concentration (18, 19), we speculate that regression to the mean may explain at most 3 g/L of the hematologic changes observed in women with relatively high or low hemoglobin values at enrollment.

These and other limitations could have been addressed by using a control group, that is, a group not receiving iron supplements or receiving a placebo during pregnancy. The use of a placebo was not possible within the programmatic context in which we were working, and this remains a limitation to the interpretation of our results. However, it is possible to compare our results with those reported in placebo-controlled trials of iron supplementation to provide plausible evidence that the observed hematologic changes are likely due to iron supplementation. The results from past trials indicate the following (20–26). First, regardless of supplementation, both hemoglobin and serum ferritin concentrations drop to a nadir around 28 wk because of hemodilution as well as other physiologic changes during pregnancy. Second, hemoglobin and serum ferritin concentrations do not rise again at the end of pregnancy without supplemental iron, even in women with adequate iron stores at the beginning of pregnancy. Third, among women entering preg-

nancy with hemoglobin concentrations <110 g/L, both hemoglobin and serum ferritin concentrations may rise continually throughout pregnancy—but only if supplemental iron is provided. Fourth, despite iron supplementation, 10–60% of women entering prenatal care with anemia will still have anemia at the end of pregnancy (26). Furthermore, among women with hemoglobin concentrations >110 g/L at entry into prenatal care, 10–20% will likely have hemoglobin concentrations <110 g/L by the end of pregnancy and 10–50% will likely have iron deficiency (24, 26). Thus, the hematologic changes we described in Peruvian women who were provided supplemental iron during pregnancy

TABLE 4

Risk factors for anemia (hemoglobin <110 g/L) at 37–38 wk gestation in Peruvian women who consumed iron and folic acid supplements with or without zinc during pregnancy¹


Risk factor	OR (95% CI)
Hemoglobin at enrollment (g/L)	
130	0.60 (0.51, 0.70)
120	1.00
110	1.67 (0.43, 1.95)
100	2.79 (2.04, 3.80)
Supplements (per 20 tablets)	0.75 (0.66, 0.86)
Duration of supplementation (per week of supplementation)	1.13 (1.07, 1.19)
Parity	
0	1.00
1	2.21 (1.35, 3.63)
2–3	3.27 (1.74, 6.26)
≥4	4.68 (1.81, 12.05)
Age (y)	
15	1.88 (1.15, 3.05)
20	1.37 (1.07, 1.75)
25	1.00
30	0.73 (0.57, 0.93)
35	0.53 (0.33, 0.87)
Married or in consensual union	
Yes	1.00
No	1.94 (1.13, 3.34)

¹ OR, odds ratio.

are consistent with the changes estimated in randomized, placebo-controlled studies of iron supplementation.

As expected, hematologic indicator values in the newborns were higher than those observed in the mothers (20, 21). Although no differences were observed according to the initial hematologic status of the mother, a small positive correlation ($r = 0.20$) was observed between neonatal and maternal serum ferritin late in pregnancy. This is consistent with the findings of another study reported in the literature (24).

Compliance with daily iron supplementation in this study, conducted through the regular public health care system of a shantytown, was 85%, a value higher than the 40–65% reported in other regions (27, 28). We speculate that this achievement was due to the lower dosage of iron used in this study than in other studies, the accessibility of the health care system, a dependable tablet supply, provision of educational materials, and the presence of a motivated staff and at-home follow-up with health promoters.

Despite iron supplementation, the overall prevalence of anemia did not decline by the end of pregnancy. As discussed, this finding is consistent with the results of previous randomized, placebo-controlled trials and was not due to noncompliance. Furthermore, it was not likely due to the lower daily dosage of iron, because the efficacy of iron supplementation during pregnancy does not differ greatly depending on whether the supplements contain 60 or 120 mg Fe/d (26, 29). Although the folate status of these women was improved by supplementation, continued folate deficiency and vitamin B-12 deficiency may have been factors contributing to their anemia (15). Hemodilution may have also contributed to the finding of anemia at the end of pregnancy regardless of iron status. Thus, it may be necessary to assess iron status after pregnancy to measure improvements in iron status in women due to prenatal iron supplementation. We showed that women who are generally considered to be at high social or obstetric risk (those who are younger, multiparous, or without a partner) are more likely to end pregnancy with anemia, irrespective of initial hemoglobin status and compliance with supplementation. Because these characteristics are generally known to practitioners when women enter prenatal care, they could be used to identify women who would benefit from higher doses of iron prenatally; however, the effectiveness of such an approach would need to be evaluated in future studies. It may be that no tolerable dosage of oral iron, initiated once pregnancy has begun, is able to make up the deficit of prepregnancy iron depletion and meet gestational iron needs even if dietary intake and compliance with supplementation are optimal. Programs to improve iron status outside of pregnancy may be needed in addition to available and accessible routine prenatal supplementation to redress the burden of anemia in underprivileged women in developing countries. 

We sincerely thank the women who volunteered to participate in this study; Alberto Figueroa, Juanita Callali, and the rest of the research team for their excellent medical care of these women; the staff of the IIN laboratory; the director and personnel of the obstetric ward at the Hospital Cesar Lopez Silva in Villa El Salvador, Lima, Peru, for their collaboration on the project; and the Health Authorities and Departmental Health Unit of the Ministry of Health for allowing us to use their medical facilities.

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