

# Lack of hemoglobin response to iron supplementation in anemic Mexican preschoolers with multiple micronutrient deficiencies<sup>1-3</sup>

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## ABSTRACT

**Background:** In developing countries, incomplete resolution of anemia with iron supplementation is often attributed to poor compliance or inadequate duration of supplementation, but it could result from deficiencies of other micronutrients.

**Objective:** Our objective was to assess children's hematologic response to supervised, long-term iron supplementation and the relation of this response to other micronutrient deficiencies, anthropometry, morbidity, and usual dietary intake.

**Design:** Rural Mexican children aged 18–36 mo ( $n = 219$ ) were supplemented for 12 mo with either 20 mg Fe, 20 mg Zn, both iron and zinc, or placebo. Children were categorized as iron-unsupplemented (IUS;  $n = 109$ ) or iron supplemented (IS;  $n = 108$ ). Hemoglobin, hematocrit, mean corpuscular volume, mean cell hemoglobin, plasma concentrations of micronutrients that can affect hematopoiesis, anthropometry, and diet were assessed at 0, 6, and 12 mo; morbidity was assessed biweekly.

**Results:** At baseline, 70% of children had low hemoglobin ( $\leq 115$  g/L), 60% had low hematocrit, 48% were ferritin deficient, 10% had deficient and 33% had low plasma vitamin B-12 concentrations, 29% had deficient vitamin A concentrations, and 70% had deficient vitamin E concentrations. Iron supplementation increased ferritin from  $11 \pm 14$   $\mu\text{g/L}$  at baseline to  $31 \pm 18$   $\mu\text{g/L}$  after 6 mo ( $P < 0.001$ ) and  $41 \pm 17$   $\mu\text{g/L}$  after 12 mo. However, anemia persisted in 30% and 31% of supplemented children at 6 and 12 mo, respectively, and was not significantly different between the IUS and IS groups at 12 mo. Initial plasma vitamin B-12, height-for-age, and dietary quality predicted the hematopoietic response to iron.

**Conclusion:** Lack of hemoglobin response to iron was associated with indicators of chronic undernutrition and multiple micronutrient deficiencies. *Am J Clin Nutr* 2000;71:1485–94.

**KEY WORDS** Iron supplementation, anemia, iron deficiency, vitamin B-12, retinol, tocopherol, anthropometry, dietary quality, children, micronutrient deficiencies, hemoglobin response, Mexico

## INTRODUCTION

Iron deficiency anemia is a common problem worldwide, affecting  $\approx 50\%$  of individuals in high-risk groups such as preschool children and women of childbearing age (1). Its consequences include impaired school performance (2, 3), work

performance (4), motor and mental development (5, 6), and possibly growth (7). It is generally accepted that iron deficiency is the most common cause of low hemoglobin concentrations; therefore, iron deficiency is the main focus of programs that try to alleviate anemia. However, iron deficiency may be accompanied by other micronutrient deficiencies because both can result from high rates of infection, diarrhea, anorexia, and poor dietary quality and nutrient bioavailability. For example, the present investigation was conducted in a rural Mexican region where the percentage of preschoolers (18–36 mo) with inadequate intakes of various nutrients was estimated to be as follows in a previous study: vitamin A, 68%; vitamin E, 92%; vitamin C, 63%; riboflavin, 52%; vitamin B-12, 8%; iron, 89%; and zinc, 68% (8, 9). In addition, a high prevalence of deficient and marginal plasma vitamin B-12 concentrations was documented in men, women of childbearing age, preschoolers, and school-age children in these communities (10, 11).

The coexistence of these micronutrient deficiencies and iron deficiency may increase the risk of anemia and limit the hematologic response to iron supplementation. The common failure of iron supplementation to cure anemia completely is often ascribed to poor compliance or an inadequate duration of supplementation. In a recent meta-analysis of the efficacy of intermittent iron supplementation in developing countries, the authors concluded, "there is a suggestion in the data, not well documented except in a couple of studies, that something other than iron may be operating to limit hemoglobin response and anemia control" (12). If deficiencies of other micronutrients are responsible, this would suggest that supplements should contain additional micronutrients to improve the hematologic response.

In the same rural Mexican region where the studies discussed above were conducted, we subsequently performed a randomized,

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controlled trial to measure the effect of supplementation with iron, zinc, or both on preschoolers' growth and morbidity (13). Data on hematology, micronutrient status, and dietary intake were collected and are the focus of this article. The purpose of this article is to describe the hematologic response of preschool children to 12 mo of supplementation with iron, zinc, or both and the association of this response with other micronutrient deficiencies, anthropometric measurements, morbidity, and usual dietary intake.

## SUBJECTS AND METHODS

### Subjects and location

This study was conducted in 5 rural communities with a low socioeconomic status in the Valley of Solfs, located in the central highland plateau of the state of Mexico  $\approx$ 150 km northwest of Mexico City. The study was approved by the Human Subjects Research Committee of the University of California at Davis and the Committee on Biomedical Research in Human Subjects of the National Institute of Nutrition in Mexico. The parents of the subjects gave verbal informed consent in the presence of a community witness. People living in this area are employed in agriculture, predominantly maize production, with most men migrating to Mexico City or elsewhere to support their families. The diet is typical of that of rural Mexico, with tortillas contributing 51–66% of the total energy intake of adults and children. Animal product consumption is low and plant foods provide >90% of dietary energy (14).

The subjects were 219 children aged 18–36 mo. We invited the parents ( $n = 290$ ) of these children to give consent for their children's participation, and 76% accepted. The child's age was the only selection criterion. Once selected, the children were stratified on the basis of age and sex and were randomly assigned to 1 of the 4 treatment groups. A sample size of 48 subjects per group was required to detect a 1-cm difference in height resulting from supplementation, assuming an SD for change in height of 1.5 cm and a statistical power of 80%.

### Study design

The study was described in detail elsewhere (13). Briefly, for 6 d/wk over 12 mo, one group received an iron supplement (20 mg Fe as ferrous sulfate; iron group), a second group received a zinc supplement (20 mg Zn as zinc methionine; zinc group), a third group received a combination of iron and zinc (20 mg of each; iron + zinc group), and a fourth group received a placebo (a sweet syrup with citrus flavor to which the mineral supplements were added for the other groups). Supplements were carried to the children's homes each day and their consumption was observed by the fieldworkers.

Blood samples were obtained at baseline and 6 and 12 mo later for measurement of hemoglobin, hematocrit, red blood cell (RBC) count, erythrocyte glutathione reductase activity coefficient (EGRAC), and plasma ferritin, vitamin B-12, vitamin A, vitamin E, and C-reactive protein (CRP) concentrations. Anthropometric measurements were obtained at 0, 6, and 12 mo by one examiner who followed standard procedures (15). Morbidity was evaluated twice weekly by using a recall questionnaire administered to the mother by trained fieldworkers who visited each child at home. Symptoms were classified by a physician as indicating the presence or absence of upper and lower respiratory disease, diarrhea, fever, and other illness, as described in more detail elsewhere (13). Neither hookworm nor malaria occurs in this area of Mexico.

### Hematology and plasma ferritin, C-reactive protein, and zinc

Hematologic measurements included hemoglobin concentrations, hematocrit values, and RBC counts, which were measured with a Coulter counter. Plasma ferritin concentrations were analyzed in duplicate by using an enzyme-linked immunosorbent assay (Coat-A-Count Ferritin immunoradiometric assay; Diagnostic Products Inc, Los Angeles). The cutoffs for anemia were as follows: hemoglobin (increased by 0.5 g/L for the altitude of 2300 m) <115 g/L (16) and hematocrit (increased by 2% for altitude) <35% (16). The cutoff for iron deficiency was a ferritin concentration <10  $\mu$ g/L (17). Cutoffs for low and elevated mean corpuscular volume (MCV) were <73 and >87 fL, respectively (18). Cutoffs for low and elevated mean cell hemoglobin (MCH) were <27 and >32 pg, respectively—the 95th percentiles from the second National Health and Nutrition Examination Survey for children 1–2 y of age (19).

CRP concentrations were measured by using laser nephelometry (Behring Diagnostics Inc, Somerville, NJ) with an anti-serum specific for CRP; concentrations >0.3 mmol/L indicate an infection or inflammatory process (Behring Diagnostics Inc), which may increase plasma ferritin concentrations. The numbers of children with elevated CRP values in the IUS and IS groups, respectively, were 23 and 19 at baseline, 18 and 21 at 6 mo, and 7 and 12 at 12 mo. All samples were accompanied by standards and control sera.

### Plasma concentrations of vitamins B-12, A, and E and riboflavin

Plasma vitamin B-12 concentrations were measured by using a microparticle enzyme intrinsic factor assay (IMx B-12 assay; Abbott Laboratories, Abbott Park, IL). Concentrations  $\leq$ 147.7 pmol/L indicated deficiency and concentrations of 147.7–221.3 pmol/L were considered to be low (20).

Plasma retinol and  $\alpha$ -tocopherol concentrations were measured by using HPLC (21). Retinol and  $\alpha$ -tocopherol concentrations <0.7 (22) and <11.6 (23)  $\mu$ mol/L, respectively, indicated deficiency. The vitamin E cutoff was based on data reported by Karr et al (24), who defined age-specific intervals for vitamin E concentrations in a healthy population of Australian children aged 9–62 mo. Riboflavin status was determined on the basis of the EGRAC (25), with deficiency defined as an EGRAC >1.2 (26). The EGRAC was not assessed at baseline because the erythrocyte samples were lost.

### Dietary information

A semiquantitative questionnaire was used twice to collect dietary information: once between baseline and 6 mo and again between 6 and 12 mo. The mother of the child was asked to report how frequently her child ate specific foods during the past week. Foods were grouped as meat, eggs, cheese, legumes, tortillas, cereals, fruit, and vegetables. Because vitamin B-12 may be destroyed when milk is processed (27), the type of milk consumed was recorded as raw cow milk, powdered milk, pasteurized milk, or evaporated milk.

### Statistical analyses

Statistical analyses were performed by using PC-SAS, release 6.04 (SAS Institute Inc, Cary, NC; 28). The results are presented as means  $\pm$  SDs. Log transformation was used to normalize the distributions of plasma ferritin, vitamin B-12, retinol, and tocopherol concentrations. The children were divided into 2 groups:

an iron-unsupplemented group (IUS;  $n = 109$ ), which included the zinc and placebo groups, and an iron-supplemented group (IS;  $n = 108$ ), which consisted of the iron and iron + zinc groups. Zinc supplementation was used as a covariate. The prevalence of deficiency for each nutrient was expressed as the percentage of children below the appropriate cutoff value.

The analyses included descriptive statistics on hematologic measures and nutrient status at 0, 6, and 12 mo and Spearman's correlation coefficients between the hematologic and biochemical variables and anthropometry. Student's  $t$  test was used to determine whether the children with elevated CRP concentrations had significantly higher plasma ferritin concentrations. A two-way repeated-measures analysis of covariance (ANCOVA) with SAS PROC GLM was used to analyze changes in concentrations of biochemical variables; the explanatory variables were treatment group, time, time-by-treatment interaction, and baseline value of the response variable. Because of limitations in the program's ability to do post hoc comparisons with the repeated-measures procedure, subanalyses were carried out when the time-by-iron-supplementation interaction was significant. Specifically, a separate repeated-measures analysis was done for each treatment group to examine changes over time and separate ANCOVAs were done at 6 and 12 mo to examine differences between treatments. Bonferroni corrections were used to adjust the  $P$  values of these comparisons. The same approach was used to determine whether iron supplementation had an effect on the prevalence of anemia or iron deficiency, except that a categorical procedure (SAS PROC CATMOD) was used in lieu of ANCOVA.

Stepwise linear regression models were used to determine whether plasma ferritin or vitamin B-12 concentrations predicted MCV and MCH. Stepwise linear regression models (29) were also used to identify the main predictors of the changes in hemoglobin, hematocrit, and ferritin. Possible predictors included biochemical variables, dietary intake, anthropometric measures (including weight and height gain), morbidity, age, and iron and zinc supplementation. Because dietary information was collected once between baseline and 6 mo and again between 6 and 12 mo, the dietary intake variables from the first and second time points were tested for associations with the biochemical variables at 6 and 12 mo, respectively. Baseline concentrations of biochemical variables were controlled for in the multiple regression models.

To investigate the high prevalence of anemia in the IS group at 6 and 12 mo, we used 2 ways of defining responders to iron supplementation: 1) the commonly used definition of individuals whose hemoglobin increased by 10 g/L, and 2) individuals whose values fell above the regression line of change in hemoglobin, with initial hemoglobin and ferritin concentrations and change in ferritin as explanatory variables. Student's  $t$  test was then used to identify biochemical, dietary, age, anthropometric, and morbidity variables at baseline that were significantly different between those who responded to iron supplementation and those who did not, by both definitions. ANCOVA was used to evaluate whether the slopes of the regression lines of change in hemoglobin versus initial hemoglobin were significantly different between the IUS and IS groups. The same type of analysis was also used to test for differences between the IS and IUS groups in change in hemoglobin from baseline to 6 and 12 mo. Possible determinants of hemoglobin response to iron supplementation between baseline and 6 and 12 mo were investigated with stepwise logistic regression by using the biochemical,

dietary, age, anthropometric, or morbidity variables at baseline for both definitions of response to iron supplementation. A  $P$  value  $< 0.05$  was considered statistically significant.

## RESULTS

### Subject characteristics at baseline

At baseline for the IUS and IS groups, respectively, mean age was  $2.4 \pm 0.7$  and  $2.3 \pm 0.7$  y, weight-for-age  $z$  score was  $-1.4 \pm 0.8$  and  $-1.4 \pm 1.0$ , length-for-age  $z$  score was  $-1.7 \pm 1.0$  and  $-1.5 \pm 1.2$ , and weight-for-length  $z$  score was  $-0.4 \pm 0.7$  and  $-0.5 \pm 0.8$ . None of the baseline characteristics were significantly different between the children in the IUS and IS groups, except for plasma vitamin B-12 concentrations ( $237.6 \pm 98.2$  and  $261.2 \pm 118.9$  pmol/L, respectively; **Table 1**). Also, no baseline characteristic differed significantly between children in the zinc-supplemented and zinc-unsupplemented groups. There was no significant difference in mean plasma ferritin concentrations between those children with elevated and those with normal CRP concentrations at any time point.

### Changes in hematologic measures and micronutrient status during the study

On the basis of the repeated-measures analysis, there was a clear improvement in the nutritional status of these children as they aged from 2.4 to 3.4 y on average, regardless of the effect of iron supplementation. For example, children had significantly higher mean hemoglobin concentrations, hematocrit values, RBC counts, MCVs, and vitamin B-12 and tocopherol concentrations at 6 and 12 mo than at baseline. At 12 mo, retinol concentrations were significantly higher and MCH concentrations were significantly lower than at baseline. The only variables for which there was a significant time-by-iron-supplementation interaction were hemoglobin and plasma ferritin concentrations. Both groups had significantly higher hemoglobin values at 6 and 12 mo than at baseline, but the value in the IS group was significantly higher than that of the IUS group at 6 mo only. In the IUS group, ferritin was significantly different from baseline only at 12 mo, but in the IS group it was significantly different from baseline at both 6 and 12 mo. Thus, at the end of a year of supplementation with iron, only plasma ferritin was significantly different between the IUS and IS groups.

### Prevalence of anemia and micronutrient deficiencies

The prevalences of anemia, low ferritin concentration, elevated and low MCV, and elevated and low MCH are presented in **Figures 1** and **2**. For iron deficiency, there was a significant time-by-treatment interaction ( $P < 0.0001$ ); iron deficiency was significantly less prevalent in the IS group at 6 mo and in both groups at 12 mo compared with baseline. Despite the fact that the prevalences of low hemoglobin and low hematocrit were so different, there was a significant association between these measures ( $n = 219$ ; **Table 2**).

Anemia persisted in about one-third of the children supplemented with iron for 6 and 12 mo. In fact, the prevalence of anemia in the IS group was not significantly different from that in the IUS group (30% compared with 38%, respectively, at 6 mo and 31% compared with 33%, respectively, at 12 mo). Hematocrit was low in  $\approx 60\%$  of the children at baseline. Compared with baseline, significantly fewer children in both the IS and IUS groups had low

**TABLE 1**

Hematology and nutritional status at baseline and 6 and 12 mo in the iron-supplemented (IS;  $n = 108$ ) and iron-unsupplemented (IUS;  $n = 109$ ) rural Mexican preschool children<sup>1</sup>

	Baseline		6 mo		12 mo	
	IUS	IS	IUS	IS	IUS	IS
Hemoglobin (g/L) <sup>2,3</sup>	108.3 ± 13.1	107.6 ± 11.9	117.2 ± 9.8 <sup>4</sup>	120.6 ± 9.8 <sup>4</sup>	117.8 ± 8.1 <sup>4</sup>	118.5 ± 8.8 <sup>4</sup>
Hematocrit	0.339 ± 0.028	0.336 ± 0.030	0.381 ± 0.028 <sup>4</sup>	0.389 ± 0.032 <sup>4</sup>	0.388 ± 0.026 <sup>4</sup>	0.391 ± 0.030 <sup>4</sup>
RBC count (× 10 <sup>12</sup> /L)	3.9 ± 0.5	3.9 ± 0.5	4.3 ± 0.5 <sup>4</sup>	4.2 ± 0.7 <sup>4</sup>	4.6 ± 0.5 <sup>4</sup>	4.6 ± 0.5 <sup>4</sup>
MCH (pg)	27.9 ± 3.3	27.9 ± 3.6	27.5 ± 2.9	28.9 ± 3.1	25.3 ± 2.5 <sup>4</sup>	26.1 ± 2.4 <sup>4</sup>
MCV (fL)	88.4 ± 8.7	87.7 ± 9.3	91.8 ± 8.8 <sup>4</sup>	93.2 ± 9.4 <sup>4</sup>	85.0 ± 7.3 <sup>4</sup>	85.9 ± 8.1 <sup>4</sup>
Ferritin (μg/L) <sup>2,5,6</sup>	11.8 ± 15.7	11.0 ± 13.8	11.8 ± 19.2	31.0 ± 17.9 <sup>4</sup>	16.9 ± 19.7 <sup>7</sup>	40.5 ± 17.3 <sup>4</sup>
Vitamin B-12 (pmol/L) <sup>6</sup>	237.6 ± 98.2	261.2 ± 118.9 <sup>8</sup>	336.5 ± 141.7 <sup>9</sup>	317.3 ± 149.0 <sup>9</sup>	316.8 ± 120.7 <sup>9</sup>	306.7 ± 172.1 <sup>9</sup>
Retinol (μmol/L) <sup>6</sup>	0.94 ± 0.6	0.88 ± 0.5	1.12 ± 0.4	1.12 ± 0.4	1.16 ± 0.4 <sup>4</sup>	1.17 ± 0.4 <sup>4</sup>
Tocopherol (μmol/L) <sup>6,10</sup>	8.0 ± 5.6	7.6 ± 4.7	9.1 ± 7.5 <sup>4</sup>	11.4 ± 7.7 <sup>4</sup>	10.5 ± 6.7 <sup>4</sup>	11.8 ± 6.9 <sup>4</sup>
EGRAC <sup>11</sup>	—	—	1.13 ± 0.1	1.14 ± 0.2	1.04 ± 0.1	1.02 ± 0.1

<sup>1</sup> $\bar{x} \pm$  SD. RBC, red blood cell; MCH, mean cell hemoglobin; MCV, mean corpuscular volume; EGRAC, erythrocyte glutathione reductase activity coefficient. All variables were analyzed with two-way repeated-measures analysis of covariance.

<sup>2</sup>Significant time-by-treatment interaction,  $P < 0.05$ .

<sup>3,10</sup>Significant treatment effect at 6 mo: <sup>3</sup> $P < 0.01$ , <sup>10</sup> $P < 0.05$ .

<sup>4,7</sup>Significantly different from baseline: <sup>4</sup> $P < 0.001$ , <sup>7</sup> $P < 0.01$ .

<sup>5</sup>Significant treatment effect at 6 and 12 mo,  $P < 0.001$ .

<sup>6</sup>Geometric mean ± estimated SD of log-transformed variables.

<sup>8</sup>Significantly different from IUS group at baseline,  $P < 0.05$ .

<sup>9</sup>Significantly different from baseline after initial vitamin B-12 concentrations were controlled for,  $P < 0.001$ .

<sup>11</sup>Not assessed at baseline.

hematocrit values at 6 and 12 mo, but the prevalence of low hematocrit was unaffected by iron supplementation. In contrast, only one iron-supplemented child at 6 mo and none at 12 mo had a low ferritin concentration. The prevalence of elevated MCV values at baseline (52%) increased significantly to 71% and 77% at 6 mo and then decreased to 34% and 44% at 12 mo in the IUS and IS groups, respectively. The prevalence of low MCV values (6% at baseline, 3% and 0% at 6 mo, and 6% and 6% at 12 mo in the IUS and IS groups, respectively) was lower than that of high values. The prevalence of elevated MCH at baseline (12%) changed to 8% and 13% at 6 mo and fell to 0% and 1% at 12 mo in the IUS and IS groups, respectively. MCH values were low in 37% of children at baseline, 35% and 26% at 6 mo, and 80% and 64% at 12 mo in the IUS and IS groups, respectively. Neither MCV nor MCH was affected by iron supplementation.

The prevalences of deficiencies of vitamins B-12, A, and E and riboflavin in the children at the 3 time points are presented in **Figure 3**. The prevalence of vitamin B-12 deficiency, as indicated by plasma concentrations, was 10% at baseline; at 6 mo it was 5% in the IUS group and 1% in the IS group and at 12 mo it was 8% in the IUS group and 2% in the IS group. In addition, low plasma vitamin B-12 values were found in 33% of the children at baseline, 22% at 6 mo, and 29% at 12 mo (data not shown). Plasma retinol concentrations indicating deficiency were found in 26% of the IUS group and in 33% of the IS group at baseline and in 6% of the IUS group and 4% of the IS group at both 6 and 12 mo. At baseline, 70% of the children had low plasma vitamin E concentrations. This prevalence fell to 43% in the IUS group and to 33% in the IS group at 6 mo and to 32% in the IUS group and to 31% in the IS group at 12 mo. An elevated EGRAC was found in 31% of the IUS group and in 34% of the IS group at 6 mo, but in only 4% of the IUS group and in 2% of the IS group at 12 mo.

### Dietary intake

The mean servings of food consumed per day are shown in **Table 3**. Although the children consumed animal products

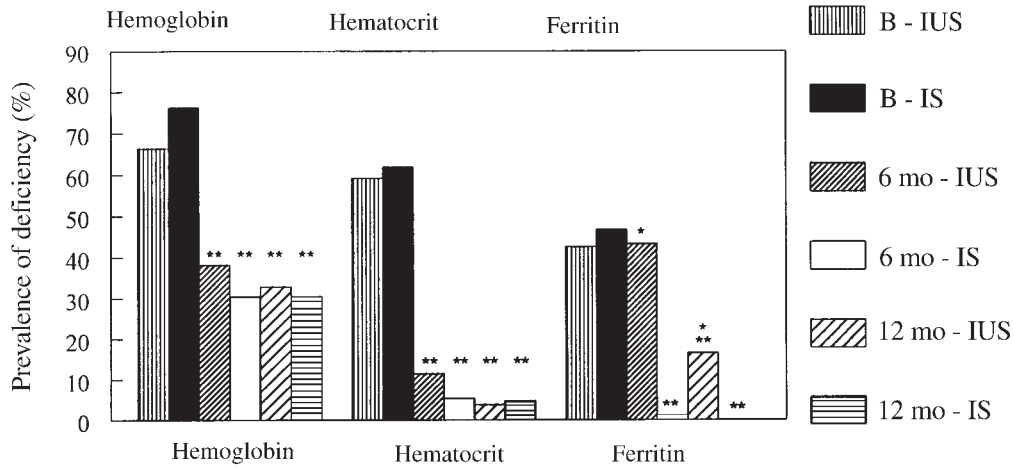
3 times/d on average, the amounts eaten were found to be very small in our previous studies of children of the same age in this community (14). Dairy products and cereals were each consumed  $\approx 2$  times/d, and  $\approx 5$  tortillas were eaten daily on average. The latter would supply  $\approx 75\%$  of the energy requirements of the children.

### Determinants of change in hematologic measures and iron status

The bivariate relations between hematologic measures, nutritional status, age, and anthropometric variables are shown in **Table 2**. Plasma ferritin, vitamin B-12, retinol, and tocopherol, and weight- and height-for-age were generally positively and significantly correlated with the hematologic measures.

The stepwise linear regression models showed that the change in hemoglobin values from baseline to 6 mo was predicted by initial hemoglobin, height-for-age at 6 mo, and age ( $P < 0.03$ ; **Table 4**). For the change in hemoglobin from baseline to 12 mo, the main predictors were initial hemoglobin and initial vitamin B-12 concentrations ( $P < 0.03$ ); the possible determinants tested were biochemical variables, dietary intake, anthropometric measures (including weight and height gain), morbidity, age, and iron and zinc supplementation. Change in hematocrit from baseline to 6 mo was predicted by initial hematocrit and incidence of illness ( $P < 0.001$ ). The main predictors of change in hematocrit from baseline to 12 mo were initial hematocrit, initial ferritin concentration, cereal intake, and age ( $P < 0.03$ ), with the same explanatory variables as possible determinants. Iron supplementation and initial ferritin concentration were the main predictors ( $P < 0.001$ ) of the change in ferritin concentration from baseline to both 6 and 12 mo. Change in ferritin concentration from baseline to 12 mo was also predicted by weight-for-age at 12 mo and fruit intake. Hemoglobin and hematocrit were so highly correlated that they were not used simultaneously in any regression model.

The high prevalence of elevated MCV values, and to a lesser extent MCH values, and their significant negative correlations with



**FIGURE 1.** Prevalence of anemia and iron deficiency at baseline (B), 6 mo, and 12 mo in the iron-unsupplemented (IUS;  $n = 109$ ) and iron-supplemented (IS;  $n = 108$ ) groups. \*Significantly different from the respective IS group. \*\*Significantly different from the respective baseline value,  $P < 0.05$  (repeated-measures categorical procedures).

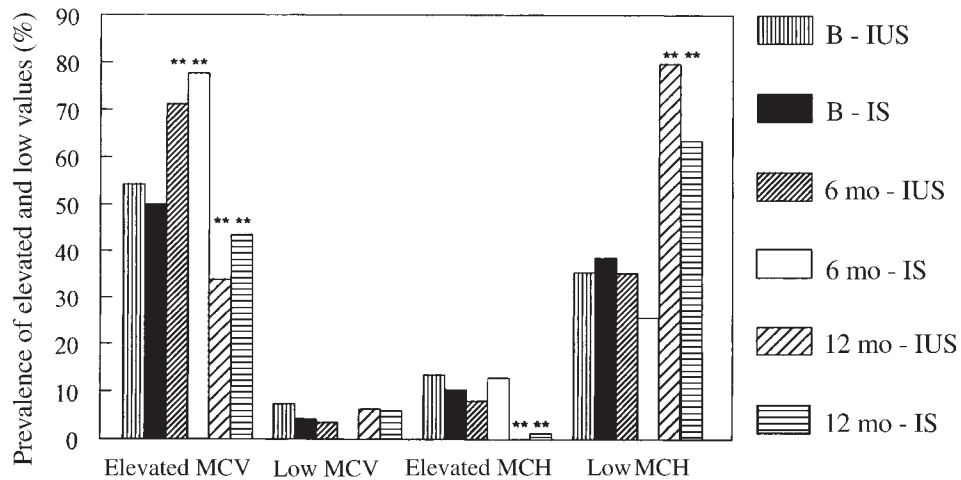
plasma vitamin B-12 concentrations at baseline suggested the possibility that vitamin B-12 deficiency is affecting hematopoiesis in these children. Because iron and vitamin B-12 have opposite effects on MCV and MCH, plasma ferritin was included in multiple regression models that showed that only ferritin predicted MCV (positive correlation), whereas MCH was predicted by both ferritin (positive correlation) and vitamin B-12 (negative correlation).

**Predictors of change in hemoglobin in response to iron supplementation**

To compare the changes in hemoglobin concentration in the IUS and IS groups, we plotted the frequency distribution of the change in hemoglobin concentrations from baseline to 6 mo and baseline to 12 mo, assuming a normal distribution in each group (Figure 4). The frequency distributions illustrate the finding that after 6 mo, but not after 12 mo, the mean change was significantly greater in the children who received iron.

The persistence of anemia despite improved iron status after iron supplementation could have been an artifact of using a hemoglobin cutoff that was too high. To examine this possibility, the regression between change in hemoglobin from baseline to 6 mo and initial hemoglobin concentration was examined for the IUS versus IS groups. The regression line of the IS group was significantly higher than that of the IUS group ( $P < 0.004$ ; Figure 5). However, the slopes of these regression lines were the same, which indicated that the effect of either an iron supplement or a placebo was the same regardless of the initial hemoglobin value, representing regression to the mean in both groups. This suggests that there was no cutoff below which iron supplementation had an effect on hemoglobin, and the persistence of anemia cannot be attributed to an inappropriate hemoglobin cutoff.

To take regression to the mean into account, children were classified as responders to iron supplementation according to 2



**FIGURE 2.** Prevalence of elevated and low mean corpuscular volume (MCV) and mean cell hemoglobin (MCH) at baseline (B), 6 mo, and 12 mo in the iron-unsupplemented (IUS;  $n = 109$ ) and iron-supplemented (IS;  $n = 108$ ) groups. \*\*\*Significantly different from the respective baseline value,  $P < 0.05$  (repeated-measures categorical procedures).

**TABLE 2**Spearman's correlation coefficients between hematologic and biochemical variables and anthropometry in 219 rural Mexican preschool children at baseline<sup>1</sup>

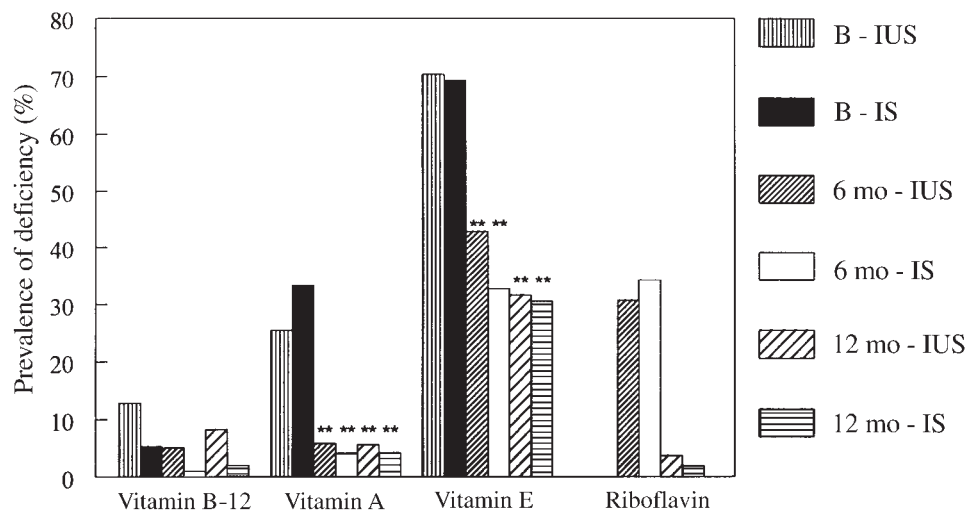
	Hematocrit	RBC count	MCH	MCV	Ferritin	Plasma vitamin B-12	Retinol	Tocopherol	Age	Wt-for-age	Ht-for-age	Wt-for-ht
Hemoglobin	0.76 <sup>2</sup>	0.42 <sup>2</sup>	0.42 <sup>2</sup>	0.04	0.33 <sup>2</sup>	-0.05	0.17 <sup>3</sup>	0.02	0.20 <sup>4</sup>	0.21 <sup>4</sup>	0.20 <sup>4</sup>	0.11
Hematocrit		0.57 <sup>2</sup>	0.05	0.05	0.23 <sup>4</sup>	0.20 <sup>3</sup>	0.33 <sup>2</sup>	0.21 <sup>4</sup>	0.23 <sup>4</sup>	0.29 <sup>2</sup>	0.34 <sup>2</sup>	0.07
RBC count			-0.63 <sup>2</sup>	-0.77 <sup>2</sup>	-0.02	0.27 <sup>2</sup>	0.14	0.10	0.08	0.22 <sup>4</sup>	0.17 <sup>3</sup>	0.11
MCH				0.82 <sup>2</sup>	0.22 <sup>4</sup>	-0.28 <sup>2</sup>	0.01	-0.09	0.08	-0.07	-0.01	-0.04
MCV					0.18 <sup>3</sup>	-0.17 <sup>3</sup>	0.08	0.04	0.06	-0.06	0.04	-0.10
Ferritin <sup>5</sup>						-0.22 <sup>4</sup>	0.05	0.05	0.32 <sup>2</sup>	0.05	0.03	0.04
Plasma vitamin B-12 <sup>5</sup>							0.10	0.03	-0.08	0.11	0.12	0.02
Plasma retinol <sup>5</sup>								0.32 <sup>2</sup>	0.16 <sup>3</sup>	0.17 <sup>3</sup>	0.15	0.11
Plasma $\alpha$ -tocopherol <sup>5</sup>									0.23 <sup>4</sup>	0.10	0.06	0.08
Age										0.00	-0.03	0.03
Wt-for-age											0.72 <sup>2</sup>	0.71 <sup>2</sup>
Ht-for-age												0.05

<sup>1</sup>RBC, red blood cell; MCH, mean cell hemoglobin; MCV, mean corpuscular volume; wt, weight; ht, height.<sup>2</sup> $P < 0.001$ .<sup>3</sup> $P < 0.05$ .<sup>4</sup> $P < 0.01$ .<sup>5</sup>Log-transformed variable.

definitions. On the basis of the first definition of response, an increase in hemoglobin of  $>10$  g/L from baseline to 6 and 12 mo in the IS group, 37% of the children (35 of 94) were classified as responders and 63% were classified as nonresponders at 6 mo. Responders had lower initial hemoglobin concentrations than did nonresponders ( $99.8 \pm 8.3$  and  $108.6 \pm 5.2$  g/L, respectively;  $P < 0.001$ ) and lower initial hematocrit values ( $0.32 \pm 0.03$  and  $0.34 \pm 0.02$ , respectively;  $P < 0.05$ ). At 12 mo, 45% of the children (38 of 84) were classified as responders and 55% were nonresponders. Responders consumed more cereal ( $2.2 \pm 0.7$  compared with  $1.7 \pm 0.8$  servings/d;  $P < 0.01$ ) and fewer tortillas ( $4.3 \pm 2.5$  compared with  $5.9 \pm 3.1$  servings/d;  $P < 0.03$ ) and had lower initial hemoglobin concentrations ( $101.5 \pm 9.2$  versus  $114.9 \pm 10.8$  g/L;  $P < 0.001$ ) and lower initial hematocrit values ( $0.33 \pm 0.03$

versus  $0.35 \pm 0.03$ ;  $P < 0.01$ ) than the nonresponders. The logistic regression showed that lower initial hemoglobin concentration was the only significant determinant ( $P < 0.001$ ) of hemoglobin response from baseline to 6 mo. The significant predictors of positive response from baseline to 12 mo were lower initial hemoglobin concentration ( $P < 0.001$ ) and lower tortilla intake ( $P < 0.01$ ).

A second and more specific definition of response to iron supplementation was added because of the change in hemoglobin concentration from baseline to 6 and 12 mo in the IUS group. This definition of response was a change in hemoglobin, either from 0 to 6 mo or from 0 to 12 mo, that was above the regression line in the multiple regression models of change in hemoglobin with initial hemoglobin, initial ferritin, change in ferritin, and zinc supplementation included in the model. On the basis of



**FIGURE 3.** Prevalence of micronutrient deficiencies at baseline (B), 6 mo, and 12 mo in the iron-unsupplemented (IUS;  $n = 109$ ) and iron-supplemented (IS;  $n = 108$ ) groups. \*\*Significantly different from the respective baseline value,  $P < 0.05$  (repeated-measures categorical procedures). Riboflavin status was not assessed at baseline.

**TABLE 3**  
Mean daily intake of food groups by 219 rural Mexican preschool children

Food group	Value	
	<i>servings/d</i>	
Animal products		
Raw milk	0.76 ± 1.26 <sup>1</sup>	0.00 <sup>2</sup>
Powdered milk	0.13 ± 0.55	0.00
Pasteurized and evaporated milks	0.87 ± 1.27	0.00
Eggs	0.62 ± 0.35	0.64
Meat	0.47 ± 0.27	0.41
Cheese	0.15 ± 0.23	0.07
Total	3.00 ± 1.46	2.65
Plant products		
Fruit	1.08 ± 0.93	1.11
Cereal	2.09 ± 0.85	1.93
Vegetables	0.67 ± 0.50	0.72
Legumes	0.48 ± 0.31	0.43
Tortillas	4.52 ± 3.32	4.50
Total	9.64 ± 2.95	9.23

<sup>1</sup> $\bar{x} \pm$  SD.<sup>2</sup>Median.

this definition, 48% of the children (42 of 88) were classified as responders and 52% were classified as nonresponders at 6 mo. Responders consumed milk more often ( $1.9 \pm 1.0$  compared with  $1.4 \pm 0.9$  servings/d;  $P < 0.02$ ), ate fewer tortillas ( $4.4 \pm 2.9$  compared with  $5.7 \pm 3.0$  servings/d;  $P < 0.05$ ), had higher initial plasma vitamin B-12 concentrations ( $344.7 \pm 153.7$  compared with  $261.3 \pm 101.9$  pmol/L;  $P < 0.02$ ), and were taller ( $z$  scores of  $-1.3 \pm 1.3$  compared with  $-1.8 \pm 1.1$ ;  $P < 0.03$ ) than nonresponders. At 12 mo, 39% of the children (31 of 79) were classified as responders and 61% were classified as nonresponders. Responders consumed milk more often ( $2.1 \pm 1.0$  compared with  $1.4 \pm 1.0$  servings/d;  $P < 0.005$ ) and ate fruit more often ( $1.6 \pm 0.7$  compared with  $1.1 \pm 0.9$  servings/d;  $P < 0.05$ ), but none of the biochemical variables differed significantly between the responders and the nonresponders. The indicators of morbidity (prevalence and incidence) were not significantly different

between the responders and the nonresponders at either 6 or 12 mo (data not shown). The logistic regression showed that initial plasma vitamin B-12 concentration was the only significant determinant ( $P < 0.04$ ) of hemoglobin response from baseline to 6 mo, and age was the only significant determinant ( $P < 0.03$ ) of change from baseline to 12 mo.

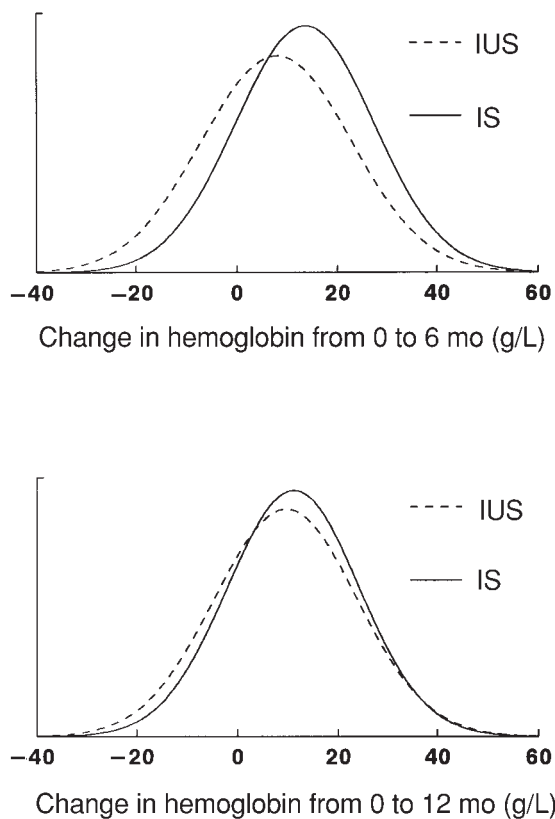
## DISCUSSION

In recent years, nutrition research in developing countries has focused primarily on iron, vitamin A, and iodine deficiencies and there has been relatively little investigation of the prevalence of other micronutrient problems. It is recognized that anemia affects most infants, young children, and women in developing countries and it is generally assumed that anemia is caused by iron deficiency. Thus, it is assumed that the many existing, large-scale iron-supplementation programs would be effective for the prevention and treatment of anemia if adequate amounts of iron were consumed. However, the predicted and actual prevalences of deficiencies of other micronutrients required for hemoglobin synthesis are high in population groups such as the rural Mexican children studied here, who consume low amounts of animal products and have frequent episodes of intestinal illness and parasitic infection. In these Mexican children, deficiencies of iron, vitamin A, vitamin B-12, vitamin E, and riboflavin were common, especially at baseline when the children were 28 mo old on average. The prevalences of anemia and deficiencies of iron, vitamin A, vitamin E, and riboflavin decreased considerably as the children aged, suggesting that the higher nutrient requirements of younger children, poor complementary feeding, or both place them at greater nutritional risk.

After 1 y of supervised iron supplementation, the children's hemoglobin concentrations were not significantly higher than those of unsupplemented children. Many iron-supplemented children remained anemic (30% at 6 mo and 31% at 12 mo) despite improved iron status and eradication of the iron deficiency that was highly prevalent at baseline. Clearly, the lack of hemoglobin response to iron in this study cannot be attributed to noncompliance, an inadequate duration of supplementation, or lack of iron absorption. Moreover, high prevalences of elevated MCV and low MCH were found at the end of the supplementation period.

**TABLE 4**  
Determinants of change in iron-status variables from baseline to 6 and 12 mo in 219 rural Mexican preschool children

Iron-status variable	Baseline to 6 mo				Baseline to 12 mo			
	Predictor	Regression coefficient ± SE	<i>P</i>	<i>R</i> <sup>2</sup>	Predictor	Regression coefficient ± SE	<i>P</i>	<i>R</i> <sup>2</sup>
Hemoglobin	Initial hemoglobin	-0.09 ± 0.01	0.001	0.61	Initial hemoglobin	-0.09 ± 0.01	0.001	0.65
	Height-for-age at 6 mo	0.28 ± 0.07	0.001		Initial vitamin B-12	0.39 ± 0.16	0.02	
	Age	0.26 ± 0.11	0.02					
Hematocrit	Initial hematocrit	-71.0 ± 7.5	0.001	0.41	Initial hematocrit	-68.2 ± 7.4	0.001	0.41
	Incidence of illness	0.09 ± 0.02	0.001		Initial ferritin	-0.76 ± 0.23	0.001	
					Cereal intake	0.09 ± 0.04	0.02	
					Age	0.79 ± 0.34	0.03	
Ferritin	Initial ferritin	-6.63 ± 1.30	0.001	0.34	Initial ferritin	-8.27 ± 1.60	0.001	0.52
	Iron supplementation	18.26 ± 2.34	0.001		Iron supplementation	25.48 ± 2.83	0.001	
					Weight-for-age at 12 mo	4.46 ± 1.92	0.03	
					Fruit intake	0.52 ± 0.24	0.04	



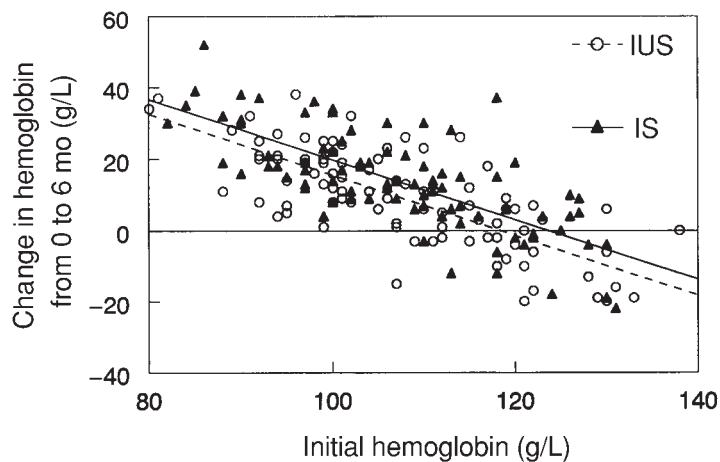
**FIGURE 4.** Estimated probability density curve of the change in hemoglobin concentrations from baseline (0 mo) to 6 mo and from baseline to 12 mo in children in the iron-unsupplemented (IUS;  $n = 109$ ) and iron-supplemented (IS;  $n = 108$ ) groups. The mean change was significantly greater in the IS group than in the IUS group at 6 mo but not at 12 mo,  $P < 0.01$ .

Although 70% of these children were anemic (low hemoglobin) at baseline, low hematocrit values were much less common. The strong correlation between hemoglobin and hematocrit values ( $r = 0.76$ ,  $P < 0.001$ ) argues against a methodologic explanation for the relatively normal hematocrit concentra-

tions. One possible explanation is that the elevated MCV increased the volume of packed red cells. Hematocrit is also less sensitive than is hemoglobin to iron deficiency; Graitcer et al (30) found that in early cases of moderate iron deficiency, a marginally low hemoglobin concentration was associated with a near-normal hematocrit.

The 45–80% prevalence of elevated MCV and the 0–14% prevalence of elevated MCH during the study are remarkable. Because of the high prevalence of vitamin B-12 deficiency, it was logical to explore whether low plasma vitamin B-12 concentrations might explain the high MCV and MCH values. Regression analyses controlling for iron status as a potential confounder showed that plasma vitamin B-12 concentrations were inversely related to MCH values, but not to MCV values. Folate deficiency could also increase MCV and MCH. However, folate status was not measured in this study because previous research in this community indicated normal plasma folate concentrations in preschoolers (8) and adults (9) and the predicted prevalence of inadequate intakes by preschoolers was zero given the amounts consumed (10).

Other investigators have reported incomplete recovery of hemoglobin after iron supplementation of anemic children (31). In a study by Palupi et al (31), a once-weekly iron supplement was provided for 9 wk to 289 preschoolers in West Java. The prevalence of anemia decreased from 37% to 18% in the supplemented group, compared with a decrease from 36% to 27% in the control group. The incomplete hemoglobin recovery was attributed to the short length of the study or to deficiencies of other micronutrients, such as vitamin A. In several studies, the inclusion of other micronutrients in iron supplements did improve the hemoglobin response. For example, the increase in hemoglobin was greater in anemic pregnant Indonesian women supplemented with both vitamin A and iron daily for 8 wk than in those supplemented with iron or vitamin A alone (32). Vitamin A supplements alone produced a  $9.3 \pm 5.6$  g/L increase in the average hemoglobin concentration of anemic Guatemalan children aged 1–8 y (33). In The Gambia, the hemoglobin response of men was greatly improved after supplementation with riboflavin in addition to iron, especially in men who were most anemic initially (34), and riboflavin supplementation alone for 14 d improved hemoglobin compared




**FIGURE 5.** Relation between initial hemoglobin concentration and change in hemoglobin concentration from baseline (0 mo) to 6 mo in children in the iron-unsupplemented (IUS;  $n = 109$ ) and iron-supplemented (IS;  $n = 108$ ) groups.

with a placebo (35). In lactating Guatemalan women, we found that adding riboflavin to iron supplements improved plasma ferritin marginally more than did iron supplementation alone, although hemoglobin concentrations were not increased after 2 mo of supplementation with iron, riboflavin, or both (LH Allen, MT Ruel, JE Casterline, and LM Rogers, unpublished observations, 2000). Zinc supplementation alone or in combination with iron did not affect hemoglobin synthesis in this study.

In the Mexican children in the present study, anemia and poor hemoglobin response to iron supplementation were associated with generalized undernutrition and poor dietary quality. Hemoglobin was lower in children who were shorter at baseline, and the change in hemoglobin from baseline to 6 mo was predicted by height-for-age in the group as a whole; taller children had a stronger hemoglobin response to iron supplementation between baseline and 6 mo. In this community, dietary quality predicted height-for-age and growth (36) and also may have affected the children's hemoglobin response to iron supplements. Animal product intake was positively correlated with hemoglobin response; children who were more dependent on plant foods such as tortillas and legumes had a poorer hemoglobin response. In this region, an increase in the consumption of animal products increases the intakes of nutrients such as absorbable iron, vitamin A, vitamin B-12, and riboflavin and displaces foods high in phytate and fiber that reduce mineral bioavailability (37).

Children with higher initial vitamin B-12 concentrations were more likely to respond to the iron supplements with improved hemoglobin concentrations. About one-third of the children had deficient or low plasma vitamin B-12 concentrations at each time point. In a previous study in this same location, vitamin B-12 deficiency was found in all age groups studied, with a prevalence of 19–42% (8). We also reported a high percentage (47%) of Guatemalan lactating women with plasma vitamin B-12 concentrations that were low or indicated deficiency (38) and the same percentage (47%) of schoolchildren with *Giardia lamblia* infection who had vitamin B-12 deficiency or low B-12 concentrations (39). The association between hemoglobin response and low plasma vitamin B-12 may indicate that this vitamin deficiency is limiting erythropoiesis, or it may be a proxy for low intakes of animal products and other micronutrients. We are currently conducting a trial on the benefits of adding vitamin B-12 to iron supplements for the treatment of anemia in this community.

In conclusion, there was a lack of hemoglobin response in a high percentage of anemic children who received iron supplements for 12 mo, even though none of the children were iron-deficient at the end of this period. Remarkably, hemoglobin was not higher after children received iron supplements for a year, despite their improved iron status. We attribute the lack of hemoglobin response in this study to a general syndrome of undernutrition, manifested by poor dietary quality and growth and possibly to vitamin B-12 deficiency specifically. Public health interventions usually deliver iron supplements or iron with folate, or fortify food supplies with iron alone, without regard for the possible need for other nutrients necessary for hematopoiesis. In many locations, there may be a need to include other micronutrients in the iron supplements that are currently being provided. Hemoglobin response to iron supplements with or without other micronutrients should be assessed in this and other locations where multiple micronutrient deficiencies are suspected. The ethical problem of providing iron alone to children with a high risk of multiple micronutrient deficiencies also needs to be considered; in most sit-

uations, the addition of other micronutrients to iron supplements would add little to the cost of supplement delivery. 

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