

# School-administered weekly iron-folate supplements improve hemoglobin and ferritin concentrations in Malaysian adolescent girls<sup>1-3</sup>

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See corresponding editorial on page 1069.

## ABSTRACT

**Background:** Iron deficiency and its consequent anemia constitute the commonest micronutrient deficiency in the world.

**Objective:** We investigated whether long-term, weekly iron-folate supplements administered at school would improve hemoglobin and ferritin concentrations in adolescent girls, including those with mild-to-moderate anemia and hemoglobin concentrations indicating borderline anemia.

**Design:** Subjects were 266 girls with hemoglobin concentrations of 80–119.9 g/L (group A) and 358 girls with hemoglobin concentrations of 120–130 g/L (group B) who were otherwise healthy. Two hundred sixty-six girls in group A and 268 girls in group B were randomly assigned to receive either 60 or 120 mg Fe plus 3.5 mg folic acid weekly for 22 wk. Ninety of the girls in group B were randomly assigned to receive only 5 mg folic acid weekly. Capillary hemoglobin and plasma ferritin were measured at baseline and after 12 and 22 wk of supplementation.

**Results:** By the end of the study, 2% of the girls had dropped out and >96% had taken  $\geq 20$  of the 22 tablets; side effects were minimal. Mean plasma ferritin increased significantly in all iron-supplemented groups, independently of initial hemoglobin values and iron doses. Ferritin concentrations decreased in the girls supplemented with folic acid only. As expected, hemoglobin responses to iron were higher in group A than in group B and increases were positively correlated with initial plasma ferritin. Hemoglobin failed to respond to folate supplementation if initial plasma ferritin concentrations were low. Mean hemoglobin increased significantly and consistently in relation to the length of treatment.

**Conclusion:** Long-term, weekly iron-folate supplementation was found to be a practical, safe, effective, and inexpensive method for improving iron nutrition in adolescent schoolgirls. *Am J Clin Nutr* 1999;69:1249–56.

**KEY WORDS** Iron nutrition, iron-folate supplements, effectiveness, safety, adolescents, schoolgirls, nutritional anemia, hemoglobin, Malaysia

## INTRODUCTION

Iron deficiency and its consequent anemia constitute the commonest micronutrient deficiency in the world, affecting an estimated 2 billion people in developing and developed countries

(1), whereas folate deficiency often accompanies iron deficiency in pregnancy in developing countries (2), including Malaysia (3–5). Antenatal iron-supplementation programs in developing countries have been reported to have had limited biological effect in reducing the prevalence of iron deficiency and iron deficiency anemia. These programs have been ineffective, partly because side effects limit compliance, operational failures are the rule (eg, inadequate supplies and poor packaging and presentation of supplements), and health workers and recipients lack appropriate information and motivation (6, 7). Additionally, these programs traditionally have targeted only pregnant women—the thought process being that pregnancy is an isolated event in the life of a woman—and have not paid sufficient attention to the iron status, including iron reserves, of nonpregnant women of childbearing age despite their high risk of iron deficiency and iron deficiency anemia (8, 9).

In experimental animals, the absorption of supplemental iron is greatest when it is administered at times of intestinal mucosal renewal, so that each dose is received by new cells. Thus, inhibition of iron absorption is minimized because of the iron overload in intestinal cells, which occurs with daily iron supplementation (10, 11). Studies conducted in preschool children and pregnant women showed that iron supplementation once (12, 13) or twice (14) per week can be as effective as daily supplementation, whereas the frequency of side effects was 9 times higher (36% compared with 4%) with daily than with weekly supplementation in children (12) and  $\approx 7$  times higher (40% compared with 6%) with daily than with weekly supplementation in pregnant women (13).

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**TABLE 1**  
Iron and folate doses by subject group

Group	Initial hemoglobin <sup>1</sup> g/L	Iron mg/wk	Folate mg/wk
A1 (n = 134)	80–119.9	60	3.5
A2 (n = 132)	80–119.9	120	3.5
B1 (n = 133)	120–130.9	60	3.5
B2 (n = 135)	120–130.9	120	3.5
B3 (n = 90)	120–130.9	0	5.0

<sup>1</sup>An initial hemoglobin concentration of 80–119.9 g/L indicated mild-to-moderate anemia and of 120–130 g/L indicated borderline anemia.

Surveys conducted by the Institute for Medical Research of the Ministry of Health, Kuala Lumpur (IMR), in secondary schools of the 2 Malaysian states of Selangor and Sarawak showed moderate-to-high prevalences of anemia on the basis of World Health Organization (WHO) criteria (2) in adolescent schoolgirls (E-S Tee, unpublished observations, 1994). On the basis of these findings and as part of a multicenter study promoted by the United Nations University, WHO, and the United Nations Children's Fund, the IMR—in collaboration with the Division of Family Health of the Ministry of Health, Malaysia; the Sarawak Health Department; the WHO Regional Center for Research and Training in Tropical Diseases and Nutrition, Kuala Lumpur; and the Universities of California (Berkeley), Queensland (Australia), and Milano (Italy)—conducted a study of weekly iron-folate supplementation in Sarawakian adolescent schoolgirls.

The general objective of this study was to assess the effectiveness, safety, and feasibility of weekly iron-folate supplementation for improving the iron nutrition, specifically iron reserves, of 12–17-y-old schoolgirls, including those with mild-to-moderate anemia (hemoglobin: 80–119.9 g/L) and those with hemoglobin concentrations indicating borderline anemia (120–130 g/L). The specific objectives were to 1) determine the effect of weekly administration of 60 or 120 mg Fe plus 3.5 mg folic acid on plasma ferritin and hemoglobin concentrations, 2) assess the frequency of side effects of weekly iron-folate supplementation, and 3) assess the social feasibility of weekly iron and folate supplementation under the supervision of schoolteachers.

## SUBJECTS AND METHODS

### Screening and selection of subjects

We used the results of preliminary surveys to select 3 secondary schools in the Samarahan district of Sarawak, Malaysia. This site was selected because anemia had not been studied intensively there. The 3 schools—Sekolah Menengah Kebangsaan (SMK, State Middle School) Asajaya, SMK Semera, and SMK Muara Tuang—were chosen because they were within a 2-h drive from the state capital, Kuching. Girls enrolled in forms 1–4 (ie, secondary school; ages 12–17 y) each had a finger-prick capillary blood sample taken for hemoglobin determination. Those with a hemoglobin value indicating acute or chronic diseases (<80 g/L) that could affect iron metabolism or cause anemia through mechanisms other than nutritional deficiencies were excluded. Malaria is not endemic in this district.

Sample size calculations showed that ≥116 subjects per group were required to distinguish a difference in hemoglobin concen-

trations between any 2 groups or between any 2 times of blood sampling of ≥5 g/L at a 5% comparison-wise significance level (one sided) and with a power of ≥90%, assuming an SD of the change in hemoglobin concentration within each group of ≤13 g/L on the basis of a previous study (15). Estimating a drop out rate of 15–20%, we aimed to have ≈135 girls per group at baseline. Considering that the study had 5 different initial status by supplementation groups (Table 1), the power to detect a 5-g/L difference between groups was ≈63%; differences of 6 and 6.75 g/L could be detected with a power of 80% and 90%, respectively.

One thousand four hundred eight girls were screened (87.5% of all girls in forms 1–4 of the 3 schools) and divided into 4 groups depending on their hemoglobin concentration: 3 girls (0.2%) in group X (<80 g/L, severe anemia), 266 girls (18.9%) in group A (80–119.9 g/L, mild-to-moderate anemia), 358 girls (25.4%) in group B (120–130 g/L, borderline anemia), and 781 girls (55.5%) in group C (>130 g/L, no anemia). The girls in group X were excluded from the study and referred to the Kuching Medical Department for further diagnostic tests and treatment. We randomly selected 135 subjects from group C. This group received no supplements and was not followed up, but their baseline hemoglobin concentration was used to study the correlation of hemoglobin concentration with anthropometry and school performance (to be reported elsewhere). In total, 624 subjects participated in this supplementation trial. Girls in group A were randomly assigned to groups A1 and A2 and those in group B to groups B1, B2, and B3.

The headmasters and teachers of the 3 schools involved were given a detailed briefing on the study protocol. Consent forms were distributed to all girls in the 3 schools and were signed by the girls' parents or guardians, or the headmaster. Fact sheets explaining the project were distributed to all schoolgirls. The protocol of the study was approved by the Research Review Committee and the Ethical Committee of the Ministry of Health, Malaysia.

### Iron and folate supplementation and side effects

The various treatment groups and the amounts of supplementary iron and folate provided are shown in Table 1. Approximately 135 girls were allocated to groups that would receive both iron (60 or 120 mg as ferrous sulfate/wk) and folate (3.5 mg/wk) supplements (A1, A2, B1, and B2); the remaining 90 girls were assigned to group B3 (control group). The girls in group B3 had borderline anemia and received 5.0 mg folate/wk (3.5-mg tablets were unavailable at the time) and no iron; the folate supplements were provided by the Government Medical Store of the Ministry of Health, Malaysia. A similar control group for the anemic girls was not used because it was considered unethical to withhold iron-folate supplements from anemic subjects. The girls who participated were informed that they would be given different types of supplements to establish which was most effective for preventing and treating anemia.

The iron and folate supplements were prepared for this multicenter study by Unifarm, a German-Swiss enterprise based in Chur-Suiza, Guatemala, through arrangements with the Institute of Nutrition of Central America and Panama. The iron and folate contents of the tablets were confirmed by analyses performed at the Division of Human Nutrition of the IMR. Both the 60- and 120-mg Fe tablets had the same shape and size; however, the 120-mg tablet appeared slightly darker yellow than the 60-mg tablet.

The tablets for each girl were packed in a small, sealable plastic bag clearly labeled with the girl's name, class, and school,



and were given to the supervising schoolteacher, who verified that the tablets were ingested. Supplements were taken with a glass of water between meals to optimize iron absorption, either before the midmorning break or at 1500. Students were asked to not drink tea or coffee when taking the tablets (an uncommon practice among secondary school students), which could have inhibited iron absorption. The supplements were given every Monday, from 18 April to 12 September 1994, for 22 wk.

Ingestion of the supplements was supervised by a teacher and recorded on a form along with any reported symptoms (abdominal discomfort, bloating, nausea, heartburn, diarrhea, or constipation) in the previous week that may have been related to the iron supplement. If any girl reported any of these symptoms during the 24 h after taking a supplement she was encouraged to continue the study but to take the supplement with a snack, if necessary. During a 2-wk holiday, the tablets were ingested at home without supervision; the tablets were packed separately with instructions on when to take them and to record any unpleasant side effects during this time. After the children returned to school, the teacher in charge recorded this information on the master form. At the end of the study, all records of supplement intake and side effects were collected and checked together with the number of remaining tablets.

#### Biochemical and hematologic analyses

Capillary blood samples were collected before the supplementation trial began (time  $t_1$ ) and again after 12 ( $t_2$ ) and 22 ( $t_3$ ) wk. Hemoglobin was determined in 0.02 mL blood by using the cyanmethemoglobin method. The blood was collected into a capillary tube calibrated to measure exactly that volume, immediately dropped into a test tube containing 5 mL Drabkin's reagent (Sigma Diagnostics, St Louis), mixed well, and allowed to stay in the dark for  $\geq 10$  min; the color developed was read in a colorimeter at 540 nm. The CV of hemoglobin determinations, based on 2 consecutive, separate, finger-prick samples collected from 68 schoolgirls in July and September 1994 by 4 laboratory staff, was 3.9%.

Additional blood was collected into 2 capillary tubes and processed in the evening of the same day to obtain the hematocrit value and plasma samples. The plasma samples were frozen and stored at  $-20^\circ\text{C}$  for measurement of plasma ferritin concentrations, which was done at the end of the study. Each ferritin assay was performed in duplicate with 10  $\mu\text{L}$  plasma by using the immunoradiometric assay method of the North East Thames Region Immunoassay laboratory, St Bartholomew's Hospital, London.

The quality-control procedure for ferritin concentrations was conducted on 23 d over 2 mo at the same time as plasma ferritin concentrations were measured. Three pools of serum samples [low (25.67  $\mu\text{g/L}$ ), mid (64.93  $\mu\text{g/L}$ ), and high (378.37  $\mu\text{g/L}$ ) plasma ferritin concentrations] were provided by the assay manufacturer. On each day, 8 repeated analyses were carried out for each of the 3 pools. Day-to-day variation (expressed as CV) was 14%, 18%, and 27% for the low, mid, and high pools, respectively. The corresponding mean bias values were  $-3.5$   $\mu\text{g/L}$  ( $-12\%$ ), 2.5  $\mu\text{g/L}$  (4%), and 20.4  $\mu\text{g/L}$  (6%), respectively.

A set of 50 frozen control samples was analyzed blindly for ferritin concentrations at the IMR with the North East Thames Region Immunoassay and in Viteri's laboratory at the Department of Nutritional Sciences, University of California, Berkeley, by using the S-22 enzyme-linked immunoassay method (Ramco

Laboratories, Inc, Houston). The correlation coefficient between the results obtained with these 2 methods was 0.982.

#### Other data collected

A sample of 124 girls, or 20% of all study subjects in groups A and B, was randomly selected for dietary assessment. The number of boarding and nonboarding schoolgirls included was proportional to the existing ratio in the schools. Nurses from a government clinic in Kuching, after being trained for this survey, interviewed subjects once using a food-frequency questionnaire and 3 times using a 24-h recall questionnaire referring to 2 different weekdays and 1 Sunday.

Stature and weight measurements were taken for all study subjects, including those in group C. After the school year ended in November 1994, school authorities provided the grades obtained by all girls participating in the study (including those in group C) for 3 academic subjects (English language, Malay language, and mathematics) as well as the average score for all subjects. These grades were provided for semester 1, ending  $\approx 1$  mo after the study started and for semester 2, which ended shortly after the study ended. The number of days absent from school was also recorded for all subjects. The results of this part of the study will be reported separately.

#### Statistics

Data were processed and analyzed by using the SAS statistical package (SAS Institute Inc, Cary, NC). Individual time profiles of hemoglobin values were fitted by 2 multivariate analysis of variance (ANOVA) models. The terms included in the first model were main factors: hemoglobin group (anemic or borderline anemic), treatment (folate only, 60 mg Fe + folate, and 120 mg Fe + folate), and their interaction. This model was also fitted to plasma ferritin profiles after log transformation. The terms included in the second model were main factors: plasma ferritin group (below first tertile, intermediate, above second tertile), treatment (as above), and their interaction.

With the first model, larger increases in hemoglobin were expected in anemic than in borderline-anemic girls because hemoglobin groups, by definition, were identified on the basis of initial hemoglobin concentration. This regression to the mean effect could not be estimated directly because ethical concerns did not allow us to treat anemic girls with folate only. However, the extent of this effect, estimated from the ratio of intra- to interindividual variability, was by far lower than the increases in hemoglobin observed during the study (**Appendix A**).

In the second model, groups were defined on the basis of initial plasma ferritin concentration. This model aimed to estimate how initial iron status (as measured by plasma ferritin concentration) affected the hemoglobin response to iron and folate supplements in groups of girls with similar baseline hemoglobin concentrations. Subjects were classified as having initially low, mid, or high plasma ferritin concentrations; tertiles were used as cutoffs (29.5 and 58.5  $\mu\text{g/L}$ ). In the 63 subjects in whom baseline plasma ferritin concentrations were not available, the value at 12 wk was used ( $n = 14$  in group A1, 10 in group A2, 7 in group B1, 17 in group B2, and 15 in B3). Only subjects with all 3 hemoglobin values available and  $\geq 1$  ferritin value (either at  $t_1$  or  $t_2$ ) were included in the analysis of hemoglobin profiles, for a total of 591 cases. The analysis carried out after exclusion of the 63 subjects without baseline plasma ferritin concentrations resulted in similar results.



**TABLE 2**

Hemoglobin concentration at baseline (*t*1) and after 12 (*t*2) and 22 wk (*t*3) of supplementation with iron and folate or folate only<sup>1</sup>

Group	Baseline ( <i>t</i> 1)	After 12 wk ( <i>t</i> 2)	After 22 wk ( <i>t</i> 3)
	<i>g/L</i>		
A1 ( <i>n</i> = 133)	110.4 ± 0.56	128.5 ± 1.15 <sup>2</sup>	131.8 ± 0.96 <sup>2,3</sup>
A2 ( <i>n</i> = 121)	109.5 ± 0.58	127.4 ± 1.20 <sup>2</sup>	132.6 ± 1.01 <sup>2,3</sup>
B1 ( <i>n</i> = 122)	125.2 ± 0.58	132.6 ± 1.20 <sup>2</sup>	136.6 ± 1.00 <sup>2,3</sup>
B2 ( <i>n</i> = 128)	125.2 ± 0.57	135.6 ± 1.17 <sup>2</sup>	138.2 ± 0.98 <sup>2,3</sup>
B3 ( <i>n</i> = 87)	125.1 ± 0.69	131.2 ± 1.42 <sup>2</sup>	134.4 ± 1.19 <sup>2,3</sup>

<sup>1</sup> $\bar{x} \pm SE$ . A1: mild-to-moderate anemia, 3.5 mg folate + 60 mg Fe; A2: mild-to-moderate anemia, 3.5 mg folate + 120 mg Fe; B1: borderline anemia, 3.5 mg folate + 60 mg Fe; B2: borderline anemia, 3.5 mg folate + 120 mg Fe; B3: borderline anemia, 5.0 mg folate.

<sup>2</sup>Significantly different from baseline,  $P < 0.001$ .

<sup>3</sup>Significantly different from after 12 wk,  $P < 0.01$ .

The number of weeks with complaints of side effects during the trial (out of 22 wk) was fitted by an ANOVA model after arc sine transformation because this variable is assumed to have binomial distribution. The terms included in this model were main factors: hemoglobin group, treatment, and the interaction between hemoglobin group and treatment.

## RESULTS

### Compliance and side effects

Only 13 girls (2.1%) dropped out of the study, because they were transferred to other schools, leaving 611 study subjects in groups A and B ( $\approx 38\%$  of all students in the 3 schools). More than 96% of the girls took  $\geq 20$  of the 22 iron-folate tablets provided, 2–3% took 18–19 tablets, and 1% took less. The mean number of weeks for which side effects were reported in each group was as follows: 1.7 for group A1, 2.2 for group A2, 1.6 for group B1, 2.1 for group B2, and 0.8 for group B3. The girls who took 60 or 120 mg Fe plus folate complained, on average, 2 and 3 times more frequently, respectively, than did those who took folate only. The frequency of complaints was similar in the anemic and borderline-anemic subjects who took the same dose of iron.

The ANOVA showed no significant difference between the anemic and borderline-anemic girls in the number of weeks for which complaints were reported. The girls who took 120 mg Fe had significantly ( $P = 0.024$ ) more complaints than did those who took 60 mg Fe, independently of hemoglobin group (no significant interaction between hemoglobin group and treatment). Of the borderline-anemic girls, both those who took 60 mg Fe and those who took 120 mg Fe complained of side effects more frequently than did those who took folate only ( $P = 0.047$  and  $P < 0.001$ , respectively).

Complaints were reported for  $\leq 5$  wk (out of 22 wk) by 87–92% of the girls who took both iron and folate and by all the girls who took folate only. Complaints decreased rapidly after the first few weeks in all groups. Of the girls who took both iron and folate, 25–34% reported side effects in the first week, whereas only 13–16% still had complaints after 4 wk and 1–6% after 20 wk.

### Changes in hemoglobin and plasma ferritin

Mean hemoglobin concentrations in the 5 study groups and changes over time are shown in **Table 2** and **Figure 1**. Concentra-

tions for all girls and for girls categorized by having low, mid, or high initial plasma ferritin concentrations are shown in **Figure 1**.

At baseline, the only difference in mean hemoglobin concentrations was between the anemic and the borderline-anemic girls, a consequence of the girls having been divided them into these 2 groups. Within each of the 5 study groups, all increases in hemoglobin between *t*1 and *t*2 and between *t*2 and *t*3 were highly significant ( $P < 0.01$ ). The greatest increases occurred during the first 12 wk: 18.1 g/L in group A1, 17.9 g/L in group A2, 7.4 g/L in group B1, 10.4 g/L in group B2, and 6.1 g/L in group B3. In the following 10 wk, smaller hemoglobin increases occurred: 3.3 g/L in group A1, 5.2 g/L in group A2, 4.0 g/L in group B1, 2.6 g/L in group B2, and 3.2 g/L in group B3.

After 12 wk of supplementation, the hemoglobin increase observed in the anemic girls was significantly higher ( $P < 0.001$ ) than that observed in the borderline-anemic girls, regardless of the iron dose (ie, there was no significant interaction between hemoglobin group and treatment group, 60 mg Fe compared with 120 mg Fe). Of the borderline-anemic girls, those who took 120 mg Fe plus folate had greater hemoglobin increases than did those who took no iron ( $P = 0.018$ ), whereas there were no significant differences in the hemoglobin increases between those who took 60 mg Fe plus folate and those who took folate only or between those who took 120 mg Fe and those who took 60 mg Fe. These findings were confirmed when changes over the entire 22-wk period were considered. There was no significant difference in hemoglobin increases between *t*2 and *t*3 between the anemic and borderline-anemic girls who took iron or in relation to the effect of different doses of iron.

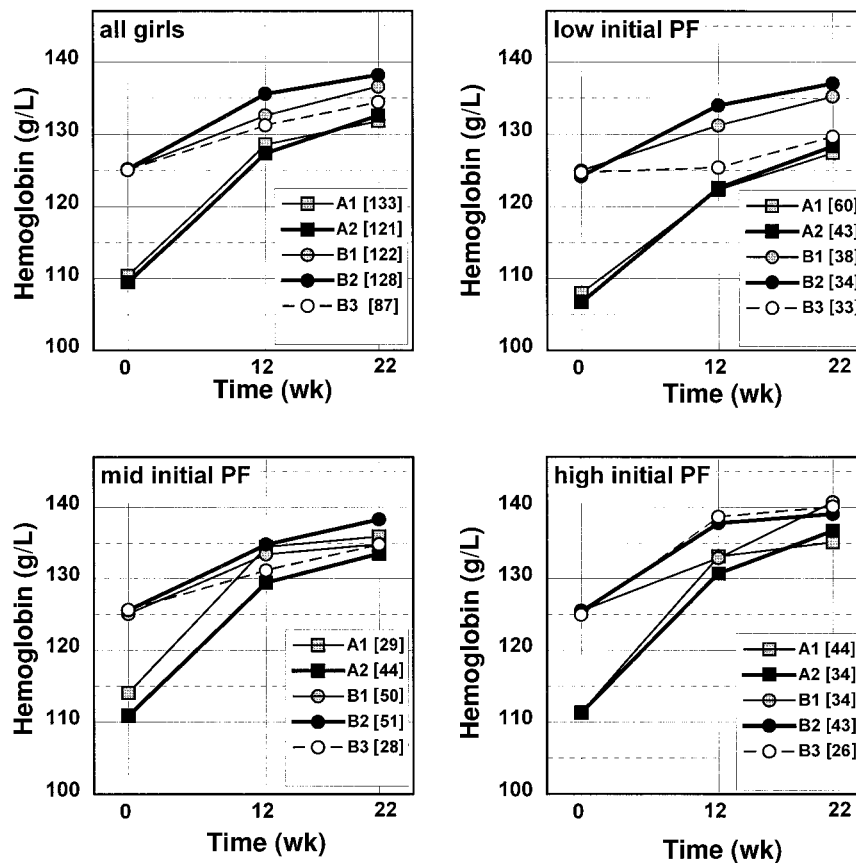
We subsequently considered together all girls with hemoglobin values between 80 and 130 g/L, ie, without distinguishing between anemic and borderline-anemic subjects, and divided them by using tertiles of initial plasma ferritin concentration as cutoffs. Nine groups were thus created on the basis of 3 doses of iron supplements (0, 60, and 120 mg) and 3 initial plasma ferritin concentrations (low, mid, and high) (**Table 3** and **Figure 2**). During the first 12 wk, all groups who took 60 or 120 mg Fe plus folate had highly significant increases in mean hemoglobin concentrations, varying between 11.2 and 15.5 g/L ( $P < 0.001$ ). Hemoglobin increases were related to initial plasma ferritin concentrations, with smaller increases in the groups with lower initial plasma ferritin concentrations (**Table 3**). When plasma ferritin was low (0.7 g/L) there was a negligible increase in hemoglobin in the girls who took folate only; when plasma ferritin was mid or high there were significant increases in hemoglobin, 5.5 g/L and 13.7 g/L, respectively, the latter being comparable with that of girls who took iron plus folate (**Table 3**). Over the duration of the study (22 wk), the findings were similar, but hemoglobin increases were larger.

The number and percentage of borderline-anemic girls who became anemic or normal during the study are shown in **Table 4**. In all 3 groups, the proportion of normal subjects was consistently higher after 22 wk than after 12 wk of treatment. Conversely, in all 3 groups there was a certain number of girls who became anemic. The proportion of anemic subjects in group B3 who took folate only was much higher than the proportions of anemic subjects in groups B1 and B2.

As for the girls who were anemic initially, 81% in group A1 and 78% in group A2 were no longer anemic after taking iron-folate supplements for 12 wk. After 10 more wk of the same supplementation schedule, 83% in group A1 and 89% in group A2 were







**FIGURE 1.** Mean hemoglobin concentrations over the 22-wk study by treatment group [A1 (mild-to-moderate anemia), 60 mg Fe + 3.5 mg folate; A2 (mild-to-moderate anemia), 120 mg Fe + 3.5 mg folate; B1 (borderline anemia), 60 mg Fe + 3.5 mg folate; B2 (borderline anemia), 120 mg Fe + 3.5 mg folate; and B3 (borderline anemia), 5.0 mg folate] and hemoglobin and plasma ferritin (PF) status. *n* in brackets.

no longer anemic. Geometric mean ( $\pm$ SE) plasma ferritin concentrations at  $t_1$ ,  $t_2$ , and  $t_3$  are shown in **Table 5**. Plasma ferritin increased in all 4 groups taking iron plus folate, whereas it decreased in the group taking folate only. At baseline, the ANOVA showed that the borderline-anemic girls had significantly higher plasma ferritin concentration than the anemic girls ( $P = 0.008$ ).

In group A1, mean increases in plasma ferritin after 12 and 22 wk were 3.2 and 7.7  $\mu$ g/L, respectively ( $P = 0.117$  and  $P < 0.001$ , respectively). In group A2, the respective increases were 6.7 and 7.2  $\mu$ g/L ( $P = 0.002$  and  $P < 0.001$ , respectively). There were no significant differences in plasma ferritin increases after 12 and 22 wk between those taking 120 mg Fe and those taking 60 mg Fe.

Similar increases occurred after 12 and 22 wk in the borderline-anemic girls who took iron supplements ( $P \leq 0.003$ ). By contrast, in the borderline-anemic girls who took folate only, plasma ferritin decreased by 6.6  $\mu$ g/L after 12 wk ( $P = 0.010$ ); a less prominent decrease was still present after 22 wk (4.8  $\mu$ g/L,  $P = 0.058$ ). Plasma ferritin time profiles of the girls treated with 60 or 120 mg Fe were significantly different from those of girls who took folate only ( $P < 0.001$ ).

## DISCUSSION

Baseline plasma ferritin concentrations in the anemic girls were not as low as expected for an iron-deficient population. Iron deficiency, however, was indicated by the following: 1)

**TABLE 3**

Changes in hemoglobin (Hb) concentration after 12 wk of treatment, with subjects grouped by type of supplement and initial plasma ferritin concentration tertile

Supplement and baseline plasma ferritin	Baseline Hb g/L	Hb change from baseline g/L
5.0 mg folate only		
Low ( $n = 33$ )	124.7 $\pm$ 1.62 <sup>1</sup>	0.7 $\pm$ 2.35
Mid ( $n = 28$ )	125.6 $\pm$ 1.76	5.5 $\pm$ 2.55 <sup>2</sup>
High ( $n = 26$ )	124.9 $\pm$ 1.82	13.7 $\pm$ 2.65 <sup>3</sup>
3.5 mg folate + 60 mg Fe		
Low ( $n = 98$ )	114.6 $\pm$ 0.94	11.2 $\pm$ 1.36 <sup>3</sup>
Mid ( $n = 79$ )	121.0 $\pm$ 1.05	12.8 $\pm$ 1.52 <sup>3</sup>
High ( $n = 78$ )	117.5 $\pm$ 1.06	15.5 $\pm$ 1.53 <sup>3</sup>
3.5 mg folate + 120 mg Fe		
Low ( $n = 77$ )	114.4 $\pm$ 0.95	13.2 $\pm$ 1.54 <sup>3</sup>
Mid ( $n = 95$ )	118.8 $\pm$ 0.89	13.5 $\pm$ 1.39 <sup>3</sup>
High ( $n = 77$ )	119.2 $\pm$ 0.95	15.5 $\pm$ 1.54 <sup>3</sup>

<sup>1</sup> $\bar{x} \pm$ SE. Effects of initial plasma ferritin, iron, and interaction between plasma ferritin and iron concentrations on changes in hemoglobin concentration are as follows: initial plasma ferritin ( $P < 0.001$ , linear trend;  $P = 0.447$ , departure from linear trend), iron ( $P < 0.001$ , linear trend;  $P = 0.020$ , departure from linear trend), plasma ferritin  $\times$  iron ( $P = 0.010$ , linear trend  $\times$  linear trend;  $P = 0.617$ , other components of the interaction).

<sup>2,3</sup>Significant change: <sup>2</sup> $P = 0.03$ , <sup>3</sup> $P < 0.001$ .

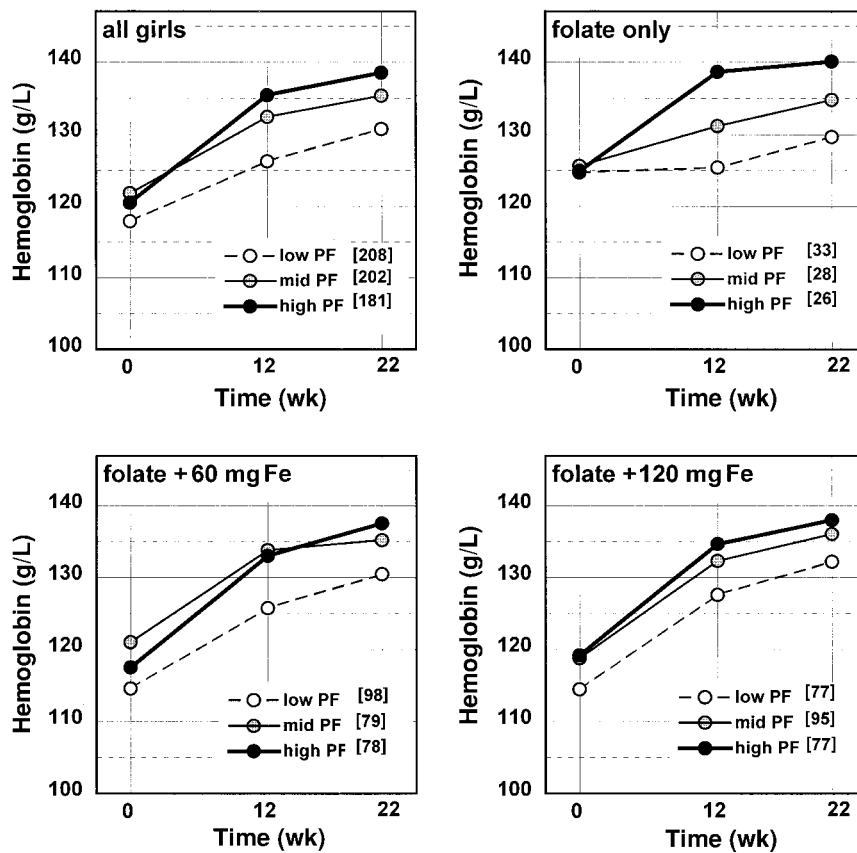


FIGURE 2. Mean hemoglobin concentrations over the 22-wk study by treatment and plasma ferritin (PF) status. *n* in brackets.

baseline ferritin concentrations were significantly lower in groups A1 and A2 than in groups B1, B2, and B3, and 2) the response of ferritin concentrations to the 2 iron doses in groups A1, A2, B1, and B2 was in the expected direction (ie, they increased), whereas ferritin concentrations decreased over the course of the study in the group that received no iron (group B3).

In each of the 5 study groups, mean hemoglobin increased considerably in the first 12 wk and to a lesser extent in the following 10 wk. About 45% of this change in the anemic group and  $\approx 30\%$  in the borderline-anemic group was attributed to regression to the mean (Appendix A). The second model used for data analysis (Table 3 and Figure 2) clearly confirmed that increases in hemoglobin were strongly related to iron status on the basis of initial plasma ferritin concentrations, the response to iron supple-

mentation, or both. This was particularly evident in group B3, who did not receive iron supplements, because the increase in hemoglobin concentrations in response to the folate supplement was directly proportional to iron stores. Note that the girls in this group (Table 3) had initially higher hemoglobin concentrations ( $\approx 125$  g/L) than the girls who took iron and folate supplements; therefore, their hemoglobin increases were less affected by regression to the mean. The mean hemoglobin concentration was 131.8 g/L in the whole sample studied (1408 girls in 3 schools).

The mean hemoglobin increase was  $\approx 2$  times greater in the anemic girls (groups A1 and A2) than in the borderline-anemic girls (groups B1 and B2) during the first 12 wk ( $P < 0.001$ ); this difference was attributed in part to a greater efficiency of iron absorption as a result of the iron deficiency because the mean

TABLE 4

Number and percentage of girls who were anemic (hemoglobin  $< 120$  g/L) or borderline anemic (hemoglobin = 120–130 g/L) at baseline and who became (or remained) anemic, borderline-anemic, or normal (hemoglobin  $> 130$  g/L) after 12 (*t*2) and 22 wk (*t*3) of supplementation with iron and folate or folate only<sup>1</sup>

Group	After 12 wk ( <i>t</i> 2)			After 22 wk ( <i>t</i> 3)		
	Anemia	Borderline anemia	Normal	Anemia	Borderline anemia	Normal
A1	25 (18.8)	39 (29.3)	69 (51.9)	22 (16.5)	23 (17.3)	88 (66.2)
A2	27 (22.3)	43 (35.5)	51 (42.2)	13 (10.7)	34 (28.1)	74 (61.2)
B1	14 (11.5)	40 (32.8)	68 (55.7)	2 (1.6)	29 (23.8)	91 (74.6)
B2	11 (8.6)	33 (25.8)	84 (65.6)	5 (3.9)	17 (13.3)	106 (82.8)
B3	15 (17.2)	30 (34.5)	42 (48.3)	9 (10.3)	20 (23.0)	58 (66.7)

<sup>1</sup>Percentage in parentheses. A1: mild-to-moderate anemia, 3.5 mg folate + 60 mg Fe; A2: mild-to-moderate anemia, 3.5 mg folate + 120 mg Fe; B1: borderline anemia, 3.5 mg folate + 60 mg Fe; B2: borderline anemia, 3.5 mg folate + 120 mg Fe; B3: borderline anemia, 5.0 mg folate.

TABLE 5

Plasma ferritin concentration at baseline (*t*<sub>1</sub>), and after 12 (*t*<sub>2</sub>) and 22 (*t*<sub>3</sub>) wk of supplementation with iron and folate or folate only<sup>1</sup>

Group	Baseline ( <i>t</i> <sub>1</sub> )	After 12 wk ( <i>t</i> <sub>2</sub> )	After 22 wk ( <i>t</i> <sub>3</sub> )
	μg/L		
A1 ( <i>n</i> = 115)	34.3 ± 2.49	37.5 ± 2.49	42.0 ± 2.88 <sup>2</sup>
A2 ( <i>n</i> = 108)	33.4 ± 2.50	40.1 ± 2.75 <sup>3</sup>	40.6 ± 2.88 <sup>2</sup>
B1 ( <i>n</i> = 110)	38.5 ± 2.85	47.3 ± 3.21 <sup>2</sup>	46.1 ± 3.24 <sup>3</sup>
B2 ( <i>n</i> = 108)	44.0 ± 3.30	52.6 ± 3.60 <sup>3</sup>	54.7 ± 3.87 <sup>2</sup>
B3 ( <i>n</i> = 71)	37.9 ± 3.50	31.3 ± 2.65 <sup>3</sup>	33.1 ± 2.89

<sup>1</sup>Geometric  $\bar{x} \pm SE$ . A1: mild-to-moderate anemia, 3.5 mg folate + 60 mg Fe; A2: mild-to-moderate anemia, 3.5 mg folate + 120 mg Fe; B1: borderline anemia, 3.5 mg folate + 60 mg Fe; B2: borderline anemia, 3.5 mg folate + 120 mg Fe; B3: borderline anemia, 5.0 mg folate.

<sup>2,3</sup>Significantly different from baseline: <sup>2</sup> $P < 0.001$ , <sup>3</sup> $P < 0.01$ .

plasma ferritin concentration was significantly lower ( $P = 0.008$ ) in the anemic than in the borderline-anemic girls.


The fact that the hemoglobin concentration increased in all 5 groups, whereas the plasma ferritin concentration increased in the 4 groups who took iron plus folate and decreased in the group who took folate only, suggests that the folate supplement stimulated the synthesis of hemoglobin, thereby resulting in the utilization of existing iron stores and leading to a decrease in plasma ferritin concentrations in group B3. The finding that hemoglobin concentrations increased after the folate supplement alone emphasizes the importance of supplementation with both iron and folate.

In conclusion, this study showed that the long-term (ie, 22 wk), weekly supplementation of adolescent schoolgirls with iron and folate resulted in a significant improvement in their iron nutrition and hemoglobin concentrations. The positive changes included a slow and progressive increase in iron reserves in the iron-supplemented girls, regardless of whether their initial hemoglobin concentration was 80–119.9 (mild-to-moderate anemia) or 120–130 g/L (borderline anemia). In contrast, plasma ferritin concentrations decreased significantly in the girls who received folate supplements only. The hemoglobin response to iron-folate supplements was significant and varied between 11.2 and 15.5 g/L, depending on whether initial plasma ferritin concentrations were high or low. About 55% of this change was independent of the regression to the mean effect; therefore, an increase of  $\geq 6.2$ –8.5 g/L was attributed to the effect of the iron-folate supplements.

The fact that the hemoglobin response to the iron-folate supplements was highest in girls with the highest baseline plasma ferritin concentrations and that the increase in hemoglobin was related to initial plasma ferritin concentrations only in the girls receiving folate only suggests that when iron stores are adequate, folate may limit erythropoiesis somewhat. This possibility needs to be explored further and justifies the use of a combination of iron and folate in schoolgirls aged 12–17 y, particularly because prepregnancy folic acid deficits have been shown to increase the risk of neural tube defects in this population.

Compliance with taking the tablets was good. Side effects were minimal in the long term and in no case stopped any of the girls from taking the supplements. This suggests that both levels of supplementation were safe and that the regimen used did not cause poor adherence or rejections. Considering that the 2 levels of supplementation produced similar increases in hemoglobin and plasma ferritin in the anemic subjects and that the frequency of side effects was lower with the smaller dose of iron, we suggest that weekly

supplements of 60 mg Fe plus 3.5 mg folate be given to adolescent schoolchildren to improve their iron and folate reserves and thus prevent iron deficiency anemia and folate deficiency in areas with a moderate-to-high prevalence of iron deficiency anemia.

Improving iron and folate nutrition and preventing iron deficiency anemia in adolescent girls has a direct benefit on the well-being of this population. In addition, improved iron and folate reserves in these girls is important because of the high proportion of teenage pregnancies in the developing world. Thus, long-term community-based prevention of iron and folate deficiencies and related anemias through weekly iron-folate supplementation of adolescent girls, including one or more future pregnancies, should be implemented. Because such an intervention is safe, well-tolerated, and inexpensive ( $\approx \$0.04$  per person per school year), it would be more cost-effective than periodic screening and selective therapy or than the proposed supplementation schedule (3 mo/y) of the WHO (2, 16). 

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## APPENDIX A

With regard to the problem of regression to the mean, let us consider a random variable  $x$  describing the distribution of hemoglobin. Each value  $x$  may be expressed as

$$x = \mu + \eta + \varepsilon \quad (A1)$$

where  $\mu$  is the mean hemoglobin value in the population,  $\eta$  is a random term [with expectation  $E(\eta) = 0$  and variance  $V(\eta) = \sigma_\eta^2$ ] denoting interindividual variability, and  $\varepsilon$  is a random term [with expectation  $E(\varepsilon) = 0$  and variance  $V(\varepsilon) = \sigma_\varepsilon^2$ ] denoting intraindividual variability (biological variability and measurement error). Let us consider 2 measures taken on the same subject at baseline ( $x_1$ ) and after 12 wk of iron supplementation ( $x_2$ ). On the basis of the hypothesis that the treatment has no effect, we get

$$E(x_1) = E(x_2) = \mu \quad (A2)$$

$$V(x_1) = V(x_2) = \sigma_\eta^2 + \sigma_\varepsilon^2 \quad (A3)$$

$$\text{Cov}(x_1, x_2) = \sigma_\eta^2 \quad (A4)$$

and that the regression of values recorded after treatment on basal values is

$$E(x_2 | x_1) = \mu + \frac{\sigma_\eta^2}{\sigma_\eta^2 + \sigma_\varepsilon^2} (x_1 - \mu) \quad (A5)$$

According to the above expression, the conditional expectation of the second measure is lower than the first measure if the first measure is higher than the population mean and, conversely, is higher than the first measure if the first measure is lower than the population mean. The larger the ratio of intraindividual vari-

ance to the interindividual variance, the larger the effect of the regression to the mean.

In the present study, it was possible to obtain an estimate  $s_2^2$  of  $(\sigma_\eta^2 + \sigma_\varepsilon^2)$  from the distribution of basal hemoglobin values (1408 observations,  $s_2^2 = 222$ ) and an estimate  $s_1^2$  of  $2\sigma_\varepsilon^2$  from the distribution of the differences  $x_2 - x_1$ , (591 observations,  $s_1^2 = 168$ ). The mean hemoglobin concentration in the whole sample studied was 131.8 g/L (1408 girls in 3 schools). Under these conditions, girls in the anemic and borderline-anemic groups would be expected to show mean hemoglobin increases of  $8.3 \pm 0.2$  and  $2.6 \pm 0.1$  g/L, respectively, as can be easily computed from the expression

$$x_2 - x_1 = (\bar{x} - x_1) \left( 1 - \frac{s_2^2 - s_1^2/2}{s_2^2} \right) \quad (A6)$$

where  $\bar{x}$  is the mean of the distribution of baseline hemoglobin values. These expected values are by far (and significantly) lower than the increases observed after 12 wk of treatment: from  $17.9 \pm 1.8$  to  $18.1 \pm 1.3$  g/L in the anemic groups (A2 and A1, respectively) and from  $6.1 \pm 1.6$  to  $10.4 \pm 1.3$  g/L in the borderline-anemic groups (B3 and B2, respectively). This indicates that most of the increase in hemoglobin concentration was attributable to the treatment and not to the regression to the mean. Moreover, mean hemoglobin increased consistently and significantly in relation to the length of treatment, especially in the anemic girls, but also in the borderline-anemic groups. This would not be expected if the regression to the mean was the major source of the effect, nor if some unlikely or unknown factors had exerted their effects on the iron status of the girls included in the sample.

