Concomitant supplemental vitamin A enhances the response to weekly supplemental iron and folic acid in anemic teenagers in urban Bangladesh[1–3]

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ABSTRACT

Background: Iron deficiency is the most common micronutrient deficiency and affects >2 billion persons worldwide, leading to anemia in >40% of women of reproductive age in the developing world.

Objective: The objective was to determine whether weekly supplementation with iron and folate would reduce the frequency of anemia in teenage women in urban Bangladesh before they became pregnant.

Design: Participants with a hemoglobin concentration of 80–120 g/L were entered into a randomized, double-blind, placebo-controlled trial and received supplements of placebo, vitamin A, iron + folic acid, or iron + folic acid + vitamin A weekly for 12 wk. The supplements contained 2.42 mg vitamin A (retinol) as retinyl palmitate, 120 mg elemental Fe as ferrous sulfate, and 3.5 mg folic acid.

Results: Hemoglobin concentrations increased significantly more after supplementation with iron + folic acid or iron + folic acid + vitamin A than after either the placebo or vitamin A alone. There was a significantly greater increase in hemoglobin after iron + folic acid + vitamin A than after iron + folic acid, but the additional effect disappeared after adjustment for baseline hemoglobin, serum vitamin A, and ferritin and the number of supplements taken. Those with the lowest baseline hemoglobin had the greatest increase in hemoglobin. Compared with the placebo, iron + folic acid + vitamin A reduced anemia by 92%, iron deficiency by 90%, and vitamin A deficiency by 76%.

Conclusion: There may be significant health benefits from a program that enhances the nutritional status of iron, folate, and vitamin A in poor urban young women before they become pregnant. *Am J Clin Nutr* 2001;74:108–15.

KEY WORDS Retinol, vitamin A, iron, folate, folic acid, supplementation, adolescence, hemoglobin, teenagers, Bangladesh

INTRODUCTION

Iron deficiency is the most common micronutrient deficiency and affects >2 billion persons worldwide (1). It leads to anemia in >40% of women of reproductive age in the developing world (2). Iron deficiency is associated with an increase in complications in pregnant women and with poorer fetal growth and survival (3). Recent evidence relates poor iron status during pregnancy with an increased risk of chronic disease later in life (4). There are more insidious social costs associated with iron deficiency, eg, impaired work performance and a stunting of intellectual development, that together can constrain social and economic development (5).

The demand for iron increases during periods of growth. One of the oldest intervention strategies is daily supplementation with iron tablets. However, without close control and supervision of consumption, these programs are ineffective because of irregular tablet distribution or poor compliance (6, 7). A practical alternative would be weekly supplementation, which was shown to be effective in different age groups (8–12). Vitamin A may play a part in the hematologic response to iron (13–17), and individuals deficient in vitamin A are more likely to be unresponsive to dietary supplementation with iron (18). After supplementation with vitamin A, hematologic indexes and other measures of iron status improve (18–21). Thus, in a population in which mild-to-moderate vitamin A deficiency is common, iron supplementation alone may fail to improve hemoglobin concentrations unless vitamin A status is also improved. A pregnancy during adolescence, when the mother's own growth and maturation are still occurring, imposes an additional nutritional burden with the potential for long-term adverse effects on the unborn fetus (4). One critical objective should be to prepare the mother's nutritional state in anticipation of any pregnancy, thereby enhancing the quality of each individual pregnancy, reducing the rate of complications and problems, and maximizing the survival of both the mothers and infants.

In Bangladesh, the estimated economic consequence of iron deficiency is a loss of productivity of US$4.19/person annually,

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≈2% of the gross domestic product (22). About 26% of maternal deaths are related to nutritional anemia and postpartum hemorrhage (23). It is usual for the first pregnancy to take place during the teenage years and both vitamin A deficiency and nutritional anemia have been identified as major public health problems (24), especially among women of reproductive age (25). For relatively affluent children or teenage girls, those with the lowest vitamin A concentrations also have low concentrations of hemoglobin and serum iron (15, 16). In adolescent females from the poorest section of the society, 44% are anemic, 56% have marginal vitamin A status, and 14% have vitamin A deficiency (26).

We conducted a prospective intervention study to determine whether weekly supplementation with iron + folic acid would reduce the frequency of anemia in adolescents before they became pregnant. Furthermore, we sought to determine whether concomitant weekly supplementation with a low dose of vitamin A would provide further benefit.

SUBJECTS AND METHODS

Subjects

The subjects were postmenarcheal, nonpregnant teenagers aged 14–19 y who worked in garment factories in Dhaka City, Bangladesh. An open meeting was held at each garment factory to explain the purpose of the study to the female workers. Verbal consent to participate in the study was sought and each potential subject provided a sample of venous blood to screen for anemia. Those with a hemoglobin concentration between 80 and 120 g/L were recruited for the study and randomly allocated to 1 of 4 supplementation groups. Those with a hemoglobin concentration < 80 g/L were excluded from the study and referred for treatment. Those with clinical manifestations of chronic or infectious disease were excluded from the study.

The sample size for the study was calculated on the basis of a difference of 5 g hemoglobin/L between 2 groups with a power of 80% at the 5% significance level. We estimated the SD of the hemoglobin concentration for the population to be 11 g/L (26). Because 2 of the 4 groups were to receive no iron supplementation, an allowance was made for this in the power calculation on the basis of the findings of Suharno et al (27). The study population was highly transitional with a high rate of migration; therefore, we anticipated that a large proportion of the subjects would fail to complete the protocol and thus an allowance of 40% was made for losses. Hence, 120 subjects were recruited to each of the 4 groups to give a total study population of 480 subjects. The study protocol was approved by the ethical committee of the Medical Research Council, Dhaka, Bangladesh. The study was conducted from March to September 1998.

Study design and interventions

A randomized, double-blind, placebo-controlled experimental design was used in a 2 × 2 factorial design and the subjects were randomly assigned to 1 of 4 groups: placebo (a placebo for vitamin A and for iron and folic acid), vitamin A only (2.42 mg retinol as retinyl palmitate and a placebo for iron and folic acid), iron + folic acid (120 mg elemental Fe as ferrous sulfate, 3.5 mg folic acid, and a placebo for vitamin A), and iron + folic acid + vitamin A. All subjects received the preparations weekly for 12 wk. The weekly dose of iron + folic acid was chosen to be similar to that found to be effective in producing a significant increase in hemoglobin in adolescent schoolgirls and is similar to the amount usually used as a supplement during pregnancy (10). The dose of vitamin A was similar to that shown previously to be effective in treating vitamin A deficiency (28). An independent person coded the preparations and the code was not broken until all the data had been entered into the computer.

Compliance

The enrolled subjects were followed at weekly intervals for 12 wk. The field staff made ≥2 visits each week to each of the factories to supervise the consumption of the supplements to ensure maximum compliance. There was some variability in the administration of the supplements, depending on the factory management. In some factories, supplements were given before lunch and in some they were given after lunch. Many subjects came to work after having eaten little or no breakfast and ate only a small lunch. The subjects were offered biscuits (50 g) just before being given the supplement to ensure that it was not taken on an empty stomach. The field staff ensured that the supplements were taken with a glass of water and that all the supplements were swallowed. The field staff maintained a written weekly record of consumption of the supplement for each subject as a measure of compliance.

Data collection

During screening, the field staff obtained a personal history from the subjects and recorded anthropometric measurements. In the presence of a physician, 2 trained nurses collected a 5-mL venous blood sample with a disposable syringe from each subject. An appropriate aliquot of blood was placed directly into Drabkin's solution (5 mL) for hemoglobin measurement. The remainder of the blood sample was placed in an acid-washed glass test tube and kept on ice until centrifugation for serum separation. Blood was transported to the laboratory and hemoglobin was measured within 4 h of collection. Serum was obtained by centrifugation (1125 × *g*, 15 min, 4°C) and stored at −20°C. Within 2 d of screening, the field staff visited the selected subjects to collect information about their health and socioeconomic status and then supplementation commenced.

After the 12-wk period of supplementation, health-related information was recorded again and 5-mL venous blood samples were drawn again from each subject and handled in the same way as were the first samples. At the end of the trial, all of the subjects were provided with a 3-mo supply of multivitamins that contained 60 mg elemental Fe in a commercial preparation.

Analytic procedures

Hemoglobin concentration was measured by using a commercial kit (Boehringer Mannheim, Mannheim, Germany). Serum iron and total-iron-binding capacity (TIBC) were measured by the bathophenanthroline method without deproteinization by using a commercial kit (Boehringer Mannheim). Serum transferrin saturation was calculated by dividing the serum iron concentration by TIBC. Serum ferritin was measured in duplicate by using an enzyme-linked immunosorbent assay method with a kit (GenZyme, Kent, United Kingdom) and the interassay variation was 9.1%. C-reactive protein was measured by using an enzyme-linked immunosorbent assay method with a commercial kit (Eurogenetics, Tessenderlo, Belgium) and the interassay variation was 3.1%. Serum retinol (vitamin A) concentrations were measured as described elsewhere (26) and the interassay variation was 2.7%.

TABLE 1
Anthropometric and sociodemographic characteristics of those who completed the supplementation period and those who did not

	Supplementation not completed (n = 191)		Supplementation completed (n = 289)	
	Mean (95% CI)	Range	Mean (95% CI)	Range
Age (y)	16.1 (15.9, 16.3)	14–19	16.2 (16.1, 16.4)	14–19
Height (m)	1.49 (1.48, 1.50)	1.34–1.63	1.49 (1.48, 1.49)	1.32–1.62
Weight (kg)	40.1 (39.4, 40.8)	30.3–55.5	39.9 (39.4, 40.5)	28.5–56.2
Body mass index (kg/m^2)	18.1 (17.8, 18.3)	14.3–23.5	18.1 (17.9, 18.3)	12.9–25.2
Midupper arm circumference (cm)	21.4 (21.1, 21.7)	18.0–29.5	21.4 (21.2, 21.6)	16.5–27.0
Triceps skinfold thickness (mm)	11.0 (10.5, 11.6)	5.0–20.1	11.1 (10.7, 11.5)	4.0–24.0
Hemoglobin (g/L)	111.5 (110.1, 112.9)	80–120	111.8 (110.8, 112.9)	80–120
Income (taka/mo)[1]	1015 (954, 1076)	500–2200	1182 (1129, 1234)	500–2800

[1] 49 taka = US$1.

Statistical analysis

Statistical analysis was carried out by using SPSS (version 8; SPSS Inc, Chicago) with univariate analysis of simple-frequency distributions of the selected variables. For each biochemical variable, the normality of the distribution was assessed with the Kolmogorov-Smirnov goodness-of-fit test. The social, personal, and health-related data for the 4 treatment groups were compared with the chi-square test by one- or two-factor analysis of variance and with a post hoc Bonferroni test when applicable. To assess the change in prevalence of anemia, iron deficiency, and vitamin A deficiency, logistic regression analysis was used after correction for baseline hemoglobin, ferritin, and retinol concentrations, respectively.

In populations in whom infection is common, it may be difficult to use serum ferritin, which is an acute phase reactant, to determine iron status. Therefore, the serum concentration of C-reactive protein was measured to identify those subjects who might have inflammation at the time of the study. A secondary analysis was conducted in a subpopulation (n = 219) of the study group who had no evidence of infection on the basis of a normal C-reactive protein concentration (≤50.0 μg/L) at the start and the end of the supplementation period and who were clearly iron deficient on the basis of low iron status (serum ferritin <12.0 μg/L): placebo group, n = 58; vitamin A group, n = 44; iron + folic acid group, n = 59; and iron + folic acid + vitamin A group, n = 58. A multivariate analysis was used to adjust for the effect of possible confounding factors on the changes in biochemical concentrations in the blood at the start and the end of the supplementation period.

To assess whether subjects with different degrees of anemia responded differently to supplementation, the subjects were divided into 3 groups on the basis of the distribution of the baseline hemoglobin concentration (<110, 110–118, and >118–120 g/L). It is estimated that an increase in plasma ferritin of 1 μg/L represents an increase in stored iron of 10 mg (29). On the assumption that blood volume is 70 mL/kg body wt and that the iron content of hemoglobin is 4 mol/mol, the iron content of any increase in hemoglobin concentration can be estimated for each individual (30). Thus, the total iron retained in the body as hemoglobin and stored ferritin after 12 wk of supplementation was estimated for the individuals selected on the basis of low initial ferritin and reference C-reactive protein concentrations. The increase in total body iron was expressed as a percentage of the iron administered as the supplement and comparisons were made between groups according to both baseline hemoglobin concentrations and supplementation.

RESULTS

Of the 480 women recruited to the study, 289 subjects completed the 12-wk study protocol: 59% of the placebo group, 62% of the iron + folic acid group, 56% of the vitamin A group, and 65% of the iron + folic acid + vitamin A group. One hundred ninety-one subjects were lost to follow-up for the following reasons: 75% left their job or moved to another factory, 3% became pregnant, 5% refused to give a second blood sample, 12% were absent on the day of blood collection, and 5% did not take the supplements for the full 12-wk supplementation period. The number of subjects lost to follow-up (chi-square test) was not significantly different between groups.

A comparison of the subjects who completed the study and those who did not is given in **Table 1**. The mean age, height, weight, body mass index (BMI; in kg/m^2), midupper arm circumference, and triceps skinfold thickness of the subjects who left the study were similar to those of the subjects who completed the study. There were no significant differences between groups (data not shown). The hemoglobin concentration of the subjects who left the study was comparable with that of the subjects who completed the study; however, those subjects who left the study had hemoglobin concentrations lower than those of the iron + folic acid, iron + folic acid + vitamin A, and vitamin A groups.

On the basis of the compliance record, 68% of subjects in the placebo group received all 12 doses, 24% received 11 doses, 6% received 10 doses, and 2% received 9 doses. In the iron + folic acid group, 60% received 12 doses, 24% received 11 doses, 14% received 10 doses, and 2% received 9 doses. In the vitamin A group, 64% received 12 doses, 25% received 11 doses, 9% received 10 doses, and 2% received 8 doses. In the iron + folic acid + vitamin A group, 57% received 12 doses, 30% received 11 doses, 9% received 10 doses, 3% received 9 doses, and 1% received 8 doses. There was no significant difference in the number of doses received by treatment group. The biochemical data (*see* below) indicated very high levels of compliance.

The mean age, body weight, BMI, midupper arm circumference, and triceps skinfold thickness of each of the 4 supplementation groups were similar (**Table 2**). The subjects in the iron + folic acid group were significantly shorter than those in the vitamin A and iron + folic acid + vitamin A groups. Monthly incomes and food expenditures were not significantly different between the 4 treatment groups. There were no significant differences in family size, marital status, or education level between the 4 treatment groups (data not shown).

Of the 289 subjects who completed the study protocol, 88% were anemic (hemoglobin concentration <120 g/L), 79.5% were iron deficient (serum ferritin <12 μg/L), and 70.5% were vitamin A defi-

TABLE 2
Anthropometric and sociodemographic characteristics of the study participants at baseline by treatment group[1]

	Placebo (n = 71)	Vitamin A (n = 67)	Iron + folic acid (n = 74)	Iron + folic acid + vitamin A (n = 77)	Total (n = 289)
Age (y)	16.3 ± 1.5	16.3 ± 1.6	16.1 ± 1.5	16.1 ± 1.4	16.2 ± 1.5
Body weight (kg)	39.4 ± 4.3	40.1 ± 5.2	39.9 ± 4.7	40.4 ± 4.3	39.9 ± 4.6
Height (cm)[2]	148.3 ± 5.1[a,b]	149.5 ± 4.0[a]	147.5 ± 4.9[b]	149.2 ± 4.7[a]	148.6 ± 4.7
BMI (kg/m^2)	17.9 ± 1.7	18.0 ± 2.2	18.3 ± 2.0	18.1 ± 1.4	18.1 ± 1.9
MUAC (cm)	21.3 ± 1.7	21.2 ± 2.1	21.6 ± 1.9	21.2 ± 1.5	21.3 ± 1.8
SFT (mm)	10.7 ± 2.8	10.7 ± 3.8	11.8 ± 3.5	10.8 ± 2.8	11.0 ± 3.2
Family size (n)	4.3 ± 1.8	4.0 ± 1.7	4.5 ± 2.0	4.8 ± 2.0	4.4 ± 1.9
Income (taka/mo)[3]	1200 ± 457	1238 ± 415	1114 ± 438	1182 ± 467	1182 ± 445
Food expenditure (taka/mo)[3]	738 ± 170	788 ± 251	761 ± 203	835 ± 227	788 ± 218

[1] $\bar{x}$ ± SD. MUAC, midupper arm circumference; SFT, triceps skinfold thickness.
[2] Values with different superscript letters are significantly different, $P < 0.05$ (ANOVA with post hoc Bonferroni correction).
[3] 49 taka = US$1.

cient (serum retinol <1.05 μmol/L) on the basis of World Health Organization criteria. At baseline, there were significant correlations between hemoglobin and ferritin concentrations (r = 0.155, P = 0.008) and between hemoglobin and retinol concentrations (r = 0.282, P = 0.000). Serum retinol concentrations were also marginally correlated with serum ferritin (r = 0.11, P = 0.06).

There were no significant differences in baseline hemoglobin concentrations between the 4 treatment groups (**Table 3**). After the 12-wk supplementation period, the hemoglobin concentration was significantly different between the placebo group and the other 3 groups. Furthermore, the hemoglobin concentration was significantly different between the vitamin A group and the iron + folic acid and iron + folic acid + vitamin A groups. When the results were analyzed as the change in hemoglobin concentration after 12 wk of supplementation (Table 3), the increase in hemoglobin was significantly greater in the iron + folic acid group (9.1 g/L) than in the placebo group (1.2 g/L). The hemoglobin concentration increased by

TABLE 3
Hemoglobin and serum measures of iron status and vitamin A in the study participants at baseline and after the 12-wk supplementation period and the differences between baseline and postsupplementation by treatment group[1]

					P[2]		
Variable	Placebo (n = 71)	Vitamin A (n = 67)	Iron + folic acid (n = 74)	Iron + folic acid + vitamin A (n = 77)	Within groups over time	Between groups	Time × group interaction
Hemoglobin (g/L)							
Baseline	111 ± 9	113 ± 9	113 ± 8	111 ± 10			
Postsupplementation	112 ± 10[a]	116 ± 12[b]	122 ± 7[c]	123 ± 9[c]	0.001	0.001	0.001
Baseline − postsupplementation	1.2 ± 7.7[a]	3.3 ± 7.3[a]	9.1 ± 7.8[b]	12.2 ± 10[c]			
Iron (μmol/L)							
Baseline	5.7 ± 2.9	6.8 ± 3.7	6.6 ± 3.5	6.7 ± 3.5			
Postsupplementation	6.2 ± 3.6[a]	7.0 ± 4.0	8.1 ± 3.7[b]	7.9 ± 3.8[b]	0.001	0.009[3]	0.41
Baseline − postsupplementation	0.5 ± 3.5	0.2 ± 4.5	1.5 ± 4.2	1.3 ± 4.0			
TIBC (μmol/L)							
Baseline	54.5 ± 9.3[a]	52.4 ± 9.4[b]	55.9 ± 9.0[a]	56.4 ± 9.3[a]			
Postsupplementation	55.4 ± 8.7[a]	52.5 ± 9.0[b]	49.0 ± 6.6[c]	49.9 ± 7.4[b,c]	0.001	0.42	0.001
Baseline − postsupplementation	0.9 ± 8.7[a]	0.4 ± 7.4[a]	−6.9 ± 6.1[b]	−6.6 ± 7.9[b]			
TS (%)							
Baseline	11.1 ± 6.9[a]	13.9 ± 8.4[b]	12.5 ± 7.5[a,b]	12.6 ± 7.8[a,b]			
Postsupplementation	12.0 ± 8.1[a]	14.0 ± 8.6[a,b]	16.7 ± 7.6[b]	16.4 ± 8.6[b]	0.001	0.01	0.03
Baseline − postsupplementation	0.8 ± 6.8[a]	0.1 ± 8.6[a]	4.2 ± 8.2[b]	4.0 ± 8.3[b]			
Ferritin (μg/L)							
Baseline	7.9 ± 14.2	10.7 ± 14.0	8.7 ± 13.5	7.3 ± 9.2			
Postsupplementation	5.1 ± 9.0[a]	6.8 ± 10.1[a]	11.1 ± 7.7[b]	12.2 ± 12.0[b]	0.001	0.001	0.001
Baseline − postsupplementation	−2.9 ± 8.5[a]	−3.9 ± 11.3[a]	2.3 ± 10.1[b]	5.0 ±12.6[b]			
Vitamin A (μmol/L)							
Baseline	0.88 ± 0.33	0.89 ± 0.27	0.88 ± 0.28	0.96 ± 0.29			
Postsupplementation	0.87 ± 0.29[a]	1.04 ± 0.27[b]	0.87 ± 0.27[a]	1.09 ± 0.24[b]	0.001	0.002	0.001
Baseline − postsupplementation	−0.01 ± 0.25[a]	0.15 ± 0.29[b]	−0.01 ± 0.22[a]	0.13 ± 0.27[b]			

[1] $\bar{x}$ ± SD. Values with different superscript letters are significantly different, $P < 0.005$ (Bonferroni correction). TIBC, total-iron-binding capacity; TS, transferrin saturation.
[2] Repeated-measures ANOVA with test for two-factor interaction followed by Bonferroni correction.
[3] Difference based on the main effect of the ANOVA.

TABLE 4
Prevalence of anemia, iron deficiency, and biochemical vitamin A deficiency in the study participants at baseline and after the 12-wk supplementation period

	Anemia (hemoglobin <120 g/L)				Iron deficiency (ferritin <12 μg/L)				Vitamin A deficiency (retinol <1.05 μmol/L)			
Treatment group	Baseline	Post-supplementation	OR (95% CI)[1]	*P*	Baseline	Post-supplementation	OR (95% CI)[1]	*P*	Baseline	Post-supplementation	OR (95% CI)[1]	*P*
	%	%			%	%			%	%		
Placebo (n = 71)	96	76	1.0	—	86	93	1.0	—	73	76	1.0	—
Vitamin A (n = 67)	82	55	0.44 (0.20, 0.96)	0.04	67	87	0.67 (0.16, 2.78)	NS	73	54	0.29 (0.13, 0.67)	0.003
Iron + folic acid (n = 74)	82	30	0.15 (0.07, 0.33)	0.0001	84	65	0.091 (0.025, 0.33)	0.0003	70	73	0.78 (0.34, 1.81)	NS
Iron + folic acid + vitamin A (n = 77)	92	29	0.08 (0.03, 0.19)	0.0001	81	66	0.095 (0.026, 0.34)	0.0002	66	45	0.24 (0.11, 0.53)	0.0005

[1] Odds ratio (OR) based on logistic regression analysis, allowing for baseline hemoglobin with anemia, baseline serum ferritin with iron deficiency, and baseline serum retinol with vitamin A deficiency.

3.3 g/L in the vitamin A group, but this increase was not significantly different from that in the placebo group. The hemoglobin concentration increased by 12.2 g/L in the iron + folic acid + vitamin A group, which was significantly greater than the increase in the iron + folic acid group.

The prevalence of anemia, iron deficiency, and biochemical vitamin A deficiency in the subjects at baseline and after the supplementation period are shown by treatment group in **Table 4**. The prevalence of anemia decreased from 96% to 76% in the placebo group, which we presume represents regression toward the mean (**Figure 1**) and emphasizes the importance of the placebo group when conclusions are drawn. Compared with the placebo group, the odds ratio for being anemic after the supplementation period decreased by 92% in the iron + folic acid + vitamin A group, 85% in the iron + folic acid group, and 56% in the vitamin A group; all of the decreases were significant.

At baseline, all 4 groups had similar serum vitamin A and ferritin concentrations (Table 3). After 12 wk of supplementation, the serum vitamin A concentration in the iron + folic acid + vitamin A and the vitamin A groups was significantly greater than that in the placebo and or iron + folic acid groups. The serum ferritin concentration was significantly greater in the iron + folic acid and iron + folic acid + vitamin A groups than in the vitamin A and placebo groups. There was a significant decrease in ferritin concentration in the placebo and vitamin A groups and a significant increase in ferritin concentration in the iron + folic acid and iron + folic acid + vitamin A groups. At baseline, the serum iron concentration was not significantly different between groups; additionally, after supplementation, there were no significant interactions within or between supplementation groups. At baseline, serum TIBC was significantly lower in the vitamin A group than in the other 3 groups. After 12 wk of supplementation, the change in TIBC was significantly greater in the iron + folic acid and iron + folic acid + vitamin A groups than in the placebo and vitamin A groups. Before supplementation, transferrin saturation was significantly higher in the vitamin A group than in the placebo group. The increase in transferrin saturation after 12 wk of supplementation was significantly greater in the iron + folic acid and iron + folic acid + vitamin A groups than in the placebo and vitamin A groups. There was a significant reduction in iron deficiency (≈90%) in the groups that received iron supplements and a significant reduction (≈70%) in vitamin A deficiency in the groups that received vitamin A supplements (Table 4).

In the subgroup with no evidence of infection (n = 219), the overall pattern of response to supplementation was not significantly different from that in the total population, although there were small differences in the magnitude of responses. The data were adjusted for the potential effect of possible confounding or modifying factors (baseline hemoglobin, serum ferritin, and serum retinol concentrations and the number of doses of supplements received). After adjustment, the overall effect on the biochemical indexes was not significantly different from that of the unadjusted values.

There was a significant effect of baseline hemoglobin group on the change in hemoglobin concentration ($P < 0.001$), a significant effect of supplementation group ($P < 0.001$), and a significant interaction between the 2 ($P < 0.006$). In the placebo group, there was a small increase in hemoglobin in the group with the lowest baseline hemoglobin concentration and a small decrease in those with the highest baseline hemoglobin concentration; the difference between the 2 groups was significant, indicating a regression toward the mean (Figure 1). After supplementation with vitamin A, the group with the highest hemoglobin concentration had a significantly greater hemoglobin concentration and change in hemoglobin than did the placebo group. In the iron + folic acid and iron + folic acid + vitamin A groups, the increase in hemoglobin was greater in the subgroup with the lowest baseline hemoglobin concentration than in the subgroups with higher baseline hemoglobin concentrations. There was a significant effect of supplementation group on the change in serum ferritin ($P < 0.001$) and although the change in the iron + folic acid group appeared to be greater in those with the highest baseline hemoglobin concentrations, the change was not significantly different from that in the other 2 subgroups, perhaps because the number of subjects in each of the 3 groups was relatively small (Figure 1).

The total iron retained was estimated from the increase in hemoglobin plus the increase in ferritin. The iron + folic acid + vitamin A group retained ≈14% of the dose of iron, a significantly greater proportion of the administered supplement than the iron + folic acid group, who retained ≈11% of the dose ($P = 0.025$); both of these groups retained significantly more iron than did the placebo group ($P = 0.001$). The interaction between supplementation group and baseline hemoglobin had no significant effect on the amount of iron retained. Similar analyses were carried out for the total popu-

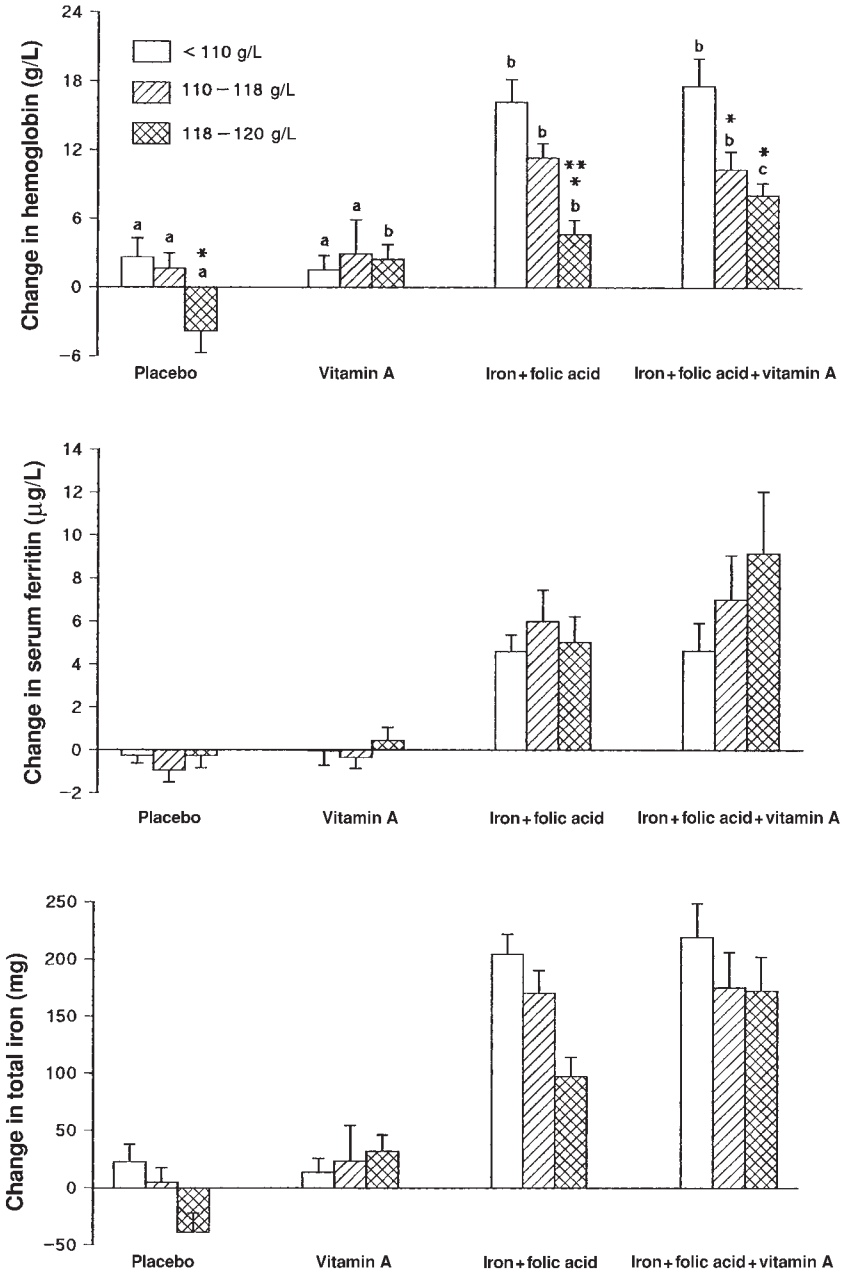

FIGURE 1. Mean (±SE) changes in hemoglobin, serum ferritin, and total iron in teenagers with anemia who were supplemented for 12 wk with placebo, vitamin A, iron + folic acid, or iron + folic acid + vitamin A. The results are presented on the basis of baseline hemoglobin concentrations: <110, 110–118, and >118–120 g/L. Two-factor ANOVA with post hoc Bonferroni correction were used to test for statistical differences between groups. There was a significant interaction ($P < 0.05$) between the change in hemoglobin and baseline hemoglobin. The changes in ferritin and in total iron were significantly greater in the iron + folic acid and iron + folic acid + vitamin A groups than in the vitamin A and placebo groups, $P < 0.05$ (on the basis of the main effects from the ANOVA). Supplementation groups with different letters are significantly different, $P < 0.05$. *Significantly different from the subgroup with hemoglobin concentrations <110 g/L, $P < 0.05$; **Significantly different from the subgroup with hemoglobin concentrations of 110–118 g/L, $P < 0.05$.

lation in the supplementation study and the patterns of results were not significantly different; however, there was greater variability in the responses obtained.

DISCUSSION

The results of the present study show that iron deficiency is common and can be effectively treated by an aggressive weekly supplementation program. Similarly, vitamin A deficiency responds effectively to weekly low-dose supplementation. We showed a substantial reduction in anemia after weekly supplementation with iron and folic acid, similar to the findings in other studies (8–10, 12). The addition of vitamin A to the supplement of iron + folic acid resulted in a further reduction in the prevalence of anemia (Table 4) and supported previous findings in pregnant women (27, 31). The increase in hemoglobin after

supplementation with iron + folic acid + vitamin A was greater than that after supplementation with iron + folic acid alone. Supplementation with vitamin A may improve iron status more in those with marginal status than in those with severe deficiency, but the number of subjects in the secondary analysis was too small to determine significance. There was a suggestion that those who received iron + folic acid + vitamin A had a greater increase in serum ferritin, and hence more stored iron, than did the group supplemented with iron + folic acid alone; this finding needs to be confirmed formally.

The implications of nutritional anemia are especially serious for women in developing countries. Iron supplementation programs have been implemented in many countries, but the effectiveness of the programs is often poor and is thus a major concern (32, 33). Iron supplementation may be less effective in the face of marginal vitamin A deficiency (16, 19, 21), and the availability of iron may be reduced in vitamin A–deficient states because plasma iron is decreased at the same time as iron stores appear elevated. The interpretation has been that despite the presence of iron in the body, it cannot be utilized effectively for red blood cell formation by the erythropoietic tissues (18, 19).

We conclude that the increase in ferritin at the end of the supplementation period probably indicates increased iron stores because increased ferritin in subjects with reference C-reactive protein concentrations excludes ongoing inflammation as a possible interpretation. This is important because by the end of the supplementation period the mean hemoglobin concentration in the iron + folic acid + vitamin A group was only 123 g/L and 29% of these subjects were still anemic. The frequency or duration of supplementation may have been too short to allow adequate red blood cell formation. However, when the groups were subdivided into thirds on the basis of baseline hemoglobin concentration, 2 findings argued against this suggestion. First, in the subgroup with the lowest baseline hemoglobin concentration, the metabolic drive to red blood cell formation was substantial and the increase in hemoglobin was 18.4 g/L compared with 8.0 g/L in the subgroup with the highest baseline hemoglobin concentration. Second, in the group with the highest baseline hemoglobin concentration, it appeared that the retained iron was placed in storage rather than used for additional hemoglobin formation. Thus, although there appeared to be a continuing need for red blood cell formation, the available iron did not seem to be used effectively for this purpose. The extent to which a limitation of this kind is likely to be of functional significance will need to be determined in future studies. If supplementation with vitamin A were to improve iron status, without necessarily increasing hemoglobin, it might suggest more complex interactions with other nutrients possibly playing a limiting role.

There was no significant difference in the response of hemoglobin to iron than to iron plus vitamin A in women who were not deficient in vitamin A at the start of the study (34). In Indonesia, weekly supplementation with iron, vitamin A, and ascorbic acid for 12 wk resulted in a significant improvement in iron status compared with a placebo (35).

The observation that those subjects with the lowest initial hemoglobin had the greatest increase in hemoglobin concentration after supplementation with either iron + folic acid or iron + folic acid + vitamin A was noted by others (6, 36). This finding emphasizes the importance of standardizing the initial conditions if comparisons are to be made between studies. Furthermore, that those with the highest baseline hemoglobin had the greatest increase in serum ferritin concentration, with a possible difference between the 2 iron-treated groups, indicates the need to be careful in the selection of outcomes, both in relation to the starting conditions and to the nature of the intervention (18, 27).

The present study was designed to determine, in a group of women who were likely to soon become pregnant, whether concomitant weekly supplementation with vitamin A and iron would be more effective in correcting iron deficiency than would iron without vitamin A. Most of the employees in the garment industry in Dhaka are young women, nearly one-half of whom are anemic and vitamin A deficient (26). In the present study, 70% of the subjects had a marginal vitamin A status (serum vitamin A <1.05 μmol/L) and 27% were deficient (serum vitamin A <0.70 μmol/L). The design of the study, which involved repeat blood sampling, increased the rate of refusal. Those subjects who failed to complete the study had hemoglobin concentrations and demographic characteristics similar to those who completed the intervention; hence, the findings likely represent the population from which they were drawn. However, relatively more married women left the study and those who left had a lower level of literacy and income (data not shown). Thus, although the differences between the participants and those who left the study were not significant, those subjects most in need of supplementation appeared to be the least likely to stay in the study.

In the present study, supplementation with iron + folic acid + vitamin A improved hemoglobin and reduced anemia, with possible further benefit on iron stores. Considerable vigilance was required to ensure good compliance. Teenage workers in the garment industry represent a captive population, and intervention with appropriate weekly supplements could significantly improve their iron status, hemoglobin concentrations, obstetric outcomes, and productivity in the workplace. Therefore, factory owners should be encouraged to provide a minimum level of health care for their employees. The present study highlights the need for appropriate and effective iron supplementation interventions in all young women before they become pregnant, with adequate attention to the details of the delivery system.

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