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ORIGINAL ARTICLE

The effect of weekly iron and vitamin A supplementation on hemoglobin levels and iron status in adolescent schoolgirls in western Kenya

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Background/Objectives: Iron deficiency anemia is a major public health problem in developing countries and may affect school performance and physical work capacity in nonpregnant adolescents, and may increase the risk of anemia during subsequent teenage pregnancies. We assessed the effect of weekly iron (120 mg elemental iron) and vitamin A (25 000 IU) supplementation on hemoglobin, iron status and malaria and nonmalaria morbidity in adolescent schoolgirls.
Subjects/Methods: A total of 279 schoolgirls aged 12–18 years from public primary schools in Kisumu, western Kenya. Double-blind randomized placebo-controlled trial using a factorial design.
Results: Five months of iron supplementation was associated with a 0.52 g dl^{-1} (0.21, 0.82) greater increase in hemoglobin relative to iron placebo. The effect was only observed in girls with iron deficiency on enrollment (1.34 g dl^{-1} (0.79, 1.88)), but not in iron-replete girls (−0.20 g dl^{-1} (−0.59, 0.18)). Similar differences in treatment effect were seen between menstruating and nonmenstruating girls. The effect of iron was independent of vitamin A. The baseline prevalence of vitamin A deficiency was low (6.7%) and no sustained increase in hemoglobin was seen with weekly vitamin A (−0.07 g dl^{-1} (−0.38, 0.25)). Incidence of malaria parasitemia was higher in the iron than iron-placebo groups (Rate ratio 1.33 (0.94, 1.88)).
Conclusions: Weekly iron supplementation results in substantial increases in hemoglobin concentration in adolescent schoolgirls in western Kenya, which may outweigh possible risks caused by malaria, but only in iron-deficient or menstruating girls and not in iron-replete and nonmenstruating girls.
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Keywords: iron; vitamin A; iron deficiency anemia; malaria; adolescents; schools

Introduction

Anemia, including iron deficiency anemia, remains one of the most widespread public health problems in developing countries, with preschool children and pregnant women most severely affected (WHO, 2001). During adolescence, requirements of iron and other micronutrients, including vitamin A, increase due to the rapid expansion of total blood volume and increase in lean body mass during the growth spurt, and following the onset of menstruation (Brabin and Brabin, 1992). In settings where additional risk factors of anemia (for example, malaria and hookworm) prevail and nutritional intake is inadequate, adolescents are at significant risk of developing anemia, with or without iron deficiency. The prevalence of anemia in adolescent girls in sub-Saharan Africa is estimated to be around 40% (DeMaeyer and Adiels-Tegman, 1985; ACC/SCN, 1997).

Adverse consequences of anemia and iron deficiency range from severe morbidity to more subtle effects on physical work capacity and deficits in cognitive development and potentially school performance (Stoltzfus, 2001). Prepregnancy hemoglobin level and iron status are also important determinants of the risk of anemia-related morbidity and mortality during pregnancy (Brabin *et al.*, 1998; Bothwell,

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2000). Furthermore, iron deficiency may be associated with retarded growth, which in turn may be associated with obstructed labor due to cephalopelvic disproportion (Harrison, 1985).

Vitamin A requirements also rise significantly during puberty reflecting the role of this nutrient in sexual maturation (Michaelsson *et al*., 1976). Vitamin A deficiency (VAD), even when subclinical, may cause anemia through the modulation of iron metabolism as reviewed by Semba and Bloem (2002). Randomized trials in anemic subjects have shown that vitamin A added to iron supplementation gave better hematological response than iron alone (Hodges *et al*., 1978; Mejia and Chew, 1988; Suharno *et al*., 1993). VAD is also recognized to play a role in growth, development and the functioning of the immune system (ACC/SCN, 1997).

There is a long-standing controversy about the safety of iron supplementation in malaria-endemic areas. Although iron deficiency causes a number of biochemical abnormalities and impaired cell-mediated immunity with increased susceptibility to infections (Fleming, 1987), concerns have also been raised that hyperferremia resulting from iron therapy may exacerbate infections, in particular malaria (Oppenheimer, 2001). The controversy has received renewed attention following the recent findings from a study in Zanzibar that reported in an increased rate of severe diseases and death in malaria-endemic Zanzibar (Sazawal *et al*., 2006). Because vitamin A supplementation raises serum iron this might also increase susceptibility to infection. On the other hand vitamin A supplementation in deficient individuals may boost the immune response and has been shown to provide protection against a number of infections (Villamor and Fawzi, 2000). High-dose vitamin A supplementation has been shown to reduce the number and severity of clinical malaria attacks in one trial (Shankar *et al*., 1999) but not in another (Binka *et al*., 1995).

School-based interventions in adolescent girls offer an opportunity to combat iron deficiency, anemia and stunting before pregnancy (Brabin and Brabin, 1992). We conducted a randomized placebo-controlled trial to determine the effect of weekly supplementation with iron alone, vitamin A alone or a combination of the two, on hemoglobin concentration and iron status in adolescent schoolgirls in western Kenya. We also determined whether the addition of vitamin A has the potential to decrease or neutralize any adverse effects of iron supplementation on morbidity both from malaria and other infections.

Methods

Study area and population

This study was conducted between April 1998 and November 1998 in the city of Kisumu (population $\sim$320 000), located on the shores of Lake Victoria, in Nyanza Province, western Kenya.

Malaria transmission in this urban area is largely uncharacterized, but is perennial with highest transmission during peak rainfall from April – July and October – December. Exposure to infective mosquitoes is likely to vary from the city center to the peri-urban areas (Robert *et al*., 2003). A study of pregnant women attending the hospital antenatal clinic reported an overall parasitemia prevalence in teenage pregnancies of 25% (van Eijk *et al*., 2001). Approximately 26% of 15- to 19-year-old girls are estimated to be infected with human immunodeficiency virus-1 (Glynn *et al*., 2001). A study of vitamin A status conducted in a rural area 60 km north west of Kisumu found the prevalence of VAD (serum retinol $\leqslant 0.70\,\mu mol\,l^{-1}$) to be 55.2 and 17.5% in preschool and primary school girls, respectively, and none with signs of xerophthalmia (Friis *et al*., 1997). A study in pregnant women receiving folate supplementation suggested that folate deficiency is not a major cause of anemia among pregnant women in this area (Ouma *et al*., 2006).

Study design and recruitment

This was a double-blinded, randomized placebo-controlled trial with a 2×2 factorial design. After consent was obtained from the parents–teachers associations of 14 public primary schools within the Kisumu municipality, all girls 12–18 years were screened for the presence of anemia using a portable Hemocue system (HaemoCue AB, Angelholm, Sweden). For logistical reasons, only schools with >10 girls fulfiling the entry criteria were considered. Girls with mild-to-moderate anemia were enrolled 6–8 weeks after screening, when a questionnaire and physical examination were completed and a repeat finger-prick blood sample taken. Pregnancy tests were conducted for girls with a history of delayed menstruation. Any girls with severe anemia (hemoglobin (Hb) $< 7.0\,g\,dl^{-1}$), evidence of severe VAD (that is, signs of xerophthalmia), pregnancy or concomitant disease requiring hospitalization were excluded.

Randomization and blinding

Balanced block randomization scheme ($N = 12$) was used. Active and placebo supplements were manufactured by Laboratory and Allied Limited (Nairobi, Kenya). The key was revealed only after completion of the study.

Interventions

Study participants were given one of the following supplementations: (1) iron and vitamin A, (2) iron and vitamin A placebo, (3) vitamin A and iron placebo or (4) iron placebo and vitamin A placebo (double placebo). Iron supplementation consisted of 120 mg elemental iron in the form of two 200 mg ferrous-sulfate tablets given once weekly. Vitamin A was given as 25 000 IU (8.3 mg) retinol in the form of a gelatin capsule once weekly, a dose considered safe in first trimester pregnancy (WHO, 1998). Tablets and capsule were given at the same time with a glass of water.

All schools were visited weekly by a study nurse for supervised administration of study supplements. If girls were absent, supplements were left with the teachers. Supervised treatment was not possible during a 3-week leave period between two school terms and a 2-week teachers' strike. Instructions were given to the girls to take the study supplements at home.

Follow-up
The study was initially designed to include a 6-month intervention period, but shortened to 5 months following an early request from the teachers because the sixth month would have fallen in the school examination month. Girls were seen monthly after enrollment for a finger-prick blood sample (for hemoglobin concentration and malaria smear), questionnaire (including medical history, menstrual history, history of potential side effects and use of medication in the prior month) and basic physical examination (including clinical impression, skin rash and axillary temperature).

At weekly treatment visits, girls were seen by the study nurse. Girls with symptomatic illness were referred to a dedicated study pediatrician. Each girl was given a health passport and girls suspecting illness could report directly to this clinic at no cost. The study pediatrician filled in a dedicated study form that was used for passive surveillance.

Laboratory methods
A Coulter Counter (Model M530, Coulter Electronics Limited, Luton, UK) was used to determine hemoglobin concentration. Daily control samples were run to determine the daily coefficient of variation, which never exceeded 7%. Blood cells and plasma were separated and stored at $-20\,^{\circ}C$, and subsequently at $-80\,^{\circ}C$ until further processing.

Ferritin and C-reactive protein (CRP) were measured on stored samples in a multiplexed bead-based assay as previously described (Coutinho *et al*., 2005). Retinol-binding protein (RBP) was measured on stored samples in a competitive binding assay using RBP (RDI, Concord, MA, USA) coupled to microspheres as target and biotinylated anti-RBP (courtesy of Dr Hix, PATH, Seattle, WA, USA) as the competitive detection Ab, essentially as described (Coutinho *et al*., 2005). RBP is known to occur as a 1:1 molar complex with retinol and can be used as a surrogate marker of serum retinol and equimolar cutoffs for RBP with similar sensitivity and specificity to predict VAD as serum retinol (Gamble *et al*., 2001). All laboratory markers for iron and vitamin A status could be assessed only for the baseline and 3 months time points, because more than 80% of stored samples taken at the 5-month time point were lost in transport.

Thick blood smears were stained with Giemsa and examined for the presence of malaria parasites with a $\times 100$ oil-emersion objective as previously described (Leenstra *et al*., 2003a).

Anthropometric data
Measurements of height and weight were performed at baseline according to standard World Health Organization procedures as described (Leenstra *et al*., 2005). Sexual maturation was assessed using a modified Tanner score based on the assessment of breast development only (Tanner, 1962).

Sample size
We estimated that a sample size of 220 participants (55 per arm) would be sufficient to detect a $0.5\,g\,dl^{-1}$. difference in the mean change in hemoglobin between the treatment groups by the end of the intervention period, with 90% power and 95% confidence, anticipating 20% dropout and assuming a standard deviation of the mean Hb level of $0.8\,g\,dl^{-1}$.

Statistical methods
All analyses were conducted in SAS (version 8.02, Statistical Application Software Institute, Cary, NC, USA). Analyses were done on an intention to treat basis. Ferritin, RBP, malaria and parasitemia were log normally distributed, and the data were Log_e transformed (Ln(value + 1)).

Changes in mean Hb, ferritin and RBP were assessed using a repeated measures linear regression model to adjust for within-subject correlation of the endpoint over time (Fitzmaurice *et al*., 2004). Means were modeled as a polynomial function of time. Models were adjusted for the baseline value of the outcome. School was included as a covariate in all models. Because ferritin and RBP levels are influenced by inflammation, models including ferritin or RBP as an outcome were adjusted for concurrent CRP level, entered into models as a time-varying covariate.

The difference in the prevalence of anemia between treatment groups was assessed using marginal log binomial regression (repeated measures logistic regression), and expressed as the prevalence ratio at the final follow-up visit, adjusted for the presence of anemia at baseline.

Differences in geometric mean parasite density between treatment groups over the course of the study period were assessed with a linear mixed effects model for parasitemic subjects only. The incidence rates of malaria parasitemia (any species) and history of illness due to malaria or other causes in the month prior to survey were calculated based on time up to the episode, the end of the main intervention period or loss to follow-up. Observation time and events occurring within 28 days of a treated malaria episode were excluded from analysis of the incidence of parasitemia. Rate ratios for incidence of malaria and clinical outcomes were obtained from a Poisson regression model, controlling for confounding factors.

Factors at baseline were introduced into initial models individually to assess possible confounding and/or effect modification. The interaction between the effects of iron and

vitamin A was tested using the log likelihood ratio test. When the interaction term was not significant the main effect of iron while adjusting for the effect of vitamin A, and vice versa, was estimated by fitting above models without the interaction term. We assessed whether iron deficiency at baseline modified the effects of iron and vitamin A using the log likelihood ratio test. Menstruation is one of the most important risk factors for iron deficiency anemia (IDA) in older teenage girls in this study area (Leenstra *et al*., 2004), and because iron deficiency is difficult to determine in practice, we also examined whether the effect of treatment on Hb concentration depended on the presence of menarche at baseline.

Results

Study number and descriptive

A total of 1615 girls aged 12–18 years were screened in 14 schools during a 6-week period in April and May 1998. Overall, 350 (21.7%) girls were found to have mild/moderate anemia ($7.0\,g\,dl^{-1} \leqslant Hb < 12.0\,g\,dl^{-1}$ (WHO, 2001)). Only 6 (0.4%) girls had severe anemia ($Hb < 7.0\,g\,dl^{-1}$ (WHO, 2001)). Of the 350 anemic girls at screening, 279 were enrolled (Figure 1). Loss to follow-up at the end of the study was 10.8% (30 of 279) and not different between the treatment groups (Figure 1). Baseline characteristics did not predict loss to follow-up (data not shown).

Treatment groups were comparable at baseline (Table 1). The mean age was 13.8 (s.d. 1.3) years. The median maturity rating (Tanner breast development stage) was 3 (IQ range 2–4) and 46.8% of the girls had passed menarche. Of the girls that were anemic at screening (using Hemocue Hb assessment), 30.5% were anemic at start of treatment 6–8 weeks later (using coulter counter Hb assessment). Baseline mean Hb was $12.8\,g\,dl^{-1}$ (s.d. 1.7). Baseline geometric mean ferritin was $13.0\,g\,dl^{-1}$ (s.d. 7.7) and prevalence of iron deficiency (ID; ferritin $< 12.0\,\mu g\,l^{-1}$ (WHO, 2001)) was 42.3%. Prevalence of VAD was 6.7%. VAD was defined as RBP $< 14.7\,mg\,l^{-1}$, which corresponds with the widely used retinol cut-off $< 0.70\,\mu mol\,l^{-1}$ (de Pee and Dary, 2002). A quarter of the girls had malaria parasitemia at baseline, a third of these had high parasite densities defined as > 500 parasites per mm^3. Only one girl presented with symptomatic malaria (presence of malaria parasites with concurrent axillary temperature $\geqslant 37.5\,^{\circ}C$). Prevalence of stunting (height-for-age *z*-score two s.d.'s below the reference median) and underweight (weight-for-age *z*-score two s.d.'s below the reference median) were 4.4 and 4.3%, respectively.

Hematological response

The adjusted differences in mean Hb concentration between the treatment groups and double placebo after 5 months of supplementation were $0.46\,g\,dl^{-1}$ (−0.00, 0.92) for iron and vitamin A combined, $0.44\,g\,dl^{-1}$ (0.01, 0.87) for iron alone and $-0.14\,g\,dl^{-1}$ (−0.59, 0.31) for vitamin A alone (Figure 2). There was no evidence for interaction between the effects of iron and vitamin A on Hb concentrations (*P*-value for the iron–vitamin A interaction = 0.89). Subsequent models were therefore developed to estimate the main effects of iron and vitamin A, while controlling for the effect of the other intervention (Table 2). Iron supplementation was associated with a $0.52\,g\,dl^{-1}$ higher mean Hb by 5 months and a 65% lower prevalence of anemia compared to placebo (Table 2). Vitamin A supplementation was not associated with a significant difference in mean Hb or prevalence of anemia, compared to vitamin A placebo by 5 months (Table 2).

Subgroup analysis indicated that the iron status at baseline significantly modified the effect of iron supplementation on Hb levels (*P*-value interaction terms < 0.0001). Iron supplementation was only effective in individuals who were iron deficient at baseline (Table 3). Baseline iron status did not modify the effect of vitamin A on Hb (*P*-value interaction term = 0.87).

At baseline, menstruating girls had a $0.64g\,dl^{-1}$ (0.21, 1.07) lower Hb compared to girls not yet menstruating ($P = 0.004$). Iron had a clear effect in menstruating girls but not in nonmenstruating girls (Table 3; *P*-value interaction term = 0.002). Menstruating girls receiving iron placebo ($N = 66$) had a $-1.03\,g\,dl^{-1}$ (−1.53, −0.54) lower Hb level at 5 months than nonmenstruating girls receiving iron placebo ($N = 75$) ($P < 0.0001$). The above effects were independent of differences in iron status at baseline (data not shown). Menstruation did not modify the effect of vitamin A on Hb (*P*-value interaction term = 0.83).

Because of the low prevalence of VAD at baseline there was insufficient power to assess whether the effects of iron or vitamin A supplementation were modified by baseline vitamin A status.

Impact on ferritin concentrations

There was no interaction between the effect of iron and vitamin A on ferritin concentrations (*P*-value interaction term = 0.35). Three months of iron supplementation was associated with a $13.3\,\mu g\,l^{-1}$ higher ferritin concentration compared to iron placebo (Table 2). Vitamin A supplementation had no effect on ferritin concentrations (Table 2).

Impact on RBP concentrations

There was no interaction between the effect of iron and vitamin A on RBP concentrations (*P*-value interaction term = 0.44). A total of 3 months of vitamin A supplementation had no significant effect on mean RBP concentration (Table 2). Similarly, iron supplementation had no effect on RBP concentrations at 3 months (Table 2).

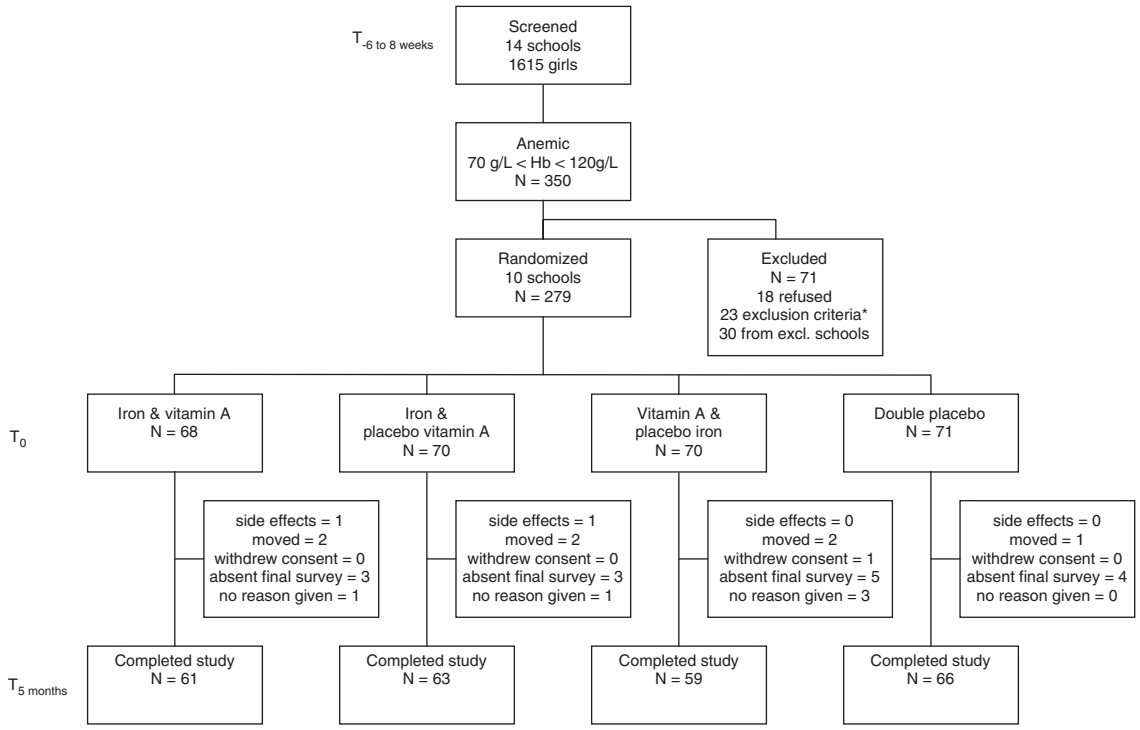

Figure 1 Trial Profile: subject distribution for a double-blind placebo-controlled trial on the effect of weekly iron and vitamin A on hemoglobin (Hb) concentration, and iron status in adolescent schoolgirls in Kisumu city, western Kenya. *Exclusion criteria; 3 developed severe anemia since screening, 3 pregnant or pregnancy could not be excluded, 2 concomitant disease (1 sickle cell disease, 1 juvenile arthritis and severe malnutrition), 3 moved away since screening, 11 were less than 12 years of age by date of birth, 1 was absent at enrollment for unknown reasons.

Table 1 Baseline characteristics, by treatment group, of 279 adolescent schoolgirls enrolled in a trial of iron and vitamin A supplementation for hemoglobin, and iron status in western Kenya

Covariates	*Intervention groups*			
	Iron and vitamin A	*Iron*	*Vitamin A*	*Placebo*
Age (years); mean (s.d.)	13.8 (1.3)	13.8 (1.3)	13.9 (1.2)	13.8 (1.3)
Menstruating; no. (%)	33/68 (48.5)	31/70 (44.3)	35/70 (50.0)	31/71 (43.7)
Hemoglobin ($g\,dl^{-1}$); mean (s.d.)	12.8 (1.9)	12.6 (2.0)	12.8 (1.4)	13.1 (1.5)
Hemoglobin $<12.0\,g\,dl^{-1}$; no. (%)	18/58 (31.0)	22/62 (35.5)	17/59 (28.8)	17/64 (26.6)
Ferritin ($\mu g\,l^{-1}$); geometric mean (s.d.)	12.3 (7.2)	13.0 (7.9)	13.9 (7.8)	12.8 (7.8)
Ferritin $<12\,\mu g\,l^{-1}$; no. (%)	19/43 (44.2)	19/47 (40.4)	21/47 (44.7)	21/52 (40.4)
CRP ($mg\,l^{-1}$); geometric mean (s.d.)	1.5 (1.0)	1.6 (1.0)	1.6 (1.1)	1.6 (1.0)
CRP $>8.2\,mg\,l^{-1}$; no. (%)	1/43 (2.3)	2/47 (4.3)	3/47 (6.4)	2/52 (3.9)
RBP ($mg\,l^{-1}$); mean (s.d.)	32.9 (19.4)	34.6 (18.0)	34.8 (15.6)	34.4 (20.0)
RBP $<14.7\,mg\,l^{-1}$; no. (%)	5/52 (9.6)	3/53 (5.7)	5/56 (8.9)	2/62 (3.2)
Malaria parasite density (mm^{-3}); geometric mean (s.d.)[a]	348 (282)	263 (216)	221 (152)	266 (148)
Any malaria parasitemia; no. (%)	16/51 (31.4)	15/55 (27.3)	9/52 (17.3)	14/55 (25.5)
Malaria parasitemia $>500\,mm^{-3}$; no. (%)	5/51 (9.8)	6/55 (10.9)	3/52 (5.8)	3/55 (5.5)
Height (cm); mean (s.d.)	156.9 (7.8)	157.0 (9.1)	157.1 (7.8)	158.0 (7.8)
Weight (kg); mean (s.d.)	43.8 (8.2)	44.8 (10.1)	45.3 (8.4)	44.4 (7.8)

Abbreviations: s.d., standard deviation; CRP, C-reactive protein; RBP, retinol-binding protein.
[a]Positive blood smears included only.

Impact on the incidence of malaria, all-cause morbidity, side effects and menstrual abnormalities

There was no evidence for interaction between the effects of iron and vitamin A on any of the malaria outcomes (data not shown); main effects incidence risk ratios are given in Table 4.

The monthly follow-up visits indicated that girls in the iron-supplemented groups were slightly more likely to be parasitemic at active follow-up visits ($P=0.10$, Table 4) compared to girls who were randomized to receive placebo iron. However, geometric mean parasite densities were not higher in this group ($P=0.99$). Vitamin A supplementation did not affect the risk of malaria (Table 4). No differences between groups were seen in any other measures of morbidity at the monthly follow-up or clinic visits (Table 4).

Passive surveillance indicated that 32 girls visited the study clinic during the follow-up period. The most common diagnosis was malaria (diagnosed eight times) followed by allergic conjunctivitis (seven times), tonsillitis (six times) and gastritis (five times). There was no difference between the groups. Anemia was diagnosed only twice; once in a girl with malaria and once in a girl with menorrhagia. None of the girls presented with severe disease.

Girls receiving iron were more likely to report constipation or dark stools than girls receiving iron placebo (Rate ratio 2.2 (1.1, 4.4) and 6.4 (1.0, 41.5), respectively). There was no difference between groups regarding the occurrence of potential side effects as nausea, vomiting or diarrhea (data not shown).

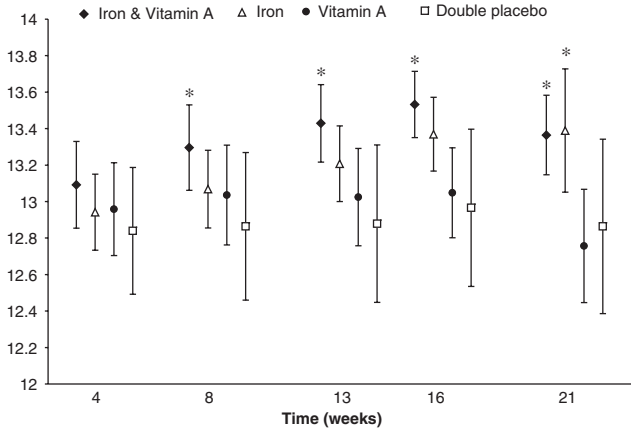

Figure 2 Repeated measures linear regression analysis of mean hemoglobin (Hb) concentration in adolescent schoolgirls in western Kenya, by treatment group over a 5-month supplementation period, using a polynomial function of time, adjusted for baseline Hb, and within-subject correlation. Error bars represent 95% CIs of the mean. Subjects were randomized to receive a weekly dose of iron (120 mg elemental iron) and vitamin A (25 000 IU), iron and vitamin A placebo, vitamin A and iron placebo or double placebo. *Significantly different from double placebo ($P<0.05$).

Discussion

This randomized controlled trial evaluated the effect of weekly iron and vitamin A supplementation on hemoglobin levels, iron status and morbidity in adolescent schoolgirls in urban Kisumu, western Kenya. Five months of weekly iron supplementation was associated with a 1.34 g dl^{-1} increase in mean hemoglobin concentrations compared to iron placebo, but only in girls who were iron deficient at baseline. No effect was seen in iron-replete girls. These results are consistent with several recent weekly iron supplementation

Table 2 Main effects of iron and vitamin A supplementation on hemoglobin, anemia, and iron and vitamin A status in adolescent schoolgirls in western Kenya

	Iron[a] (N = *138*)	*Vitamin A*[b] (N = *138*)
Hemoglobin concentration (g dl^{-1})	13.52	12.92
Difference in means (95% CI)[c]	0.52 (0.21, 0.82), $P<0.001$	−0.07 (−0.38, 0.25), $P=0.68$
Anemia (%)	9.4	27.7
Prevalence ratio (95% CI)[d]	0.35 (0.23, 0.55), $P<0.0001$	1.04 (0.70, 1.55), $P=0.84$
Serum ferritin concentration (μg l^{-1})	37.4	22.4
Difference in means (95% CI)[e]	13.3 (7.3, 20.3), $P<0.0001$	−1.7 (−5.4, 2.7), $P=0.42$
Retinol-binding protein	22.7	26.9
Difference in means (95% CI)[e]	−2.0 (−5.7, 2.4), $P=0.35$	2.3 (−2.1, 7.4), $P=0.33$

Abbreviation: CI, confidence interval.
[a]Main effect of iron is compared to iron placebo and adjusted for concurrent vitamin A supplementation.
[b]Main effect of vitamin A is compared to vitamin A placebo and adjusted for concurrent iron supplementation.
[c]Difference in means at 5 months, obtained from repeated measures linear regression analysis by treatment group, using a polynomial function of time, adjusted for baseline value of the endpoint, within-subject correlation and confounding by school.
[d]Prevalence ratio at 5 months, obtained from marginal log binomial regression analysis by treatment group, adjusted for baseline prevalence of anemia and confounding by school.
[e]Difference in means at 3 months, obtained as above, adjusted for concurrent C-reactive protein.

Table 3 Effect of iron supplementation on hemoglobin levels by 5 months, stratified by baseline iron status and menstrual status in adolescent schoolgirls in western Kenya

	Iron deficient at baseline[a] (N = 80)	*Iron replete at baseline* (N = 109)
Hemoglobin concentration ($g\,l^{-1}$)		
Difference in means (95% CI)[b]	1.34 (0.79, 1.88), $P<0.0001$	−0.20 (−0.59, 0.18), $P=0.30$
	Menstruating at baseline[c] (N = 149)	*Not menstruating at baseline* (N = 130)
Hemoglobin concentration ($g\,l^{-1}$)		
Difference in means (95% CI)[b]	1.07 (0.60, 1.55), $P<0.0001$	0.10 (−0.28, 0.49), $P=0.59$

Abbreviation: CI, confidence interval.
[a]Iron–iron deficiency interaction *P*-value <0.0001.
[b]Difference in means between iron and iron placebo, obtained from repeated measures linear regression analysis, using a polynomial function of time, adjusted for baseline hemoglobin, the effect of the vitamin A, within-subject correlation and confounding by school.
[c]Iron–menstruation interaction term $P=0.004$.

Table 4 Effect of iron and vitamin A supplementation on incidence of malaria, and clinical outcomes in adolescent schoolgirls in western Kenya

	Iron	*Iron placebo*	*Vitamin A*	*Vitamin A placebo*
Any malaria parasitemia				
Incidence rate; per 1000 person months	175	131	168	136
Rate ratio (95% CI)[a,b]	1.33 (0.94, 1.88), $P=0.10$	Reference	1.23 (0.88, 1.73), $P=0.24$	Reference
High-density malaria parasitemia				
Incidence rate; per 1000 person months	43	31	42	32
Rate ratio (95% CI)[a,b]	1.36 (0.70, 2.63), $P=0.36$	Reference	1.29 (0.68, 2.46), $P=0.44$	Reference
Any morbidity				
Incidence rate; per 1000 person months	214	182	202	193
Rate ratio (95% CI)[a,c]	1.17 (0.94, 1.47), $P=0.17$	Reference	1.05 (0.84, 1.32), $P=0.67$	Reference
Clinical malaria[d]				
Incidence rate; per 1000 person months	11	6	9	7
Rate ratio (95% CI)[a,c]	1.87 (0.39, 8.90), $P=0.36$	Reference	1.24 (0.62, 2.47), $P=0.77$	Reference
Nonmalarial illness				
Incidence rate; per 1000 person months	208	180	198	189
Rate ratio (95% CI)[a,c]	1.15 (0.92, 1.45), $P=0.22$	Reference	1.05 (0.83, 1.31), $P=0.70$	Reference

Abbreviation: CI, confidence interval.
[a]Rate ratios obtained by Poisson regression, adjusted for confounding by school.
[b]Adjusted for age and baseline parasitemia.
[c]Monthly follow-up and passive surveillance combined.
[d]Symptomatic malaria or malaria diagnosed by the study pediatrician.

trials in adolescent girls (Kätelhut *et al*., 1996; Tee *et al*., 1999; Kianfar *et al*., 2000; Zavaleta *et al*., 2000; Ahmed *et al*., 2001).

Weekly vitamin A supplementation was not associated with a sustained increase in Hb. It has long been recognized that vitamin A is essential for normal hematopoiesis and plays a role in the etiology of anemia (Semba and Bloem, 2002). In a study of iron supplementation for anemic vitamin A-depleted individuals, hematological response to iron was only observed after vitamin A status was improved (Hodges *et al*., 1978). Recent randomized controlled trials that determined the effect of vitamin A in addition to iron supplementation for the treatment of anemia showed that the combined therapy resulted in significantly better hematological recovery than iron supplementation alone (Mejia and Chew, 1988; Suharno *et al*., 1993; Mwanri *et al*., 2000; Ahmed *et al*., 2001). However, others have failed to confirm these findings (Kätelhut *et al*., 1996; Kolsteren *et al*., 1999; Muslimatun *et al*., 2001; Soekarjo *et al*., 2004). In our study, there was also no evidence that the effect of iron was dependent on the concomitant administration of vitamin A. The difference between the above named studies is not explained by differences in dosage, as most gave daily doses between 5000 and 8000 IU retinol (that is, 35 000–56 000 IU per week). Moreover, one of the studies showed a significantly

enhanced effect in adolescent girls even with a relatively low weekly dose of 8000 IU retinol for 12 weeks, which was lower than our weekly dose of 25 000 IU (Ahmed *et al*., 2001). Conversely, the differences between the above named studies might be explained by differences in baseline vitamin A status between study populations, in studies where combined supplementation was more efficacious than iron alone, there was a high (>30%) prevalence of baseline VAD (retinol $<0.70\,\mu mol\,l^{-1}$) (Suharno *et al*., 1993; Ahmed *et al*., 2001), whereas in studies where combined supplementation was not effective there was a low (<15%) prevalence of VAD (Kätelhut *et al*., 1996; Kolsteren *et al*., 1999; Soekarjo *et al*., 2004). Thus the lack of a sustained effect of vitamin A on hemoglobin levels and the lack of interaction between iron and vitamin A in our study likely reflects the low prevalence of VAD in this age group; only 6.7% of the girls were defined as VAD at enrollment.

The lack of a hematological response following iron supplementation in nonmenstruating girls suggests that iron deficiency is not a main cause of anemia during premenarche in this setting. This is consistent with our previous observations in this same age group in neighboring Bondo district, which implicated malaria and schistosomiasis as potential causes of anemia in young adolescents (12–13 years), but not in older menstruating girls (14–18 years) (Leenstra *et al*., 2004). In the older girls heavy menstruation was the only risk factor identified, presumably because resistance to parasitic infection improves with age and pubertal development (Kurtis *et al*., 2001; Leenstra *et al*., 2003b). Furthermore, insecticide-treated bed nets were found to reduce the prevalence of malaria-associated anemia in young schoolgirls aged 12–13 years, but not in the older girls (Leenstra *et al*., 2003a). These combined results suggest that the relative contribution of iron deficiency as a cause of anemia is likely to be greatest in menstruating girls and that they are the demographic group most likely to benefit from iron supplementation programs in schools. An additional explanation could be that iron absorption is upregulated in menstruating girls in anticipation of loss through menstruation and to facilitate growth.

Despite the well-recognized public health burden of anemia and the beneficial effects of iron supplementation, the use of iron therapy remains controversial in many malaria-endemic countries because of the concerns that iron therapy exacerbates infections (INACG Consensus Statement, 1999; Oppenheimer, 2001). A meta-analysis by the International Nutritional Anemia Consultative Group concluded that the overall increased risk of malaria parasitemia is small and does not outweigh the hematological benefits of iron supplementation (INACG Consensus Statement, 1999). However, recent findings that iron supplementation is associated with increased mortality and hospital admission in young preschool children in an area with intense malaria transmission has refueled the debate about the role of universal iron supplementation in malarious areas (Sazawal *et al*., 2006). In the current study, iron supplementation was associated with increased incidence of malaria parasitemia (Rate ratio 1.33 (0.94–1.88)), independent of age or baseline iron status. Although this was not statistically significant, it is in line with the findings from the previous meta-analysis (INACG Consensus Statement, 1999). A larger increase in clinical malaria (fever and parasitemia) was observed in the iron-supplemented group (Rate ratio 1.87 (0.39–8.90)), but the CIs were wide and a more substantial increase cannot be excluded. There was no evidence that weekly iron supplementation increased the risk of all-cause morbidity. Although, our study is too small to provide conclusive evidence on the risk of malaria associated with iron supplementation, the high degree of acquired immunity to malaria in older (menstruating) teenage girls (Kurtis *et al*., 2001; Leenstra *et al*., 2003b) would suggest that the public health effects of any increased risk of malaria are likely to be much smaller than in young preschool children. For example the absolute risk of clinical malaria in the teenage girls in this study was very low and all clinical episodes were mild, whereas in this same population, malaria is one of the main causes of death in infants (Phillips-Howard *et al*., 2003).

We conclude that school-based weekly iron supplementation in girls aged 12–18 is well tolerated and results in substantial increases in hemoglobin concentrations that outweigh the possible inherent risks caused by malaria but only in iron-deficient and menstruating girls, and not in iron-replete or nonmenstruating girls. This would argue in favor of targeted rather than universal iron supplementation in adolescent schoolgirls in malarious areas. However, screening for iron deficiency, or targeting iron supplementation to schoolgirls that admit to being post-menarche is unlikely to be feasible, cost effective or culturally acceptable. Alternatively, targeting the highest risk age group and the use of integrated approaches that combine universal iron supplementation with malaria prevention measures, for example, through use of insecticide-treated nets, could be considered. VAD was rare and weekly vitamin A supplementation, alone, or combined with iron, did not result in a sustained increase in hemoglobin. Further research is required to determine the long-term effects of iron alone, or combined with malaria prevention in schoolgirls of reproductive age for the prevention of anemia-related adverse outcomes in adolescent pregnancies.

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