Supplementation with iron and riboflavin enhances dark adaptation response to vitamin A–fortified rice in iron-deficient, pregnant, nightblind Nepali women[1–3]

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ABSTRACT

Background: Nightblindness affects 16–52% of pregnant women in areas of Nepal and in some cases persists after vitamin A treatment. Iron and riboflavin affect vitamin A utilization and photoreceptor function, respectively, and pilot data in the study population showed a high prevalence of iron and riboflavin deficiencies.

Objective: The objective was to assess the effect of supplemental iron and riboflavin on pupillary threshold (PT) and plasma retinol in nightblind, pregnant Nepali women given vitamin A–fortified rice.

Design: Nightblind pregnant women were randomly assigned to receive, 6 d/wk under supervision for 6 wk, a vitamin A–fortified rice curry dish providing 850 μg retinal activity equivalents/d with either a 30-mg Fe and 6-mg riboflavin (FeR + VA) capsule or a placebo control (VA only) capsule. Hemoglobin, erythrocyte riboflavin, and plasma ferritin and retinol were measured before and after the intervention. Dark adaptation was assessed by PT score.

Results: Women who were iron deficient at baseline ($n = 38$) had significantly greater improvement in PT score with iron and riboflavin supplementation than without ($P = 0.05$). Iron and riboflavin supplements significantly reduced the prevalences of riboflavin deficiency (from 60% to 6%; $P < 0.0001$), iron deficiency anemia (from 35% to 15%; $P < 0.007$), and abnormal PT (from 87% to 30%; $P < 0.05$) from baseline. Mean increases in erythrocyte riboflavin ($P < 0.0001$) and plasma ferritin ($P = 0.01$) were greater in the FeR + VA group than in the VA only group.

Conclusions: Iron deficiency may limit the efficacy of vitamin A to normalize dark adaptation in pregnant Nepali women. Further studies are needed to assess the effect of simultaneous delivery of iron and vitamin A for the treatment of nightblindness. *Am J Clin Nutr* 2007;85:1375–84.

KEY WORDS Pregnancy, impaired pupillary threshold, nightblindness, iron deficiency, riboflavin, women, Nepal

INTRODUCTION

Iron deficiency affects a large proportion of the populations of developing countries (1, 2), especially women and children. Iron deficiency can reduce hepatic retinyl ester hydrolysis or cause a shift toward hepatic retinol esterification, thereby limiting concentrations of circulating retinol (3, 4). Biochemical evidence of riboflavin deficiency was documented during the third trimester of pregnancy in Thai and Gambian women (5, 6) and during lactation in Guatemalan women (LH Allen, unpublished observations, 1999). Animal studies have shown specific riboflavin-dependent photoreceptors in the inner nuclear and ganglion layers of the retina, which are among many photoreceptors that contribute to the dark adaptation process by eliciting the neuronal signal required for pupillary response (7–10). Riboflavin also has a role as a coenzyme to mobilize iron from ferritin (11–13).

Although vitamin A deficiency is the main cause of maternal nightblindness in low-income populations in developing countries, iron and riboflavin deficiencies could increase the risk of nightblindness by affecting both vitamin A metabolism and the process of dark adaptation. In previous studies of pregnant women in the Terai region of Nepal, vitamin A supplements reduced the incidence of nightblindness by only 67% (14). Our preliminary studies in the same region showed a 40% prevalence of iron deficiency (plasma ferritin < 12 μg/L) and an 86% prevalence of riboflavin deficiency (erythrocyte riboflavin < 170 nmol/L) in pregnant women. These nutrient deficiencies often coexist with vitamin A deficiency when consumption of animal source foods is low. Thus, the objectives of the present study were to assess whether providing iron and riboflavin, as compared with a placebo, would improve nutritional status and dark adaptation among nightblind women receiving vitamin A–fortified rice. The relation between pupillary threshold (PT) score and biochemical indicators of iron and riboflavin status was also evaluated.

SUBJECTS AND METHODS

Subjects and location

This study was conducted from March 2001 to May 2002 as part of a food-based maternal nightblindness treatment trial (15).

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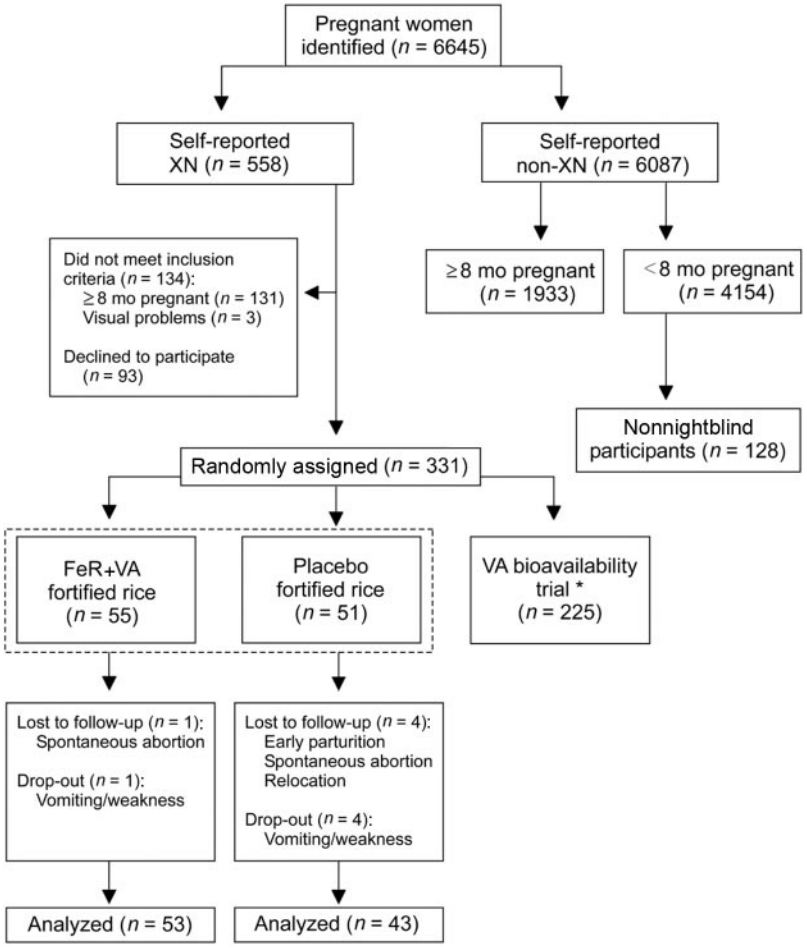

FIGURE 1. Study enrollment from March 2001 to May 2002. XN, nightblindness; FeR + VA, vitamin A (850 μg retinal activity equivalents) plus 30 mg Fe and 6 mg riboflavin; Placebo, VA only. *Of the 331 women randomly assigned to treatment groups, 225 were assigned to treatment groups associated with a food-based maternal nightblindness treatment trial as described elsewhere (15).

Fieldworkers conducted house-to-house surveys in the Saptari District of the southeastern Terai region of rural Nepal and inquired whether any pregnant women complained of *ratauni*, the local Maithili term for nightblindness. Women were eligible to participate if they were <8 mo pregnant and were without fever, underlying chronic disease, or any physical signs of xerophthalmia. Women who reported nightblindness described *ratauni* as occurring soon after dusk and requiring changes in their routine activities, such as cooking, cleaning, and tending to their families. Neighbors and close family members (mothers-in-law and children), if present, were also questioned to confirm maternal self-reports. No women reported consuming any form of supplemental vitamin A (locally known as *ratauni goti*, low-dose vitamin A capsules that enter Nepal from India, but they are not widely available) or iron and folic acid supplements.

During the study period, 6645 pregnant women were identified in Saptari, and 558 (8.4%) self-reported nightblindness (**Figure 1**). Approximately 41% of the nightblind women identified either did not meet inclusion criteria or declined to participate. One hundred six nightblind women were randomly assigned to either a supplement ($n = 55$) or placebo ($n = 51$) group while receiving vitamin A–fortified rice, and the remaining 225 women were enrolled in a vitamin A bioavailability trial occurring concurrently, as described elsewhere (15).

There is a high risk of mortality in women with nightblindness during pregnancy (16). Therefore, rather than using a placebo-control group, we recruited an untreated nonnightblind group in the same region and within the study period to serve as a local, concurrent reference group. Inclusion criteria, with the exception of nightblind status, were the same as for their nightblind counterparts participating in the trial. Of the 4154 nonnightblind women surveyed, 128 (3.1%) agreed to participate. A large proportion of these nonnightblind women refused to participate because they felt it would not be of benefit to them or their family to be involved in the study. Questionnaires, blood sampling, and dark adaptation measurements of the reference group were completed during a single visit to the field site center after enrollment.

Because of the low level of literacy in this community, the subjects' rights and the description of the study were explained orally, and their thumbprints were obtained as evidence of informed consent. The study protocol was approved by the Office of Human Research Protection at the University of California, Davis (UC Davis), and by the Nepal Health Research Council, Kathmandu, Nepal.

Study design

Eligible nightblind women were randomly assigned to receive 1 meal 6 d/wk for a period of 6 wk. The meal was vitamin

A–fortified rice [850 μg retinol activity equivalents (RAE)] served with a white gourd or cauliflower and potato curry, and it included either an iron (30 mg as ferrous sulfate) and riboflavin (6 mg) supplement (FeR + VA) or a placebo (VA only) capsule. Random assignment to masked treatment groups occurred in 8–10-wk recruitment cycles. Observed changes in PT, hemoglobin, erythrocyte riboflavin, and plasma retinol and ferritin during previous studies were used as the outcome variables for sample size calculations. A moderate effect size ≥ 0.5 SD, power of 80% (1 β), a significance level of 5% (α), and an assumed 10% attrition rate were used in the calculation, which resulted in the desired sample size of 50 women/group.

Supplement and food preparation and administration

The capsule shells used for both treatment groups were from the same batch and lot and were identical in appearance; they were dark blue and opaque to protect against light exposure. The iron and riboflavin capsules contained lactose powder as filler and were produced by the Drug Product Services Laboratory at the University of California, San Francisco (San Francisco, CA). They were stored in a dry, opaque bottle at room temperature. The placebo capsules consisted of a negligible amount of corn oil (150 μL) and were prepared on site and stored in zippered foil bags at −20 °C.

All meals were cooked fresh daily, carefully divided into aliquots, and placed into individual thermos flasks according to a written protocol. The meals and supplements were transported to each subject's home by motorbike and were served by a field motivator, who observed and recorded consumption. The meal consisted of 200 g cooked vitamin A–fortified rice (Ultra-Rice; Program for Appropriate Technology for Health, Seattle, WA) providing 850 μg RAE vitamin A and a white gourd- and potato-based curry that was low in vitamin A. The meal was cooked in 15 g (1.1 tbsp) mustard seed oil to enhance vitamin A absorption, and it provided a total of ≈370 kcal/d.

As incentive to participate, women in the nonnightblind comparison group were also offered a daily meal for up to 4 wk. Their meal consisted of local rice served with a vegetable curry low in vitamin A. The vegetable curry was the same as the preparation provided to the nightblind subjects.

Anthropometric, sociodemographic, and dietary data collection

The nightblind women were transported to the study clinic once for baseline evaluations and then weekly during the 6-wk period for follow-up assessments. Height was measured to the nearest 0.1 cm by using a stadiometer (model 214; Seca, Brooklyn, NY); weight was measured to the nearest 0.1 kg by using an electronic scale (model 890; Seca, Hamburg, Germany). The women were asked a series of questions from a standardized questionnaire that included medical history and sociodemographic information. A food-frequency questionnaire that focused on general intake of vitamin A–containing foods and categories of animal source foods was also administered during each of a subject's weekly visits to the study clinic. Animal source foods evaluated included milk, curd, eggs, poultry, meat, and organ meats. The data from the food-frequency questionnaire were stratified into 2 categories: consuming riboflavin-rich (animal source) foods >2 times/wk or ≤2 times/wk.

Pupillary threshold measurement

Each woman's visual acuity was assessed with the use of an eye chart at a distance of 4.9 m. Women who had monovision or who could not see any part of the eye chart at daytime (n = 3) were excluded from the study. The PT was measured at baseline and at the 6 weekly follow-up visits by using a hand-held illuminator (LKC Technologies Inc, Gaithersburg, MD) designed to fit over one eye (17, 18). The illuminator has 12 intensity settings at ≈0.4-log-unit intervals. These intensity settings were later converted to the corresponding level of light intensity (in units of cd/m^2) according to the baseline calibration of the illuminator. Before the testing, each subject was exposed to the flash of a battery-powered, hand-held camera to bleach the retina. Immediately after this camera flash, the subject was placed in a completely dark room for 10 min. The room was 1.8 × 1.2 m and painted black, and all corners and doorway crevices were covered by drapery to ensure that no light could enter the room. A red guidelight was used to ensure the proper fit of the illuminator over the stimulated eye and to examine the observed eye. The subject was asked to look straight ahead while the light intensity was manually increased at 4-s intervals. The light intensity that produced the first sign of pupillary constriction in the observed eye as seen through a 2.5× magnification loupe was recorded as the PT score. If the constriction was weak but definite, and if the next light intensity produced a strong constriction, the intensity at which the weak constriction occurred was recorded. Instrument drift occurred over time, which required the PT scores to be adjusted on the basis of linear regression according to the date of entry into the study, as described previously (15); this process results in the adjusted PT scores reported here. PT scores ≥–1.11 log cd/m^2 indicated impaired dark adaptation (18), and those ≤–1.24 log cd/m^2 indicated normal dark adaptation (19).

Sample collection and laboratory analyses

Blood was collected from subjects by venipuncture into heparinized tubes (Monovette; Sarstedt Inc, Newton, NC) before intervention and 6 wk after intervention to measure hemoglobin, erythrocyte riboflavin, plasma ferritin and retinol, albumin, and C-reactive protein (CRP). The samples were transported to UC Davis on dry ice and stored frozen at −20 °C until analysis. Paired baseline and final samples were analyzed in duplicate during the same analytic run.

Erythrocyte riboflavin was measured by using HPLC (1100 Series HPLC; Agilent Technologies Inc, Palo Alto, CA); plasma retinol concentrations (20, 21) were measured by using a Class VP HPLC (Shimadzu, Columbia, MD). The within-day CVs of analytes in pooled, control samples were ≤5.4% for plasma retinol and ≤10.0% for erythrocyte riboflavin. Erythrocyte riboflavin concentrations < 170 nmol/L (20) and plasma retinol concentrations < 0.70 μmol/L (22) indicated deficiency of riboflavin and vitamin A, respectively. Hemoglobin was measured by using HemoCue (HemoCue Inc, Lake Forest, CA), and plasma ferritin concentration was measured by using an immunoradiometric assay (Coat-A-Count IRMA; Diagnostic Products Corporation, Los Angeles, CA). Anemia was defined as a hemoglobin concentration < 110 g/L and iron deficiency as plasma ferritin < 12 μg/L (23). Iron deficiency anemia (IDA) was defined as combined hemogoblin < 110 g/L and plasma ferritin < 12 μg/L.

CRP concentrations were measured with the use of radial immunodiffusion (Nanorid; The Binding Site, Birmingham, United Kingdom). Concentrations >10 mg/L indicated the presence of an infection or inflammatory process, which may increase plasma ferritin concentrations and lower plasma retinol concentrations (24, 25). Only 3 women (3.1%) had elevated CRP concentrations; their mean plasma ferritin and retinol concentrations did not differ from those with normal CRP values. Therefore, results from all subjects were included in the analysis, regardless of their CRP status. Plasma albumin was measured by using the bromocresol green method (ALB plus; Roche Diagnostics, Mannheim, Germany) with the use of a Hitachi autoanalyzer (Ibaraki, Japan).

Statistical analysis

Statistical analyses were performed with the use of PC-SAS software (release 8.01; SAS Institute Inc, Cary, NC). Log transformation was used to normalize the distributions of plasma ferritin, erythrocyte riboflavin, hemoglobin-to-albumin ratio, and ferritin-to-albumin ratio. Subject characteristics were compared between the FeR + VA group and the VA only group by using Student's *t* test and a chi-square test, as appropriate. Analysis of variance (ANOVA) followed by Tukey-Kramer post hoc comparisons was used to analyze baseline biochemical measures in the 2 nightblind groups and the nonnightblind women. Pearson's correlation was used to examine associations between PT score and baseline main effects. Analysis of covariance with SAS PROC GLM was used to analyze PT score changes from baseline by treatment group with respect to measures of erythrocyte riboflavin, plasma retinol, and ferritin, after control for the baseline value of the respective response variables, pregnancy month, enrollment cycle, and frequency of consuming animal source foods; this was followed by Tukey-Kramer post hoc comparisons of group means. Nonsignificant main effects were removed systematically from the models. Interaction terms for change in PT score between treatment group and baseline status of riboflavin, iron, and vitamin A were evaluated with the use of the same procedure after control for baseline PT score. To consider the hemodilution effects of pregnancy, final hemoglobin and plasma ferritin concentrations were also evaluated jointly with albumin in a ratio by using the same analysis of covariance procedures. $P < 0.05$ was considered significant for all tests.

RESULTS

The nightblind women who were lost to follow-up or who dropped out ($n = 10$) did not differ in any characteristics from the women who completed the study. The nightblind women who remained in the study were from families with low socioeconomic status, and ≈40% had a body mass index (in kg/m^2) < 18.5 (**Table 1**). The caste distribution did not differ between the groups. Most of the women were from either the Shudra or Baishya caste, and most were illiterate. Consumption of animal source foods (mostly dairy and poultry) was infrequent and did not differ between treatment groups. None of the nightblind subjects reported eating liver. As a reference comparison group, the nonnightblind women did not differ significantly in age or gestational stage from the nightblind women participating in the trial. On average, the nonnightblind women weighed more, had a higher BMI, and consumed more chicken and curd than did their nightblind counterparts.

TABLE 1
Baseline characteristics of nightblind and nonnightblind pregnant nepali women[1]

	Nonnightblind women ($n = 128$)	Nightblind women	
		FeR + VA ($n = 55$)	Placebo ($n = 51$)
Age (y)	23.5 ± 5.0[2]	24.4 ± 5.3	24.7 ± 5.6
Gestation (mo)	5.6 ± 1.3	5.3 ± 1.0	5.3 ± 1.1
Weight (kg)	45.1 ± 5.5[3]	44.1 ± 5.9	41.9 ± 5.2
Height (cm)	150.2 ± 5.3	150.3 ± 6.0	148.9 ± 5.7
BMI (kg/m^2)	20.0 ± 2.0[4]	19.4 ± 2.0	18.8 ± 1.8
Caste distribution [*n* (%)]			
Shudra	49 (38.3)	27 (49.1)	25 (49.0)
Baishya	46 (35.9)	24 (43.6)	23 (45.1)
Muslim	25 (19.5)	3 (5.5)	3 (5.9)
Chettri	4 (3.1)	1 (1.8)	0 (0)
Brahmin	4 (3.1)	0 (0)	0 (0)
Literate (%)	25	15	16
Intake > twice in previous week [*n* (%)]			
Milk	58 (45.3)	17 (30.9)	21 (41.2)
Chicken	36 (28.6)[3]	8 (14.5)	6 (11.8)
Curd	35 (27.3)[5]	7 (12.7)	8 (15.7)
Meat	10 (7.9)	1 (1.8)	2 (3.9)
Eggs	8 (6.3)	3 (5.5)	0 (0)
Liver	1 (0.8)	0 (0)	0 (0)

[1] FeR + VA, vitamin A (850 μg retinal activity equivalents) plus 30 mg Fe and 6 mg riboflavin; Placebo, vitamin A (equal amount) only. There were no differences between the 2 groups of nightblind women.

[2] $\bar{x}$ ± SD (all such values).

[3–5] Significantly different from each of the nightblind treatment groups (general linear model ANCOVA with pregnancy months as a covariate; Tukey-Kramer test for multiple comparisons): [3]$P < 0.01$, [4]$P < 0.002$, [5]$P < 0.03$.

No significant differences at baseline were found between nonnightblind women and either nightblind treatment group for any of the biochemical measurements (**Table 2**) except erythrocyte riboflavin concentration ($P < 0.01$). The nightblind women in the VA only treatment group had a higher prevalence of low hemoglobin values at the start of the study than did the women in the FeR+VA group ($P < 0.03$) (**Table 3**).

Among nightblind women at baseline, plasma retinol and ferritin concentrations were positively associated, and both measures were negatively associated with PT score (**Table 4**). Frequency of egg consumption was positively correlated with erythrocyte riboflavin concentration, and the frequency of milk and chicken consumption was positively associated with plasma retinol concentration (data not shown).

Of the nightblind subjects, low concentrations of plasma retinol were detected in 18% of the VA only group and in 27% of the FeR + VA group at baseline (Table 3). The decrease in vitamin A deficiency from baseline was not significant in either treatment group ($P = 0.52$) (Table 3). Likewise, no significant difference was observed between treatment groups in baseline or final mean concentrations of plasma retinol, and the values increased similarly in both groups after treatment (Table 2). Because the mean change in plasma retinol concentration was small, additional tests were done to evaluate interactions between treatment group and baseline iron status. The interaction terms for difference in

TABLE 2
Baseline and final biochemical concentrations and concentration change by nightblind status and treatment group[1]

		Nightblind women	
	Nonnightblind women[2]	FeR + VA[3]	Placebo[4]
Plasma ferritin (μg/L)			
Baseline	15.2 (13.0, 17.8)	15.9 (12.8, 19.8)	16.6 (12.5, 21.8)
Final		20.4 (17.9, 23.2)[b]	15.7 (13.6, 18.2)[a]
Change		3.9 (1.4, 6.6)[b]	−0.7 (−2.7, 1.8)[a]
Erythrocyte riboflavin (nmol/L)			
Baseline	179.1 (172.9, 185.5)[a]	164.8 (154.1, 176.0)[b]	153.8 (142.2, 166.3)[b]
Final		248.6 (236.7, 261.2)[b]	150.4 (142.3, 159.0)[a]
Change		92.2 (79.7, 105.3)[b]	−4.7 (−12.6, −1.6)[a]
Plasma retinol (μmol/L)			
Baseline	1.01 ± 0.4	0.99 ± 0.4	0.97 ± 0.3
Final		1.02 ± 0.04	1.08 ± 0.05
Change		0.04 ± 0.04	0.11 ± 0.05
Hemoglobin (g/L)			
Baseline	110.6 ± 11.7	109.2 ± 14.8	105.3 ± 11.9
Final		106.7 ± 1.4	104.8 ± 1.6
Change		−2.7 ± 1.7	−0.8 ± 1.5
Albumin (g/dL)			
Baseline		3.4 ± 0.6	3.6 ± 0.4
Final		3.2 ± 0.4	3.3 ± 0.4
Ferritin:albumin			
Baseline		4.52 (3.64, 5.60)	4.80 (3.66, 6.28)
Final		6.03 (5.05, 7.20)[b]	5.06 (3.94, 6.49)[a]
Change		1.87 (1.37, 2.53)[b]	1.15 (0.82, 1.62)[a]
Hemoglobin:albumin			
Baseline		3.07 (2.94, 3.21)	3.02 (2.90, 3.15)
Final		3.24 (3.11, 3.38)	3.18 (3.04, 3.32)
Change		0.16 (0.08, 0.24)	0.13 (0.04, 0.21)

[1] Baseline values are $\bar{x}$ ± SD; final and change values are geometric $\bar{x}$ ± SEM; 95% CIs in parentheses. FeR + VA, vitamin A (850 μg retinal activity equivalents) plus 30 mg Fe and 6 mg riboflavin; Placebo, VA (equal amount) only. At baseline, values in a row with different superscript letters are significantly different, $P < 0.0005$ [Student's t test (or where nonnightblind group comparisons are made, ANOVA); Tukey-Kramer test]. At final, values in a row with different superscript letters are significantly different, $P < 0.01$ (general linear model ANCOVA with pregnancy months, study cycle, animal source food consumption, and baseline values as covariates; Tukey-Kramer test for group comparisons). For ferritin:albumin, values in a row with different superscript letters are significantly different, $P < 0.05$ (general linear model ANCOVA with pregnancy months and baseline ratio values as covariates; Tukey-Kramer test for group comparisons). For changes, values in a row with different superscript letters are significantly different, $P < 0.05$ [ANCOVA with pregnancy months, high C-reactive protein (for ferritin and retinol), and baseline values as covariates; Tukey-Kramer test for group comparisons].

[2] $n = 128$.

[3] $n = 55$ baseline and 53 final.

[4] $n = 51$ baseline and 43 final; because of insufficient sample for albumin analysis in 1 subject, $n = 50$ baseline and $n = 42$ final for albumin and both ratio values.

retinol change in iron-deficient (0.03 ± 0.05 μmol/L) and iron-nondeficient (0.09 ± 0.04 μmol/L) women were not significant ($P = 0.385$).

More than half of the subjects in the nightblindness trial had riboflavin deficiency and anemia at the start of the study (Table 3). Supplementation with iron and riboflavin was effective in increasing final erythrocyte riboflavin concentrations ($P < 0.0001$). A dramatic reduction was observed in the prevalence of riboflavin deficiency in the FeR + VA group, whereas a nonsignificant increase in prevalence, from 70.5% before treatment to 79.1% after treatment, was observed in the VA only group (Table 3).

Nightblind women at baseline had moderate prevalences of iron deficiency (36–44%) and IDA (30–35%) (Table 3). Women who received the supplements of iron and riboflavin had a significantly greater increase in their plasma ferritin concentrations than did women who did not receive the supplements ($P = 0.01$) (Table 2). The FeR + VA treatment group also had lower final prevalence of iron deficiency and IDA than at baseline ($P < 0.01$ and < 0.007, respectively) (Table 3). Paradoxically, the changes in hemoglobin concentration and prevalence of anemia were not significant.

Plasma albumin was measured to control for plasma volume expansion during pregnancy, which can lower plasma ferritin concentrations (26). Albumin concentrations fell significantly over time and equally in both groups. The change in plasma ferritin concentration by treatment group remained significant after control for albumin; the final difference in plasma ferritin-to-albumin ratio from baseline remained greater in the FeR + VA group than in the VA only group ($P < 0.05$; Table 2).

No difference was observed in the mean baseline PT score between nightblind women assigned to the FeR + VA [−0.69 log cd/m^2 (95% CI: −0.81, −0.58 log cd/m^2)] and VA only [−0.70 log cd/m^2 (95% CI: −0.86, −0.54 log cd/m^2)] groups (Table 3). However, nonnightblind women had a better mean PT

TABLE 3
Proportion of nonnightblind and nightblind women with abnormal biochemical values and pupillary threshold at baseline and at final and the mean prevalence change after treatment[1]

		Nightblind women	
	Nonnightblind women (n = 128)	FeR + VA (n = 55)	Placebo (n = 51)
	%	%	
Plasma ferritin < 12 μg/L			
Baseline	43.0 ± 4.4	43.6 ± 6.7	35.6 ± 7.2
Final		20.8 ± 5.6	37.2 ± 7.5
Change		−22.6 ± 7.4[b]	2.3 ± 6.2[a]
Erythrocyte riboflavin < 170 nmol/L			
Baseline	39.8 ± 4.3[a]	60.0 ± 6.7[b]	70.5 ± 7.0[b]
Final		5.7 ± 3.2	79.1 ± 6.3
Change		−54.3 ± 5.3[b]	8.6 ± 5.7[a]
Pupillary threshold > −1.11 log cd/m^2			
Baseline	14.8 ± 3.2[a]	87.3 ± 4.5[b]	75.6 ± 6.5[b]
Final		30.2 ± 6.4	39.5 ± 6.7
Change		−57.1 ± 7.3[b]	−36.1 ± 8.0[a]
Plasma retinol < 0.70 μmol/L			
Baseline	19.5 ± 3.5	27.3 ± 6.1	17.8 ± 5.8
Final		18.9 ± 5.4	14.0 ± 5.4
Change		−7.5 ± 6.5	−4.7 ± 6.6
Hemoglobin < 110 g/L			
Baseline	44.5 ± 4.4[a]	45.5 ± 6.8[a]	64.4 ± 7.2[b]
Final		54.7 ± 6.9	69.8 ± 7.1
Change		9.4 ± 6.2	6.7 ± 7.0
Iron deficiency anemia[2]			
Baseline	23.4 ± 3.8	34.6 ± 6.5	29.6 ± 7.0
Final		15.1 ± 5.0	33.3 ± 7.4
Change		−20.8 ± 6.8[b]	4.8 ± 5.9[a]

[1] All values are $\bar{x}$ ± SEM. FeR + VA, vitamin A (850 μg retinal activity equivalents) plus 30 mg Fe and 6 mg riboflavin; Placebo, VA (equal amount) only. At baseline, values in a row with different superscript letters are significantly different, $P < 0.03$ [logistic regression with pregnancy months and high C-reactive protein (for plasma ferritin and retinol) as covariates]. For changes, values in a row with different superscript letters are significantly different, $P < 0.05$ [logistic regression with control for pregnancy months, high C-reactive protein (for ferritin and retinol), and baseline status as covariates; Tukey-Kramer test for group comparisons].
[2] Plasma ferritin <12 μg/L and hemoglobin <110 g/L.

[−1.43 log cd/m^2 (95% CI: −1.49, −1.37 log cd/m^2), $P <$ 0.0001] than did the nightblind women at baseline.

Although all of the women reported recovery from nightblindness at the end of the treatment period, 16 women (30.2%) in the FeR + VA group and 17 women (39.5%) in the VA only group still had abnormal dark adaptation at the final timepoint. The final prevalence of poor dark adaptation after treatment remained significantly greater in both intervention groups than in the nonnightblind comparison women ($P < 0.005$). It is interesting that, although the final prevalence of impaired PT did not differ by treatment group, women in the FeR + VA group had a greater reduction in impaired PT prevalence from baseline than did

TABLE 4
Pearson correlations of enrollment cycle, gestation, biochemical measures, and pupillary threshold (PT) at baseline in nightblind women (n = 106)[1]

Variable	Plasma retinol	Plasma ferritin	Erythrocyte riboflavin	Hemoglobin	PT
Enrollment cycle	0.03	0.05	0.15	−0.23[2]	−0.13
Gestation	−0.01	−0.15	−0.22[2]	−0.02	0.01
Plasma retinol	—	0.23[2]	−0.07	0.16	−0.32[3]
Plasma ferritin	—	—	−0.15	0.36[4]	−0.34[4]
Erythrocyte riboflavin	—	—	—	−0.21[2]	0.05
Hemoglobin	—	—	—	—	−0.12
Pupillary threshold	—	—	—	—	—

[1] Plasma ferritin and erythrocyte riboflavin were log transformed to normalize distributions.
[2] $P < 0.05$.
[3] $P \leq 0.005$.
[4] $P < 0.001$.

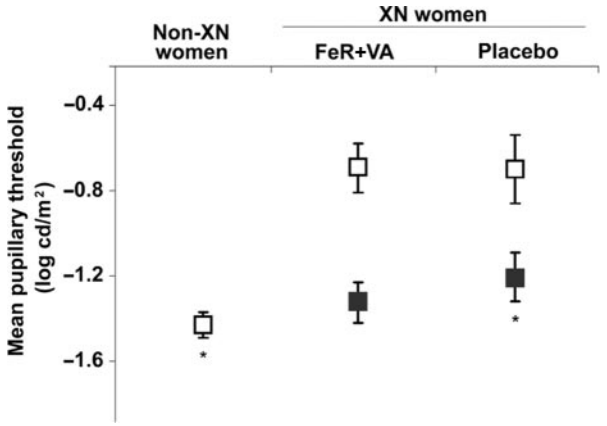

FIGURE 2. Box plot of mean (±SE) pupillary thresholds of nonnightblind (non-XN) (baseline, $n = 128$) and nightblind (XN) women at baseline. [(□) FeR + VA, $n = 55$; (■) VA, $n = 51$] and final (FeR + VA, $n = 53$; VA, $n = 43$) timepoints. FeR + VA, vitamin A (850 μg retinal activity equivalents) plus 30 mg Fe and 6 mg riboflavin; Placebo, VA only. *Nonnightblind women differed significantly from all nightblind women at baseline ($P < 0.0001$) and from the Placebo group at the end of the study ($P < 0.03$) (a general linear model ANCOVA with pregnancy months, study cycle, animal source food consumption, and baseline values as covariates; Tukey-Kramer test for group comparisons).

women in the VA only group ($P < 0.05$) (Table 3). This greater reduction in impaired PT prevalence was also reflected in the analysis of mean final PT scores in the treatment groups and in the nonnightblind reference group (**Figure 2**). Although no significant difference was observed in the mean final PT score between the treatment groups ($P = 0.17$), the mean PT score of the FeR + VA group (-1.32 log cd/m^2; 95% CI: -1.42, -1.21 log cd/m^2) at the end of the study was not significantly different from that of the nonnightblind women. In contrast, the mean final PT score of the VA only group (-1.21 log cd/m^2; 95% CI: -1.32, -1.09 log cd/m^2) remained significantly poorer than that of the nonnightblind group by the end of the study ($P < 0.03$). The PT score distributions for nightblind (final scores by treatment group) and nonnightblind (single, baseline scores) women are shown in **Figure 3**.

The change in PT score was further evaluated for interactions between treatment group and baseline status of vitamin A, iron, riboflavin, and IDA. Vitamin A ($P = 0.04$), iron ($P = 0.01$), and IDA ($P = 0.01$) status all interacted significantly with treatment group, whereas the interaction between treatment group and baseline riboflavin status was not significant ($P = 0.13$). Women with poor iron status given supplements of iron and riboflavin had a significantly larger improvement in PT score (-1.41 ± 0.20 log cd/m^2) than did women given vitamin A alone (-0.65 ± 0.25 log cd/m^2) ($P = 0.05$) (**Figure 4**). In contrast, the PT score improvement in women with adequate iron status did not differ between treatment groups and was similar to the PT score change in iron-deficient women given supplements of iron and riboflavin. Similar differences were seen in PT score change in the placebo group according to IDA status ($P < 0.01$).

DISCUSSION

To evaluate the efficacy of simultaneous administration of iron and riboflavin to improve abnormal dark adaptation and nutritional status, a randomized, masked supplementation study providing vitamin A–fortified rice was conducted in rural Nepal on pregnant women who reported being nightblind. Overall, there was no benefit of iron and riboflavin supplements in improving PT score beyond that achieved by vitamin A alone. However, a greater improvement in PT score was observed in a subgroup of women who initially were iron deficient. Supplementation with iron and riboflavin significantly reduced deficiencies of iron and riboflavin and the prevalence of IDA.

Evaluating the PT distributions between the treatment groups and nonnightblind women provided a visual explanation of how there could be such a large difference in final impaired dark adaptation prevalence, and there were only small differences in final PT means. The distribution in the group supplemented with iron and riboflavin became more centralized and similar to that in the nonnightblind women, such that more women in the FeR + VA treatment group were below the PT cutoff.

We observed a significant association between PT score and plasma ferritin at baseline, which showed a possible role for iron in the dark adaptation process. Studies in mice confirm the importance of iron and iron transport to the retina for utilization in rod cells and the inner and outer segments of photoreceptors (27). In a study by Sarici et al (28), subclinical visual impairment as measured by visual-evoked potential was attributed to IDA in otherwise healthy infants aged 7–24 mo ($n = 20$). Although dark adaptation was not assessed in those infants, iron supplementation (4 mg $FeSO_4$/kg) given daily for 12 wk significantly decreased latencies of visual-evoked potential (improved response to visual stimuli) compared with baseline.

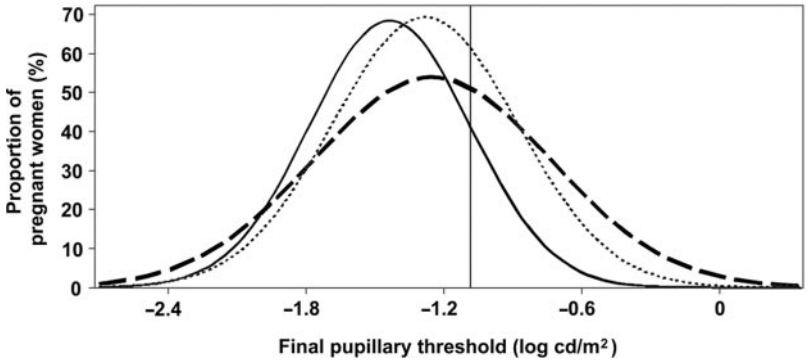

FIGURE 3. Distribution of pupillary threshold scores in nonnightblind (—; $n = 128$) and nightblind women after receiving vitamin A–fortified rice without (– – –; $n = 43$) or with (···; $n = 53$) iron and riboflavin. The vertical line represents the provisional cutoff value for abnormal dark adaptation associated with vitamin A deficiency (-1.11 log cd/m^2).

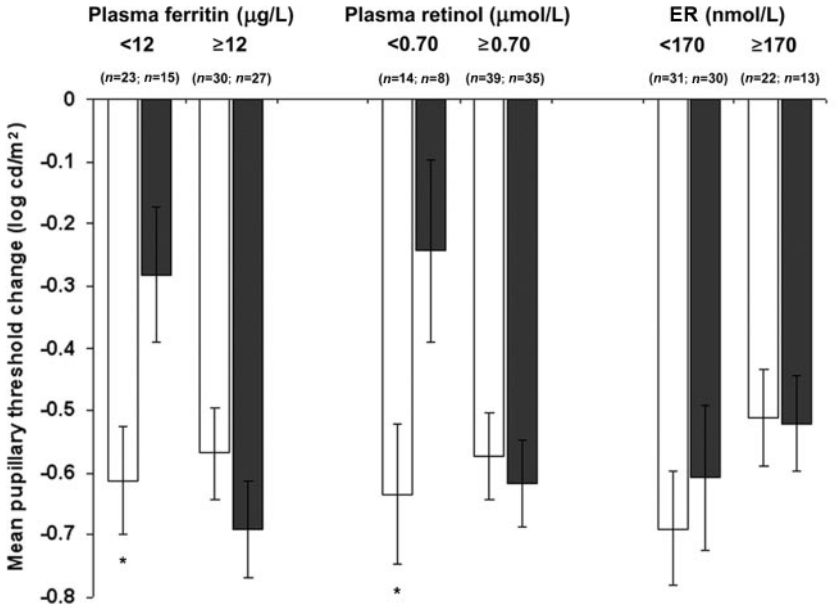

FIGURE 4. Mean (±SE) pupillary threshold changes of nightblind women after receiving vitamin A–fortified rice without (placebo; ■) or with iron plus riboflavin supplementation (FeR + VA; □) according to baseline iron, vitamin A, and riboflavin status. ER, erythrocyte riboflavin. *Significant treatment group × deficiency status interactions (a general linear model ANCOVA with baseline status and pupillary threshold as covariates): $P = 0.01$ for low plasma ferritin and $P = 0.04$ for low plasma retinol.

In further evaluation of our data, the greater change in PT score in response to treatment among the iron-deficient than among the iron-replete women also correlated with the mean change in riboflavin concentration. Although riboflavin is required for some photoreceptors, the functional importance of riboflavin in the correction of nightblindness remains unclear. The results from the present study suggest that the role of riboflavin in dark adaptation may be primarily to mobilize iron from ferritin in iron-deficient women, rather than doing so by an effect on the riboflavin-dependent photoreceptors. These issues could be addressed better if additional treatment arms, such as treatment with iron and vitamin A or riboflavin and vitamin A, were incorporated into this study.

Providing iron to these iron-deficient women was expected to increase hepatic retinyl ester hydrolysis and therefore to increase the circulating retinol concentration (4), but this was not observed. Although the prevalence of nightblindness was high in this region, the plasma retinol concentrations of these women were not as low as those seen in previous reports in nightblind women. Previous studies from other areas of Nepal have reported mean serum retinol concentrations of 0.73–0.87 μmol/L and a mean PT score of −0.84 log cd/m^2 in pregnant nightblind women (29–31). The observations in the present study were of mean concentrations of serum retinol nearer to adequacy (≥1.05 μmol/L), on average, and poorer mean dark adaptation values than those previously reported by Christian et al in other nightblind pregnant women in the Terai (31). It is interesting that the mean plasma concentration of retinol among the nonnightblind women is much lower than the serum concentrations (1.17–1.24 μmol/L) reported in healthy pregnant Nepali women in the Terai (32). Plasma vitamin A concentrations required to achieve normal dark adaptation were reported to range from 0.70 to ≥ 1.40 μmol/L (33, 34). The wide range can indicate the interindividual variability of visual sensitivity, possibly influenced by an array of nutrient deficiencies. The PT scores improved significantly in both treatment groups, even though plasma retinol concentrations increased only slightly.

A supplementation trial by Tanumihardjo et al (35) found a significant improvement in vitamin A status by the modified relative dose-response test without detecting a significant change in the serum retinol concentration of pregnant Indonesian women ($n = 27$) who had marginal vitamin A status at baseline (21–28 μg/dL) and who were given 8000 IU vitamin A plus 60 mg Fe for 8 wk. If plasma retinol concentrations do not respond to treatment when baseline values are marginal, the use of more sensitive measures of vitamin A status, such as modified relative dose response or PT score, should be more informative. However, both the current study and the trial by Tanumihardjo et al (35) may have had too small a sample size to allow detection of any significant differences in plasma retinol concentrations. The issue of small sample size may also be a factor in our not finding a significant PT difference in the post hoc analysis of the vitamin A–deficient women.

Recent developments in vitamin A assessment indicate that stable-isotope dilution provides the most accurate estimate of status. Stable-isotope dilution has been proposed as the standard of reference for other measures of vitamin A status (36). However, the mathematical model has not yet been applied or investigated in pregnant women.

At baseline, half of the women assigned to treatment groups had anemia, and nearly one-third had IDA. IDA contributes to rates of maternal morbidity and mortality and is associated with preterm delivery and low birth weight (37, 38). Anemia and iron deficiency during pregnancy have been reported frequently in Nepal (39–42). The World Health Organization considers anemia (hemoglobin < 110 g/L) to be a severe public health problem

in areas where the prevalence is >40% (43). In the present study, supplementation with iron and riboflavin significantly reduced the prevalence of IDA. Unlike previous studies that have reported positive changes in hemoglobin values and anemia when supplemental iron and riboflavin were provided (5, 44–46; LH Allen, unpublished observations, 1999), neither an improvement in hemoglobin concentrations (even after adjustment with albumin for plasma volume expansion) nor a reduction in anemia prevalence was observed in the present study. The iron dose provided in this study (30 mg) was sufficient to significantly improve iron status and reduce IDA by 50% but not to reduce the prevalence of anemia. Although the relatively short duration of treatment may have contributed to this situation, it is also possible that there are other contributors to the underlying anemia, perhaps a deficiency of vitamin B-12 or folate. Studies investigating women in this region have reported that vitamin B-12 and folate deficiencies are common. Daily supplementation from early pregnancy through 3 mo after delivery with 400 μg folic acid and 60 mg Fe reduced the prevalence of anemia by 54% in pregnant women in rural Nepal (47).

After the supplementation period, 40% of women in the VA only group and 30% in the FeR + VA group still had abnormal PT scores. Zinc deficiency may also contribute to the persistence of nightblindness in these women. Christian et al (31) found some additional curative benefit from zinc supplementation in nightblind women with low serum concentrations of zinc who were unresponsive to vitamin A treatment. Zinc status should be taken into consideration for future studies, especially in populations in which zinc deficiency is a public health concern.

Nightblindness is a debilitating disorder for pregnant women in Nepal, which negatively affects their quality of life (48) and increases the risk of mortality of their infants by 63% in the first 6 mo of life (49). This study shows that nutrient deficiencies in addition to vitamin A deficiency must be involved. Iron deficiency, in particular, may limit the efficacy of vitamin A programs in the treatment of nightblindness in pregnant women. More research is needed to test different strategies for simultaneous delivery of multiple micronutrients, especially in combination with iron, appropriate micronutrient-rich foods, or both, for the prevention and treatment of nightblindness during pregnancy.

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The authors' responsibilities were as follows—JMG: study design and management at the field site, training of field personnel, data collection, analyses and interpretation, laboratory analyses, and writing of the manuscript; MJH: study design, training of field personnel, study management, and laboratory analyses; PP: study coordinator at the field site, training of field personnel, and data collection; RKS: study design, training of personnel, and overall management of the study in Nepal; KHB: study design, study management, data analyses and interpretation, and revision of the manuscript; LHA: study design, data analysis and interpretation, and revision of the manuscript. JMG conducted this research for a doctoral thesis at the University of California, Davis. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. World Health Organization. Micronutrient deficiencies: battling iron deficiency anemia. 2003. Internet: http://www.who.int/nut/ida.htm (accessed 15 November 2005).
2. World Health Organization. Micronutrient Deficiency Information System: database on anemia. 2005. Internet: http://www.who.int/nut/db_mdis.htm, (accessed 15 November 2005).
3. Jang JT, Green JB, Beard JL, Green MH. Kinetic analysis shows that iron deficiency decreases liver vitamin A mobilization in rats. J Nutr 2000; 130:1291–6.
4. Rosales FJ, Jang JT, Piñero DJ, Erikson KM, Beard JL, Ross AC. Iron deficiency in young rats alters the distribution of vitamin A between plasma and liver and between hepatic retinol and retinyl esters. J Nutr 1999;129:1223–8.
5. Powers HJ, Bates CJ, Lamb WH. Haematological response to supplements of iron and riboflavin to pregnant and lactating women in rural Gambia. Hum Nutr Clin Nutr 1985;39:117–29.
6. Pongpaew P, Saowakontha S, Schelp FP, et al. Vitamin B1, B2 and B6 during the course of pregnancy of rural and urban women in northeast Thailand. Int J Vitam Nutr Res 1995;65:111–6.
7. Batey DW, Eckhert CD. Identification of FAD, FMN, and riboflavin in the retina by microextraction and high-performance liquid chromatography. Anal Biochem 1990;188:164–7.
8. Batey DW, Eckhert CD. Analysis of flavins in ocular tissues of the rabbit. Invest Ophthalmol Vis Sci 1991;32:1981–5.
9. Batey DW, Daneshgar KK, Eckhert CD. Flavin levels in the rat retina. Exp Eye Res 1992;54:605–9.
10. Miyamoto Y, Sancar A. Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. Proc Natl Acad Sci U S A 1998;95:6097–102.
11. Zaman Z, Verwilghen RL. Effects of riboflavin deficiency on oxidative phosphorylation, flavin enzymes and coenzymes in rat liver. Biochem Biophys Res Commun 1975;67:1192–8.
12. Zaman Z, Verwilghen RL. Effect of riboflavin deficiency on activity of NADH-FMN oxidoreductase (ferriductase) and iron content of rat liver. Biochem Soc Trans 1977;5:306–8.
13. Fairweather-Tait SJ, Powers HJ, Minski MJ, Whitehead J, Downes R. Riboflavin deficiency and iron absorption in adult Gambian men. Ann Nutr Metab 1992;36:34–40.
14. Christian P, West KP Jr, Khatry SK, et al. Vitamin A or beta-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. J Nutr 1998;128:1458–63.
15. Haskell MJ, Pandey P, Graham JM, Peerson JM, Shresta RK, Brown KH. Recovery from impaired dark adaptation in nightblind pregnant Nepali women who receive small daily doses of vitamin A as amaranth leaves, carrots, goat liver, vitamin A-fortified rice, or retinyl palmitate. Am J Clin Nutr 2005;81:461–71.
16. Christian P, West KP Jr, Khatry SK, et al. Night blindness during pregnancy and subsequent mortality among women in Nepal: effects of vitamin A and beta-carotene supplementation. Am J Epidemiol 2000; 152:542–7.
17. Congdon N, Sommer A, Severns M, et al. Pupillary and visual thresholds in young children as an index of population vitamin A status. Am J Clin Nutr 1995;61:1076–82.
18. Congdon NG, Dreyfuss ML, Christian P, et al. Responsiveness of dark-adaptation threshold to vitamin A and β-carotene supplementation in pregnant and lactating women in Nepal. Am J Clin Nutr 2000;72:1004–9.
19. Congdon NG, West KP Jr. Physiologic indicators of vitamin A status. J Nutr 2002;132(suppl):2889S–94S.
20. Graham JM, Peerson JM, Haskell MJ, Shrestha RK, Brown KH, Allen LH. Erythrocyte riboflavin for the detection of riboflavin deficiency in pregnant Nepali women. Clin Chem 2005;51:2162–5.
21. Handelman GJ, Shen B, Krinsky NI. High resolution analysis of carotenoids in human plasma by high-performance liquid chromatography. Methods Enzymol 1992;213:336–46.
22. Olson JA. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. J Natl Cancer Inst 1984;73:1439–44.
23. International Nutritional Anemia Consultative Group. Measurements of iron status. Washington, DC: The Nutrition Foundation, Inc, 1985:1–78.
24. Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal. Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. J Nutr 2002;132:3061–6.

25. Paracha PI, Jamil A, Northrop-Clewes CA, Thurnham DI. Interpretation of vitamin A status in apparently healthy Pakistani children by using markers of subclinical infection. Am J Clin Nutr 2000;72:1164–9.
26. Milman N, Graudal N, Nielsen OJ, Agger AO. Serum erythropoietin during normal pregnancy: relationship to hemoglobin and iron status markers and impact of iron supplementation in a longitudinal, placebo-controlled study on 118 women. Int J Hematol 1997;66:159–68.
27. Hahn P, Dentchev T, Qian Y, Rouault T, Harris ZL, Dunaief JL. Immunolocalization and regulation of iron handling proteins ferritin and ferroportin in the retina. Mol Vis 2004;10:598–607.
28. Sarici SU, Okutan V, Dundaroz MR, et al. The effect of iron supplementation on visual-evoked potentials in infants with iron-deficiency anemia. J Trop Pediatr 2001;47:132–5.
29. Christian P, West KP Jr, Khatry SK, et al. Night blindness of pregnancy in rural Nepal–nutritional and health risks. Int J Epidemiol 1998;27:231–7.
30. Christian P, West KP Jr, Khatry SK, et al. Vitamin A or beta-carotene supplementation reduces symptoms of illness in pregnant and lactating Nepali women. J Nutr 2000;130:2675–82.
31. Christian P, Khatry SK, Yamini S, et al. Zinc supplementation might potentiate the effect of vitamin A in restoring night vision in pregnant Nepalese women. Am J Clin Nutr 2001;73:1045–51.
32. Christian P, Jiang T, Khatry SK, LeClerq SC, Shrestha SR, West KP Jr. Antenatal supplementation with micronutrients and biochemical indicators of status and subclinical infection in rural Nepal. Am J Clin Nutr 2006;83:788–94.
33. Carney EA, Russell RM. Correlation of dark adaptation test results with serum vitamin A levels in diseased adults. J Nutr 1980;110:552–7.
34. Wachtmeister L, Björkhem I, Diczfalusy U, Emami A. Attempts to define the minimal serum level of vitamin A required for normal visual function in a patient with severe fat malabsorption. Acta Ophthalmol (Copenh) 1988;66:341–8.
35. Tanumihardjo SA. Vitamin A and iron status are improved by vitamin A and iron supplementation in pregnant Indonesian women. J Nutr 2002;132:1909–12.
36. Furr HC, Green MH, Haskell M, et al. Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. Public Health Nutr 2005;8:596–607.
37. Allen LH. Iron-deficiency anemia increases risk of preterm delivery. Nutr Rev 1993;51:49–52.
38. Ramakrishnan U. Functional consequences of nutritional anemia during pregnancy and early childhood. In: Ramakrishnan U, ed. Nutritional anemias. Boca Raton, FL: CRC Press, 2001:43–68.
39. Bondevik GT, Eskeland B, Ulvik RJ, et al. Anaemia in pregnancy: possible causes and risk factors in Nepali women. Eur J Clin Nutr 2000;54:3–8.
40. Dreyfuss ML, Shrestha JB, Khatry SK, et al. The prevalence of anaemia among pregnant and lactating women, and among their infants in Sarlahi District. J Nepali Med Assoc 1997;35:234–40.
41. Nepal Micronutrient Status Survey 1998. Kathmandu, Nepal: Ministry of Health, Child Health Division, HMG/N, New ERA, Micronutrient Initiative, UNICEF Nepal, and WHO, 1998:68–72.
42. Dreyfuss ML, Stoltzfus RJ, Shrestha JB, et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J Nutr 2000;130:2527–36.
43. World Health Organization. Iron deficiency anemia: assessment, prevention, and control: a guide for programme managers. 2001. Internet: http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf (accessed 3 August 2003).
44. Charoenlarp P, Pholpothi T, Chatpunyaporn P, Schelp FP. The effect of riboflavin on the hematologic changes in iron supplementation of schoolchildren. Southeast Asian J Trop Med Public Health 1980;11:97–103.
45. Powers HJ, Bates CJ, Prentice AM, Lamb WH, Jepson M, Bowman H. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. Hum Nutr Clin Nutr 1983;37:413–25.
46. Powers HJ. Experiment to determine the effect of riboflavin deficiency at weaning on iron economy and heme synthesis. Ann Nutr Metab 1985;29:261–6.
47. Christian P, Shrestha J, LeClerq SC, et al. Supplementation with micronutrients in addition to iron and folic acid does not further improve the hematologic status of pregnant women in rural Nepal. J Nutr 2003;133:3492–8.
48. Christian P, Thorne-Lyman AL, West KP Jr, et al. Working after the sun goes down: exploring how night blindness impairs women's work activities in rural Nepal. Eur J Clin Nutr 1998;52:519–24.
49. Christian P, West KP Jr, Khatry SK, et al. Maternal night blindness increases risk of mortality in the first 6 months of life among infants in Nepal. J Nutr 2001;131:1510–2.