

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^{1–3}

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ABSTRACT

Background: It is not known whether daily consumption of vitamin A–containing foods is efficacious for treating nightblindness.

Objective: We assessed the effect of supplementation with vitamin A from food or synthetic sources on dark adaptation and plasma retinol concentrations in nightblind pregnant Nepali women.

Design: Nightblind pregnant women were randomly assigned to 1 of 6 treatment groups to receive 6 d/wk for 6 wk either 850 μg retinol equivalents/d as retinyl palmitate, vitamin A–fortified rice, goat liver, amaranth leaves, or carrots or 2000 μg retinol equivalents/d as retinyl palmitate. Dark adaptation was assessed weekly by using the pupillary threshold (PT) test; plasma retinol concentrations were measured before and after the intervention. These outcomes were also assessed in a comparison group of nonnightblind pregnant women.

Results: In the nightblind women, the mean PT improved significantly ($P < 0.0001$) from -0.71 ± 0.04 to -1.42 ± 0.02 log cd/m², and the final mean PT did not differ significantly from that in the nonnightblind women (-1.43 ± 0.04 ; $P = 0.55$). Improvement in dark adaptation was greater in the liver group than in the vitamin A–fortified rice group ($P < 0.02$). Plasma retinol concentrations increased significantly ($P < 0.0001$) from 0.95 ± 0.05 to 1.07 ± 0.05 $\mu\text{mol/L}$. The plasma retinol response was greater in the higher-dose capsule and liver groups than in the vegetable groups and significantly greater in the liver group than in the vitamin A–fortified rice group (both: $P < 0.05$).

Conclusion: Improvement in dark adaptation did not differ significantly between women who received vitamin A as liver, amaranth leaves, carrots, or retinyl palmitate. *Am J Clin Nutr* 2005;81:461–71.

KEY WORDS Nightblindness, pregnancy, vitamin A status, supplementation, pupillary threshold, dark adaptation, Nepal, vitamin A, β -carotene, food-based interventions

INTRODUCTION

Rhodopsin is a photosensitive pigment that is required for normal adaptation to dim light. When insufficient vitamin A is available, production of rhodopsin is reduced, and this results in delayed adaptation to dim light and low visual acuity at night (1). The resulting nightblindness is a common clinical symptom of

vitamin A deficiency in children and pregnant women in less industrialized countries (2–6).

In Nepal, the prevalence of nightblindness during pregnancy ranges from ≈ 10 –40% in the lowland plains region of Terai to ≈ 50 % in the mountainous region of Jumla (7–9), and the average time of onset is approximately at 7 mo of pregnancy (5). Nepali women view nightblindness as an important illness of pregnancy because it adversely affects their child care and food preparation activities, but, because the symptoms usually disappear spontaneously after they give birth, these women also perceive nightblindness to be a transient condition, and they rarely seek treatment (5). However, the potential consequences of maternal nightblindness can be severe. Nightblind pregnant women are 5 times more likely to die of pregnancy-related complications than are nonnightblind pregnant women (10). Among rural Nepali women, weekly supplementation with 7 mg retinol equivalents (REs) during pregnancy and lactation reduced the incidence of nightblindness by ≈ 67 % (11) and reduced maternal mortality by ≈ 44 % (6). Because supplementation with vitamin A did not eliminate nightblindness in this population, it is possible that a larger dose of vitamin A— >7 mg RE/wk—is required or that deficiencies of other nutrients also may be involved in the etiology of the condition.

Because vitamin A is potentially teratogenic, the upper limit for daily intake during pregnancy is 3 mg (12). For treatment of nightblindness during pregnancy, the World Health Organization recommends supplementation with 1.5 mg vitamin A/d for 4 wk (13); however, the efficacy of that treatment has not been evaluated. Weekly supplementation with 7 mg RE has been shown to have a positive effect on maternal health, but it is logistically difficult to distribute capsules weekly to women who are at risk of deficiency because of the inadequate health care infrastructure in many countries where vitamin A deficiency occurs. Moreover, there is concern that, in those areas in which

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TABLE 1

Composition of food and capsule supplements by treatment group

	Vitamin A supplement ¹					
	High-dose	Low-dose	Fortified rice	Liver	Greens	Carrots
Plain rice (g)	200	200	—	200	200	200
Fortified rice (g)	—	—	200	—	—	—
White vegetables (g)	150	150	150	150	—	—
Green leafy vegetables (g)	—	—	—	—	150	—
Carrots (g)	—	—	—	—	—	128
Goat liver (g)	—	—	—	8	—	—
Vitamin A in corn oil, mg (in ≈200- μ L capsule)	2	0.85	0	0	0	0

¹ High-dose, 2.0 mg; low-dose, 0.85 mg.

high-dose capsule distribution programs are in place for children, pregnant women may inadvertently receive high-dose vitamin A capsules. Appropriate vitamin A-rich foods (eg, eggs, small servings of liver, green leafy vegetables, yellow or orange vegetables, and fruit) provide safe amounts of vitamin A, but it is not known whether consumption of these foods is efficacious for treating nightblindness. The purpose of this study was to assess whether daily consumption of locally available food sources of vitamin A or of vitamin A-fortified rice would be as effective as that of vitamin A supplements in improving dark adaptation, reversing symptoms of nightblindness, and improving plasma retinol concentrations in nightblind pregnant Nepali women.

SUBJECTS AND METHODS

Subjects and study site

The study was conducted in seven 6-wk cycles between July 2000 and May 2002 in a total of 60 clusters of ≈9 small villages each, with each cluster forming the Nepali structure called a Village Development Committee, in the Saptari district in the eastern Terai region of Nepal. Pregnant women aged 18–45 y were identified as nightblind by self-report during a door-to-door census conducted before each study cycle. Self-reported cases of nightblindness were confirmed by interviewing family members or neighbors (or both) to ascertain whether a woman required assistance with child-care, food preparation, or walking at dusk, any of which is a symptom of the condition. Women were eligible to participate in the study if they were <8 mo pregnant and free of fever, symptoms of underlying chronic disease, and any clinical signs of xerophthalmia.

A negative control group was not included in the study design because of the increased risk of death associated with nightblindness in pregnant Nepali women. However, a comparison group of nonnightblind pregnant women was enrolled concurrently with the nightblind women from May 2001 to March 2002. In this area of Nepal, VDCs consist of ≈9 small villages, called wards. The study area was divided into 6 contiguous geographic areas to manage the logistics of delivering the food supplements to the participants. Nightblind and nonnightblind women were enrolled from each of these 6 areas. It was not possible to select individual comparison women randomly from the study area as a whole, because the local leaders felt that it was not culturally appropriate. That is, it would be difficult to explain why some women were selected and others were not. Thus, the comparison women were enrolled from randomly selected wards among

those from which nightblind subjects were identified in each of the 6 geographic areas. Within a selected ward, all of the non-nightblind pregnant women in their 2nd or 3rd trimester of pregnancy who did not have fever or symptoms of chronic disease were allowed to participate in the study.

Oral informed consent was obtained from each of the participants, and a consent form for each woman was signed by the physician or nurse upon receiving the subject's oral consent. The study protocol was approved by the Nepal Health Research Council and by the Office for Human Research Protection at the University of California, Davis.

Study procedures

Nightblind pregnant women were randomly assigned to 1 of 6 treatment groups to receive a midday meal and capsule supplements 6 d/wk for a period of 6 wk. As shown in **Table 1**, treatments in the respective study groups consisted of 1) 200 g cooked rice, ≈150 g vegetables with low vitamin A content (curried cauliflower or white squash or white potatoes), and a high-dose capsule containing 2.0 mg vitamin A as retinyl palmitate in ≈200 μ L corn oil; 2) 200 g cooked rice, ≈150 g curried vegetables with low vitamin A content, and a low-dose capsule containing 850 μ g vitamin A as retinyl palmitate in ≈200 μ L corn oil; 3) 200 g cooked vitamin A-fortified rice containing 850 μ g vitamin A, ≈150 g curried vegetables with low vitamin A content, and a capsule containing ≈200 μ L corn oil; 4) 200 g cooked rice, ≈150 g curried vegetables with low vitamin A content, 8 g goat liver containing 850 μ g vitamin A, and a capsule containing ≈200 μ L corn oil; 5) 200 g cooked rice, ≈150 g amaranth greens containing 850 μ g RE [425 μ g retinol activity equivalents (RAE)] as β -carotene, and a capsule containing 200 μ L corn oil; and 6) 200 g cooked rice, ≈128 g carrots containing 850 μ g RE (425 μ g RAE) as β -carotene, and a capsule containing 200 μ L corn oil. For the purpose of preparing the study diets, 1 RE was defined as 1 μ g retinol or 6 μ g β -carotene. This was the recommended bioconversion factor at the time the study was conducted (14). The most recent recommended bioconversion factor is the RAE, which is defined as 1 μ g retinol or 12 μ g β -carotene (12). The RAE value of the food supplements is also presented.

Clinical assessments in nightblind women

The nightblind pregnant women were transported to the field clinic at baseline and then weekly during the 6-wk study cycle. During the initial visit, they were weighed and measured and interviewed for information on their pregnancy status, medical

history, and socioeconomic status. During the first and last visits, a venous blood sample (7.5 mL) was obtained for measurement of the hemoglobin concentration and plasma concentrations of retinol, carotenoids, α -tocopherol, ferritin, zinc, and C-reactive protein (CRP). During each of the weekly visits, women were asked whether there was any change in their symptoms of night-blindness, and dark adaptation was measured quantitatively by using the pupillary threshold (PT) test. Women were also interviewed to obtain information on the frequency of consumption of vitamin A-containing foods and any morbidity during the previous week.

Clinical assessments in nonnightblind women

The nonnightblind comparison women were transported to the field clinic on just one occasion. During that visit, they were weighed, measured, and interviewed for information on their visual status, stage of pregnancy, medical history, socioeconomic status, frequency of consumption of vitamin A-containing foods, and morbidity during the previous week. A 7.5-mL venous blood sample was drawn for the same biochemical assessments as are listed above, and the PT test was conducted to assess dark adaptation.

Food and capsule supplements

The supplemental meals were prepared daily at the field facility by using standardized recipes. The amaranth leaves, white vegetables (ie, cauliflower, white squash, and white potatoes), goat liver, and nonfortified rice were obtained from the same local suppliers throughout the study period. Carrots were obtained weekly from a supplier in Kathmandu because there was no local source in Saptari. The carrots were protected from light and stored in a refrigerator (at 4 °C) until prepared. Ultra-Rice mix was obtained from the Program in Appropriate Technology for Health (PATH, Seattle). Ultra-Rice is a vitamin A-fortified, manufactured rice product containing α -tocopherol as an antioxidant that was developed to address vitamin A deficiency in populations for whom rice is a staple food. The Ultra-Rice mix is blended with local rice to achieve the desired vitamin A concentration per serving of cooked rice for the target population. Uncooked vitamin A-fortified rice was stored in sealed black bags at -20 °C. Ultra-Rice was mixed with nonfortified local rice [1:60 (wt:wt)] before cooking. Vitamin A and placebo capsules were identical in appearance. Dark-blue capsules were used to protect against exposure to light, and the capsules were stored in sealed plastic containers at -20 °C.

Food preparation methods

The vitamin A-containing vegetables were prepared to optimize the bioavailability of β -carotene. The amaranth leaves were steamed for \approx 10 min, puréed in a food processor, and sautéed in oil with garlic and spices for 5 min. The carrots were cooked in a pressure cooker for 5 min, puréed in a food processor, and sautéed in oil with spices for 5 min. The goat liver was cut into small pieces (\approx 2.5-cm cubes) and sautéed in oil with garlic and spices for 10 min. The white vegetables were cooked in a pressure cooker for 5 min and sautéed in oil with spices for 5 min. Separate batches of fortified rice (mixture of vitamin A-fortified rice and nonfortified rice) and nonfortified local rice were prepared daily by using separate, automatic, electric rice cookers. The vitamin A content of cooked vitamin A-fortified rice and

goat liver and the carotenoid content of the cooked vegetables were determined by using HPLC to ascertain the portion sizes required to provide 850 μ g RE (425 μ g RAE) of vitamin A (see below).

Immediately after cooking, standardized portions of the foods were placed into individual thermoses for each study participant. The thermoses were packed into insulated bags to keep the foods hot. The vitamin A and placebo capsules were placed in small vials and packed in separate insulated bags with frozen ice packs to keep the capsules cool. Fieldworkers delivered the foods and capsules daily to participants at their homes and encouraged the participants to consume all of the food provided.

Vitamin A and carotenoid concentrations in test foods

According to the HPLC analyses, the vitamin A concentration of cooked, fortified rice was $4.3 \pm 0.2 \mu$ g/g. When measured in May 2000, before the study, the vitamin A concentration of cooked goat liver was $107 \pm 19.3 \mu$ g/g, and that value was used to estimate the portion size of liver. However, when measured again in July 2002, after the study, the vitamin A concentration of goat liver was found to be $340 \pm 123 \mu$ g, which was, on average, \approx 3 times the initial estimate. This variation in the concentration of vitamin in goat liver is probably related to the heterogeneous distribution of vitamin A within liver and to differences among animals because of exposure to different environmental conditions that may affect their diet. Thus, the amount of vitamin A provided by 8 g goat liver probably varied during the study period by an average of \approx 0.850–2.7 mg/d. The *all-trans* β -carotene concentration of cooked amaranth leaves was $34.2 \pm 1.5 \mu$ g/g, the concentration of *cis*-isomers of β -carotene was $13.3 \pm 0.44 \mu$ g/g (\approx 28% of total β -carotene), and the α -carotene concentration was $1.4 \pm 0.1 \mu$ g/g. The lutein content of amaranth leaves was $70.4 \pm 2.7 \mu$ g/g. The *all-trans* β -carotene concentration of cooked carrots was $40.0 \pm 2.8 \mu$ g/g, the concentration of *cis*-isomers of β -carotene was $13.1 \pm 0.8 \mu$ g/g (\approx 32% of total β -carotene), and the α -carotene concentration was $20.2 \pm 1.0 \mu$ g/g. Lutein was not detected in carrots, and β -cryptoxanthin was not detected in amaranth leaves or carrots. Portion sizes of cooked amaranth leaves (150 g) and cooked carrots (128 g), each of which contained \approx 5.1 mg β -carotene or \approx 850 μ g RE (\approx 425 μ g RAE), were based on the *all-trans* β -carotene concentration of each vegetable and on a vitamin A equivalency factor of 6:1 (6 μ g β -carotene = 1 μ g retinol). The vitamin A activity of α -carotene and that of *cis*-isomers of β -carotene were not included in the determination of portion sizes because of uncertainty about the bioavailability of α -carotene and *cis*- β -carotene relative to that of *all-trans* β -carotene. If the vitamin A activity of α -carotene and *cis*-isomers of β -carotene is estimated to be half that of β -carotene, the total vitamin A activity in a serving of cooked amaranth leaves would be \approx 1039 μ g RE (\approx 519 μ g RAE), and that in a serving of cooked carrots would be \approx 1208 μ g RE (\approx 605 μ g RAE).

Pupillary threshold

The PT was measured by using a portable hand-held device as described previously (15). Briefly, the PT test determines the intensity of light that is required to produce a pupillary contraction in a dark-adapted subject. Before the testing, each subject was exposed to a battery-powered camera flash to partially bleach the rod cells in both eyes so that all subjects started the test

at the same level of light exposure. Immediately after this camera flash, the subject was placed in a dark room for 10 min. The room had no windows, and the ceiling, walls, and door were painted black. Heavy black cloth was hung over the inside and outside of the doorway to ensure that no light could enter the room through small cracks between the door and the door frame. The hand-held device that is used to determine the PT has a yellow-green, light-emitting diode (LED) light source with 12 intensity settings at ≈ 0.4 log unit intervals, which is placed over one eye, and a red LED light source that is mounted obliquely on the device to illuminate the contralateral eye. After 10 min of dark adaptation, the subject was asked to look at a target (a fluorescent sticker in the shape of a star) at a distance of ≈ 2 m. As the subject focused on the target, the hand-held illuminator was placed over the subject's right eye, and the red LED light source illuminated the left eye. The pupil of the left eye was observed by using a 2.5 \times magnifying loupe while the right eye was exposed to light at levels of intensity increasing from the lowest level. The level of light intensity (12 different intensity settings) at which the pupil of the left eye contracted was recorded as the PT. The light intensity setting, in the range of 1 to 12, was later converted to the corresponding level of light intensity in units of cd/m^2 on the basis of the initial calibration of the device. The study nurses were trained to conduct the PT tests and were considered to be standardized observers when the PT measurements they obtained were within 1 unit of the trainer's measurements. The nurses conducting the test were unaware of the women's self-reported nightblindness status, the women's group assignments, and the women's PT scores from previous weeks.

Anthropometric measurements

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), and midupper arm circumference was measured to the nearest 0.1 cm by using a plastic measuring tape.

Collection of blood samples

Blood samples were collected by venipuncture into 7.5-mL trace metal-free tubes containing lithium heparin as an anticoagulant (Monovettes; Sarstedt, Newton, NC). The samples were centrifuged for 10 min at 3400 rpm by using a clinical tabletop centrifuge (Model 228; Fisher Scientific, Springfield, NJ) to separate plasma. Plasma was aliquotted into four 1.5-mL polypropylene screw-top vials, which were placed in opaque, covered, sample storage boxes and stored at -20° in a freezer at the field facility. All procedures were conducted in dim light. Approximately every 3–4 mo, project staff members hand-carried batches of samples, packed in coolers on frozen ice packs, to Kathmandu by air. In Kathmandu, the samples were stored in a freezer at -20°C until they were hand-carried by project staff members to the University of California, Davis, on dry ice. During the second year of the study, dry ice was no longer available in Nepal; at that time, samples were hand-carried on frozen ice-packs to Bangkok by air and packed on dry ice for transport from Bangkok to Davis.

Laboratory analyses

The plasma concentrations of retinol, lutein, α -carotene, β -carotene, and α -tocopherol were measured by using a Class

VP HPLC (Shimadzu, Columbia, MD) equipped with a photodiode array detector and auto sampler (16). Presupplementation and postsupplementation plasma concentrations were analyzed together for each subject. For quality control, a plasma pool was prepared and calibrated by using control serum (fat-soluble vitamins; National Institute of Standards, Gaithersburg, MD). Three aliquots of the plasma pool were analyzed with each set of study samples. The within-day CVs of analytes in the plasma pool samples were $\leq 5.4\%$ for retinol, $\leq 5.5\%$ for lutein, $\leq 12.9\%$ for α -carotene, $\leq 11.6\%$ for β -carotene, and $\leq 8.7\%$ for α -tocopherol. To assess accuracy, control serum from the National Institute of Standards was analyzed. The measured concentrations of retinol, lutein, α -carotene, β -carotene, and α -tocopherol were within 2.4%, 11%, 11%, 4.3% and 2.6% of the certified values, respectively. Plasma concentrations of CRP were measured by using a commercial radial immunodiffusion kit (Nanorid: The Binding Site, Birmingham, United Kingdom). Plasma ferritin concentrations were measured by using a commercial immunoradiometric assay (Coat-A-Count IRMA; Diagnostic Products Corp, Los Angeles). Plasma zinc concentrations were determined by using inductively coupled plasma mass spectrometry (ICP-MS; Thermo Jarrel Ash, Franklin, MA) (17). The retinol content of cooked foods (vitamin A-fortified Ultra-Rice and goat liver), and the carotenoid content of the cooked, puréed vegetables (amaranth leaves and carrots) were measured by using HPLC (16). The within-day CV for the retinol content of goat liver was $\leq 15\%$. The within-day CVs for the β -carotene content of amaranth leaves and carrots were $< 5\%$ and $< 7\%$, respectively.

Statistical analysis

Descriptive statistics were calculated for all variables, and variables were transformed to conform to the normal distribution if necessary. Mean initial values of PTs and biochemical tests were compared between the nightblind women and the nonnightblind women by using analysis of covariance (ANCOVA) with month of pregnancy and study cycle as the covariates. Month of pregnancy was used as a covariate because PTs and biochemical values may vary by stage of pregnancy. Study cycle was used as a covariate because of the observed "drift" in PT measurements over time. Among the nightblind women, mean changes in PT were compared by treatment group by using ANCOVA with initial values, study cycle, and month of pregnancy as covariates. Mean changes in biochemical values were compared by treatment group by using ANCOVA with initial values, study cycle, and month of gestation as covariates. CRP concentration, an indicator of subclinical infection or inflammation, was included as a covariate for the comparisons of final mean plasma retinol, zinc, and ferritin concentrations by treatment group because plasma retinol and zinc concentrations tend to decline, and plasma ferritin concentrations tend to increase when plasma CRP concentrations are elevated (18). Weekly improvement in symptoms of nightblindness (according to maternal reports) was compared by treatment group by using repeated-measures analysis of variance. Mean PTs were compared by week of observation and treatment group by using two-factor repeated-measures analysis of variance. The proportion of women with initial and final abnormal PTs was compared by using chi-square analysis and McNemar's test. The proportion of women with final abnormal PTs or biochemical values was compared by treatment group by

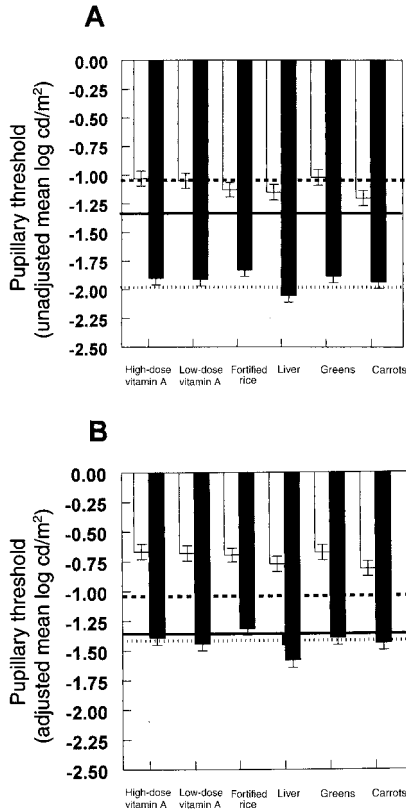


FIGURE 1. A) Initial (□) and final (■) unadjusted mean pupillary thresholds by treatment group ($n = 348$). B) Initial (□) and final (■) adjusted mean pupillary thresholds by treatment group ($n = 348$). ■■■, Proposed cutoff for abnormal dark adaptation ($> -1.11 \log \text{cd/m}^2$); —, mean pupillary threshold for nonpregnant US women ($-1.35 \log \text{cd/m}^2$); |||, mean unadjusted and adjusted pupillary thresholds in nonnightblind comparison women (A: $-2.00 \log \text{cd/m}^2$; B: $-1.42 \log \text{cd/m}^2$). Final values differed significantly between the liver group and the fortified rice group, $P < 0.02$.

using logistic regression after control for study cycle. The Tukey-Kramer test was used for post hoc group comparisons. SAS for WINDOWS software (release 8; SAS Inc, Cary, NC) was used for the statistical analyses.

The PT data were converted to light intensity in units of $\log \text{cd/m}^2$ on the basis of the initial calibration of the instrument; however, the values were lower than expected and did not make sense in relation either to the provisional cutoff value for abnormal dark adaptation of $> -1.11 \log \text{cd/m}^2$ that is associated with vitamin A deficiency (19) or to the reported value of $-1.35 \log \text{cd/m}^2$ for healthy US women (20). The values declined in response to treatment, which indicated improvement in dark adaptation, but the absolute values were much lower (ie, better) than were previously reported values for persons with normal night vision (Figure 1). As shown in Figure 2, there was a significant ($P < 0.0001$) linear decline in initial PT values in both the nightblind and the nonnightblind women during the 18-mo study period, but there was no evidence of a seasonal effect on PT values. We concluded that the calibration of the instrument had drifted over time, and we learned that this had also occurred in previous applications of the technique (N Congdon, personal communication, June 2002). Therefore, the PT values were adjusted by using the linear regression equation that was fitted to the observed relation between initial PT and the month of study.

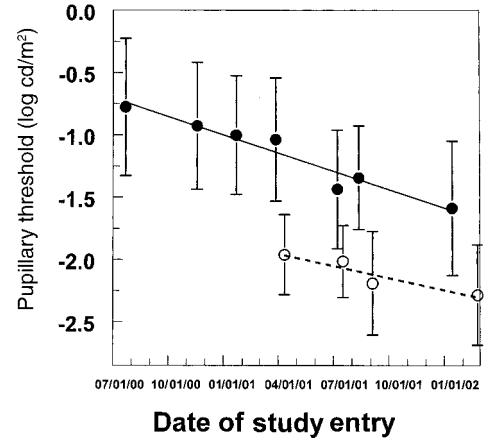


FIGURE 2. Unadjusted initial mean pupillary thresholds by month of study [$n = 380$ nightblind women (●), 128 nonnightblind women (○)]; the slopes for nightblind and nonnightblind women are significantly ($P < 0.0001$) different from zero but do not differ significantly from one another ($P = 0.13$; test for interaction between date of entry into the study and cohort).

After adjustment, the absolute mean values for PT increased, but the pattern of mean PTs across treatment groups was similar to that for the unadjusted mean values (Figure 1). Subjects with adjusted PT values $> -1.11 \log \text{cd/m}^2$ were classified as having impaired dark adaptation (19).

RESULTS

Subjects

Of the 8764 pregnant women who were identified during the study period, 704 were nightblind; of that group, 450 were eligible for the study, and 397 chose to participate (Figure 3). Forty-nine participants dropped out for various reasons, as indicated in the figure, which left a total of 348 nightblind pregnant women who completed the study protocol. The number of women who dropped out did not differ significantly by treatment group ($P = 0.78$), and the women who dropped out did not differ significantly from the study participants in age ($P = 0.65$), weight ($P = 0.47$), height ($P = 0.49$), socioeconomic status ($P = 0.18$), month of pregnancy ($P = 0.29$), initial PT ($P = 0.84$), morbidity ($P = 0.09$), hemoglobin concentration ($P = 0.57$), or

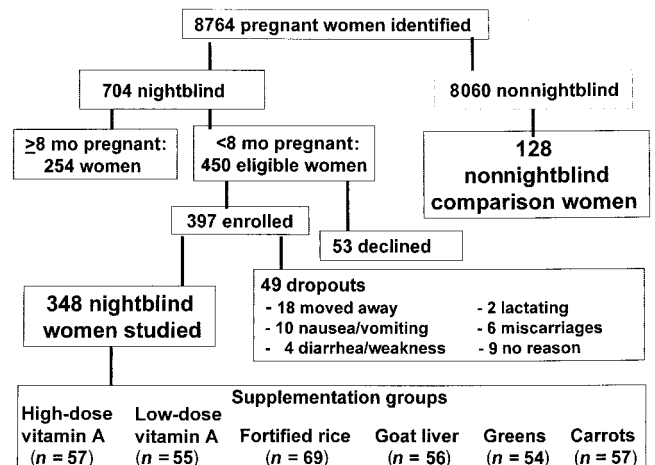


FIGURE 3. Profile of study participants.

TABLE 2
Initial characteristics of nightblind and nonnightblind comparison women¹

	Nightblind women (n = 380)	Nonnightblind women (n = 128)
Age (y)	23.8 ± 4.9	23.5 ± 5.0
Weight (kg)	43.1 ± 4.8	45.1 ± 5.4 ²
Height (cm)	149.5 ± 5.3	150.2 ± 5.4
BMI (kg/m ²)	19.3 ± 1.8	20.0 ± 2.0 ³
MUAC (mm)	21.8 ± 1.6	22.6 ± 1.6 ³
Month of pregnancy	5.6 ± 1.3	5.6 ± 1.3

¹ All values are $\bar{x} \pm$ SD. MUAC, midupper arm circumference.

^{2,3} Significantly different from nightblind women (analysis of covariance with month of pregnancy as a covariate): ² $P < 0.0001$, ³ $P < 0.001$.

plasma ferritin concentration ($P = 0.91$) (data not shown). However, the women who dropped out had significantly lower plasma concentrations of retinol ($P = 0.02$), lutein ($P < 0.01$), α -carotene ($P < 0.001$), β -carotene ($P < 0.007$), and α -tocopherol ($P < 0.0001$) than did the study participants (data not shown). One possible explanation for this is that the plasma samples from the women who dropped out were kept in a separate container in the freezer (-20°C) at the field site and were not carried to the United States until after the study was completed, so the samples were analyzed as long as ~ 2 y after they were collected. It is known that plasma concentrations of α -tocopherol, carotenoids, and retinol decline significantly when samples are stored at -20°C for > 1 y (21). In contrast, the plasma samples for the study participants were shipped to the United States for analysis approximately every 3 mo throughout the study period. Thus, it is possible that the plasma concentrations of carotenoids, α -tocopherol, and retinol were lower in the samples from the women who dropped out because of the longer storage time of those samples before analysis. There was no difference in the number of dropouts by treatment group, and thus the difference in plasma concentrations of carotenoids, α -tocopherol, and retinol did not bias the results. A total of 128 comparison women were enrolled.

The initial characteristics of the nightblind and nonnightblind comparison women are shown in **Table 2**. Mean body weight, BMI, and midupper arm circumference were significantly ($P < 0.001$) lower in the nightblind women than in the nonnightblind women. There were no significant differences between the 2 groups in mean age, height, or month of pregnancy. At their initial visit, the nightblind women reported consuming both milk and eggs significantly less frequently than did the nonnightblind women (milk: 1.8 ± 0.14 and 2.5 ± 0.25 times/wk, respectively; $P < 0.02$; eggs: 0.18 ± 0.04 and 0.38 ± 0.07 times/wk, respectively; $P = 0.03$). During the summer months (April–September), the nightblind women reported consuming dark green, leafy vegetables significantly less frequently (2.0 ± 0.15 and 3.1 ± 0.22 times/wk, respectively; $P = 0.001$) than did the nonnightblind women. The nightblind women were significantly more likely to report poor appetite ($P = 0.0002$), burning during urination ($P = 0.029$) and abdominal pain ($P = 0.002$) than were the nonnightblind women.

As shown in **Table 3**, the mean initial PT of the nightblind pregnant women was significantly ($P < 0.0001$) higher than that of the nonnightblind comparison women, which indicated significantly poorer dark adaptation in those who reported nightblindness. The initial mean plasma retinol concentration in the

TABLE 3
Initial mean pupillary threshold and biochemical values in nightblind and nonnightblind comparison women¹

	Nightblind women (n = 366)	Nonnightblind women (n = 128)
Pupillary threshold (log cd/m ²)	-0.71 ± 0.04	-1.42 ± 0.04 ²
Plasma		
Retinol ($\mu\text{mol/L}$)	0.95 ± 0.02	1.03 ± 0.03 ³
Lutein ($\mu\text{mol/L}$)	0.194 ± 0.005	0.180 ± 0.008
α -Carotene ($\mu\text{mol/L}$)	0.010 ± 0.000	0.014 ± 0.001 ⁴
β -Carotene ($\mu\text{mol/L}$)	0.094 ± 0.004	0.087 ± 0.007
α -Tocopherol ($\mu\text{mol/L}$)	14.3 ± 0.27	15.0 ± 0.48
Ferritin ($\mu\text{g/L}$)	16.1 ± 0.7	14.3 ± 1.1
Zinc ($\mu\text{mol/L}$)	7.73 ± 0.11	7.95 ± 0.18
Hemoglobin (g/L)	106 ± 1.0	109 ± 1.0 ³

¹ All values are geometric $\bar{x} \pm$ SE.

^{2–4} Significantly different from nightblind women (analysis of covariance with month of pregnancy and study cycle as covariates): ² $P < 0.0001$, ³ $P < 0.05$, ⁴ $P = 0.001$.

nightblind pregnant women was slightly but significantly lower than that in the nonnightblind comparison women ($P < 0.05$) after control for CRP concentrations. There were no significant differences in mean plasma concentrations of lutein or β -carotene between the 2 groups, but the initial mean plasma concentration of α -carotene was significantly ($P < 0.001$) lower in the nightblind women than in the nonnightblind women. There were no significant differences in mean plasma α -tocopherol, ferritin, or zinc concentrations between the 2 groups. The initial mean hemoglobin concentration was slightly but significantly ($P = 0.03$) lower in the nightblind women than in the nonnightblind women (106 ± 1 and 109 ± 1 g/L, respectively).

As expected, a significantly greater proportion of women who reported nightblindness (80%) than of those who did not (15%; $P < 0.0001$) had abnormal (> -1.11 log cd/m²) PTs (**Table 4**). There were no significant differences between the proportions of the nightblind and nonnightblind women with low plasma concentrations of retinol (< 0.70 $\mu\text{mol/L}$), ferritin (< 12 $\mu\text{g/L}$), or zinc (< 7.66 $\mu\text{mol/L}$). The proportion of women with low hemoglobin concentrations tended to be higher among the nightblind women, but the difference was not significant (nightblind: 57.3%; nonnightblind: 44.5%; $P = 0.09$) (Table 4).

TABLE 4
Nightblind and nonnightblind women with abnormal initial pupillary threshold and biochemical values

	Nightblind women (n = 366)	Nonnightblind women (n = 128)
	%	%
Pupillary threshold > -1.11 log cd/m ²	80.0	14.8 ¹
Retinol < 0.07 $\mu\text{mol/L}$	25.1	19.5
Ferritin < 12 $\mu\text{g/L}$	41.6	43.0
Zinc < 7.66 $\mu\text{mol/L}$	49.1	63.7
Hemoglobin < 110 g/L	57.3	44.5

¹ Significantly different from nightblind women, $P < 0.0001$ (logistic regression with month of pregnancy and study cycle as covariates).

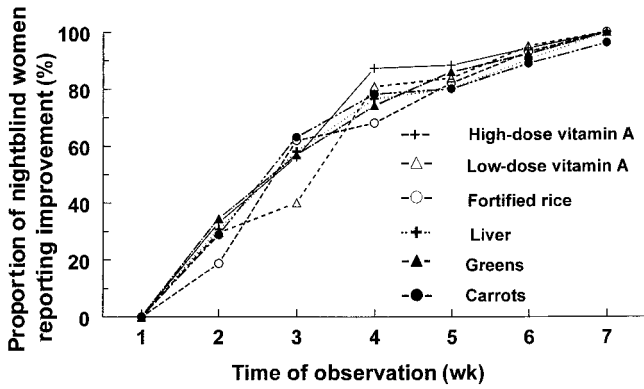


FIGURE 4. Percentage of women reporting improvement in symptoms of nightblindness by treatment group and week of observation ($n = 348$). There were no significant differences in the percentage of women reporting improvement by treatment group at any time points. $P = 0.08$ for interaction and $P < 0.0001$ for time (repeated-measures ANOVA).

Change in symptoms of nightblindness and pupillary thresholds in treated nightblind women.

The nightblind women were asked weekly if their symptoms of nightblindness had changed. Overall, the percentage of women who reported amelioration of their symptoms of nightblindness increased by weekly observation ($P < 0.0001$), but there was no significant interaction between treatment and time ($P = 0.08$). As shown in **Figure 4**, the percentage of women who reported a diminution in their symptoms did not differ significantly by treatment group at any of the time points ($P = 0.07$). At the end of the study, only 2 women (0.6%) reported that they still had symptoms of nightblindness; both were in the carrot group.

Among women who initially reported nightblindness, the initial and final mean PTs were -0.71 ± 0.04 and $-1.42 \pm 0.02 \log \text{cd/m}^2$ ($P < 0.0001$), respectively, which indicates improvement in dark adaptation after 6 wk of supplementation. The final mean PT in the nightblind women did not differ significantly from that in the nonnightblind comparison women (-1.42 ± 0.02 and $-1.43 \pm 0.04 \log \text{cd/m}^2$, respectively; $P = 0.55$). As shown in **Figure 5**, mean PTs decreased significantly ($P < 0.0001$) in all treatment groups during the 6-wk intervention, but there was no significant interaction between treatment and time ($P = 0.42$). The mean PT values dropped below the cutoff value for impaired dark adaptation ($> -1.11 \log \text{cd/m}^2$) in all treatment groups by ≈ 5 wk. The final mean PT in the liver group was significantly ($P < 0.02$) lower (ie, better) than that in the vitamin A-fortified rice group; there were no other significant differences by treatment group.

Overall, the percentage of women with abnormal ($> -1.11 \log \text{cd/m}^2$) PTs declined significantly ($P < 0.0001$) in response to the intervention, from 76.4% before the intervention to 23.6% afterward. As shown in **Figure 6**, the percentage of women with abnormal final PTs was significantly ($P < 0.05$) higher in the vitamin A-fortified rice group (39.4%) than in the goat liver group (13.5%); there were no other differences by treatment group.

Change in plasma retinol concentrations in treated nightblind women

Among the women who initially reported nightblindness, the initial and final mean plasma retinol concentrations were $0.96 \pm$

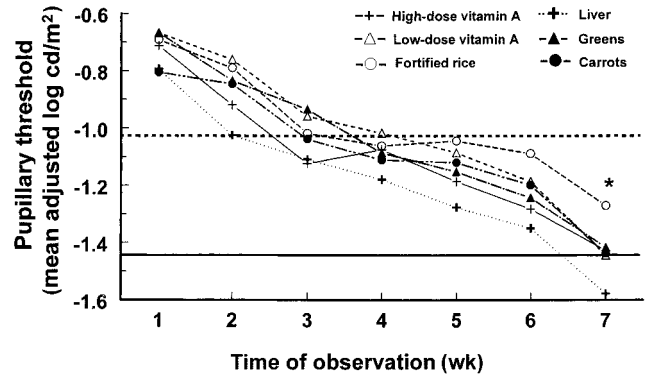


FIGURE 5. Mean adjusted pupillary thresholds by treatment group and week of observation ($n = 348$). ■■■, Proposed cutoff for abnormal dark adaptation ($> -1.11 \log \text{cd/m}^2$); —, mean pupillary threshold for nonnightblind comparison women ($-1.42 \log \text{cd/m}^2$). The overall SD = $0.38 \log \text{cd/m}^2$. *The final mean pupillary threshold in the liver group was significantly ($P < 0.02$) lower than that in the vitamin A-fortified rice group at week 7. $P = 0.42$ for interaction, $P < 0.0001$ for time (two-factor repeated-measures ANOVA; Tukey-Kramer test for group comparisons).

$0.05 \mu\text{mol/L}$ and $1.07 \pm 0.05 \mu\text{mol/L}$, respectively ($P < 0.0001$). As shown in **Figure 7**, the final mean plasma retinol concentrations in the high-dose (ie, 2 mg/d) vitamin A capsule group was significantly ($P < 0.05$) higher than those in the low-dose vitamin A capsule, amaranth greens, and carrot groups, but not significantly different from the final mean values in the goat liver ($P = 0.95$) or vitamin A-fortified rice groups ($P = 0.15$). The final mean plasma retinol concentration in the goat liver group was significantly ($P < 0.05$) higher than that in the groups that received the same prescribed amount of vitamin A ($850 \mu\text{g RE/d}$; $425 \mu\text{g RAE/d}$) but not significantly different from the final mean concentration in the high-dose capsule group. There were no significant differences in final retinol concentrations between the low-dose vitamin A capsule, vitamin A-fortified rice, amaranth greens, and carrot groups.

The percentage of women with plasma retinol concentrations $< 0.70 \mu\text{mol/L}$ declined significantly ($P < 0.05$), to $< 21.4\%$, in response to the intervention (data not shown). There were no significant differences between treatment groups in the percentage of women with low plasma retinol concentrations ($P = 0.08$).

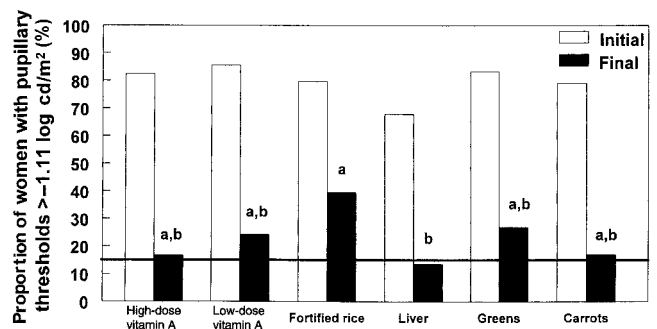


FIGURE 6. Initial and final percentages of women with abnormal pupillary thresholds by treatment group ($n = 348$). —, Percentage of nonnightblind comparison women with pupillary thresholds $> -1.11 \log \text{cd/m}^2$ (15%). Final percentages with different superscript letters are significantly different, $P < 0.05$ (logistic regression after control for study cycle; Tukey-Kramer test for group comparisons).

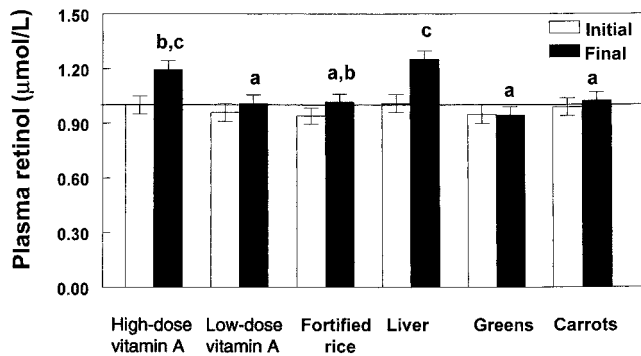


FIGURE 7. Initial and final mean plasma retinol concentrations by treatment group ($n = 345$). —, Mean plasma retinol concentration of nonnightblind comparison women, $1.03 \mu\text{mol/L}$. Final means with different superscript letters are significantly different, $P < 0.05$ (analysis of covariance with study cycle, initial values, and plasma C-reactive protein concentrations as the covariates; Tukey-Kramer test for group comparisons).

Change in plasma carotenoid, ferritin, zinc, and hemoglobin concentrations in treated nightblind women

The final mean plasma concentrations of carotenoids and α -tocopherol differed significantly by treatment group in response to supplementation (carotenoids, $P < 0.0001$; α -tocopherol, $P < 0.05$; combined group data not shown). As shown in **Table 5**, final mean plasma lutein concentrations were significantly ($P < 0.0001$) higher in the group that received amaranth leaves than in the other groups; this result was expected because green leaves are rich sources of lutein. The final mean plasma α -carotene concentrations were significantly ($P < 0.0001$) higher in the carrot group than in the other groups and significantly ($P < 0.0001$) higher in the group that received amaranth leaves than in the groups that received a source of preformed retinol. The final mean plasma β -carotene concentrations were significantly ($P = 0.011$) higher in the carrot group than in the other groups and significantly ($P < 0.0001$) higher in the amaranth leaves group than in the groups that received preformed retinol. The final mean α -tocopherol concentrations were significantly ($P < 0.03$) higher in the vitamin A–fortified rice

group than in the vegetable groups. The vitamin A–fortified rice contains α -tocopherol, an antioxidant. Overall, the final mean plasma ferritin concentrations differed significantly ($P < 0.02$) by treatment group, and, as shown in **Table 6**, final mean plasma ferritin concentrations were significantly ($P \leq 0.031$) higher in the high-dose capsule and the vitamin A–fortified rice groups than in the carrot group. There were no significant differences by treatment group in final mean plasma zinc concentrations ($P = 0.27$) or hemoglobin concentrations ($P = 0.15$).

Overall, there was no significant difference in the percentage of women with initial and final low plasma concentrations of ferritin ($< 12 \mu\text{g/L}$) or zinc ($< 7.66 \mu\text{mol/L}$; $P \geq 0.27$), but the percentage of women with low hemoglobin concentrations was significantly ($P < 0.001$) higher at the end of the intervention than at the beginning. There were no significant differences by treatment group in the percentage of women with low final plasma concentrations of ferritin ($\approx 42\%$; $P = 0.61$), zinc ($\approx 54.8\%$; $P = 0.98$), or hemoglobin ($\approx 65.8\%$; $P = 0.30$).

DISCUSSION

We assessed the effect of daily supplementation with vitamin A–containing foods on dark adaptation, symptoms of nightblindness, and plasma retinol concentrations in nightblind pregnant Nepali women. Dark adaptation improved on average in all groups of nightblind women who received small daily doses of vitamin A for 6 wk, regardless of the source of vitamin A. The final mean PT in women who initially reported nightblindness did not differ significantly from that in the nonnightblind pregnant comparison women or from that reported in a previous study of healthy, nonpregnant US women (20). The extent of improvement in dark adaptation did not differ significantly among those who received vitamin A as vitamin A capsules (0.850 or 2.0 mg/d), goat liver, amaranth leaves, or carrots. However, improvement in dark adaptation was significantly greater in the goat liver group than in the vitamin A–fortified rice group. At the end of the study, the mean vitamin A concentration of cooked liver was ≈ 3 times the initial estimate of $107 \pm 19.3 \mu\text{g/g}$, which had been used to determine the portion size of liver. Thus, the women

TABLE 5

Initial and final mean plasma carotenoid and α -tocopherol concentrations by treatment group in women who initially reported nightblindness¹

	Vitamin A supplement ²					
	High-dose ($n = 54$)	Low-dose ($n = 54$)	Fortified rice ($n = 66$)	Liver ($n = 52$)	Greens ($n = 51$)	Carrots ($n = 53$)
Lutein ($\mu\text{mol/L}$)						
Initial	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Final	0.18 ± 0.01^a	0.17 ± 0.01^a	0.16 ± 0.01^a	0.16 ± 0.01^a	0.30 ± 0.19^b	0.18 ± 0.01^a
α-Carotene ($\mu\text{mol/L}$)						
Initial	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Final	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.02 ± 0.00^b	0.17 ± 0.02^c
β-Carotene ($\mu\text{mol/L}$)						
Initial	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Final	0.06 ± 0.00^a	0.06 ± 0.00^a	0.05 ± 0.01^a	0.07 ± 0.01^a	0.13 ± 0.01^b	0.20 ± 0.02^c
α-Tocopherol ($\mu\text{mol/L}$)						
Initial	16.4 ± 0.74	13.9 ± 0.73	15.3 ± 0.68	14.9 ± 0.73	14.0 ± 0.76	13.8 ± 0.72
Final	$17.7 \pm 0.63^{a,b}$	$16.3 \pm 0.62^{a,b}$	19.2 ± 0.57^b	$16.6 \pm 0.63^{a,b}$	16.4 ± 0.64^a	15.8 ± 0.62^a

¹ All values are geometric $\bar{x} \pm \text{SE}$. Means in a row with different superscript letters are significantly different, $P < 0.03$ (analysis of covariance with initial values of month of pregnancy and study cycle as covariates and Tukey-Kramer test for group comparisons).

² High-dose, 2.0 mg; low-dose, 0.85 mg.

TABLE 6

Initial and final mean plasma concentrations of ferritin and zinc and hemoglobin concentrations by treatment group in women who initially reported nightblindness¹

	Vitamin A supplement ²					
	High-dose ³	Low-dose ⁴	Fortified rice ⁵	Liver ⁶	Greens ⁷	Carrots ⁸
Ferritin ($\mu\text{g/L}$)						
Initial	17.2 \pm 2.0	18.5 \pm 2.1	15.7 \pm 1.7	12.2 \pm 1.4	17.5 \pm 2.1	15.1 \pm 1.7
Final	17.4 \pm 2.0 ^b	14.7 \pm 1.9 ^{a,b}	17.4 \pm 1.8 ^b	14.7 \pm 1.5 ^{a,b}	16.0 \pm 1.9 ^{a,b}	12.6 \pm 1.4 ^a
Zinc ($\mu\text{mol/L}$)						
Initial	0.78 \pm 0.02	0.81 \pm 0.02	0.81 \pm 0.03	0.77 \pm 0.02	0.74 \pm 0.03	0.75 \pm 0.02
Final	0.78 \pm 0.02	0.77 \pm 0.02	0.77 \pm 0.02	0.77 \pm 0.02	0.75 \pm 0.03	0.75 \pm 0.02
Hemoglobin (g/L)						
Initial	105 \pm 2	110 \pm 2	103 \pm 2	103 \pm 2	107 \pm 2	109 \pm 2
Final	105 \pm 2	110 \pm 2	104 \pm 2	104 \pm 2	105 \pm 2	106 \pm 2

¹ All values are geometric $\bar{x} \pm \text{SE}$. Means in a row with different superscript letters are significantly different, $P < 0.031$ (analysis of covariance with month of pregnancy, study cycle, high C-reactive protein, and initial values as covariates and Tukey-Kramer test for group comparisons).

² High-dose, 2.0 mg; low-dose, 0.85 mg.

³ $n = 54$ for ferritin and hemoglobin and 49 for zinc.

⁴ $n = 54$ for ferritin and hemoglobin and 47 for zinc.

⁵ $n = 65$ for ferritin, 43 for zinc, and 56 for hemoglobin.

⁶ $n = 52$ for all.

⁷ $n = 52$ for ferritin and hemoglobin and 45 for zinc.

⁸ $n = 53$ for ferritin and hemoglobin and 49 for zinc.

may have received variable amounts of vitamin A (≈ 0.850 – 2.7 mg/d) from this source during the supplementation period, which may explain the greater response in dark adaptation in the liver group. Other nutrients in goat liver, such as iron, zinc, and animal protein, may have enhanced the effect of vitamin A. Mobilization of vitamin A from the liver is significantly lower in iron-deficient rats than in iron-replete rats (22). Protein synthesis may be impaired in zinc deficiency and protein-energy malnutrition in both animals and humans, which may result in low concentrations of retinol-binding protein (23). Low plasma concentrations of ferritin and zinc were prevalent in this population, and body weights tended to be low. Daily consumption of small amounts of highly bioavailable iron (≈ 0.82 mg), zinc (≈ 0.45 mg), and animal protein (≈ 2.0 g) in the portions of goat liver may have had a positive effect on the mobilization of vitamin A. However, there were no significant differences among groups in the change in plasma zinc concentration, which suggests that the food sources may not have had a differential effect on the women's zinc status.

Recovery from nightblindness, as assessed by self-report, was nearly universal by the end of the intervention; only 2 women (0.6%) still reported symptoms after 6 wk of supplementation with different sources of vitamin A. However $\approx 23\%$ of the women still had abnormal (> -1.11 log cd/m²) PTs after the intervention. Approximately 15% of the nonnightblind comparison women also had abnormal PTs, which suggests that the cutoff applied for the PT test is below the level at which symptoms are recognized in this population.

For ethical reasons, a negative control group was not included in the study design, so it is conceivable that recovery from impaired dark adaptation occurred for reasons other than or in addition to the treatments provided. It is possible that daily visits by project staff and provision of food supplements resulted in behavioral changes in the women that may have affected their food choices or health. However, the changes in plasma carotenoid and retinol concentrations by treatment group reflect the


carotenoid and preformed vitamin A content of the food supplements that the women received, and do not suggest that the vitamin A content of their usual diets (excluding the food supplements) changed during the intervention. Plasma carotenoid concentrations increased only in the groups that received vegetables, and plasma retinol concentrations tended to increase to a greater extent in the groups that received preformed vitamin A than in the groups receiving vegetables; this is to be expected, given that preformed vitamin A is more bioavailable than is β -carotene from plant sources. It is also possible that the night-blind women recovered spontaneously during the intervention period. However, this is unlikely because Nepali women describe nightblindness as a transient condition of pregnancy that begins typically in the 2nd or 3rd trimester and disappears only after delivery (5, 24).

Earlier accounts of maternal nightblindness in Europe, the United States, and India are consistent with this description and indicate that the condition occurs late in pregnancy and that remission is spontaneous after delivery (25). From a biological viewpoint, this is reasonable because hemodilution progresses during pregnancy, and the vitamin A demands of the fetus are greatest during the 3rd trimester (26–30). If maternal vitamin A stores are marginal at the onset of pregnancy, and if dietary vitamin A intake is low throughout pregnancy, it is conceivable that the risk of symptoms of nightblindness would increase later in pregnancy as the fetal demand for vitamin A increases. This is supported by the previous observation that PTs are significantly ($P < 0.02$) higher (ie, worse) in pregnant Nepali women during the 2nd and 3rd trimesters of pregnancy (-1.03 log cd/m²) than during the 1st trimester (-1.23 log cd/m²) (20). The spontaneous remission that is reported to occur after birth may be related to a rapid reduction in blood volume that results in an increase in the serum retinol concentration. In the current study, 76.4% ($n = 253$) of the women had normal PTs at the end of the intervention period, while they were still pregnant. It is very unlikely that

spontaneous remission would occur during pregnancy in such a large percentage of the women, given that previous reports in several different population groups indicate that spontaneous remission typically occurs after delivery.

Plasma retinol concentrations remained the same or increased in the nightblind pregnant women in response to treatment. The differences in the changes in plasma retinol concentrations by treatment group are probably related to differences in the dose and the bioavailability of vitamin A from the various sources. The plasma retinol response was significantly greater in the high-dose capsule (2.0 mg/d) and goat liver groups than in the low-dose capsule (0.850 mg/d) and vegetable groups. Because women in the high-dose capsule group received, in a highly bioavailable form, more than twice as much vitamin A as did the women in the vegetable group (2.0 and 0.85 mg/d, respectively), it is not surprising that the plasma retinol response was significantly greater in the former group than in the latter group, who received less vitamin A as β -carotene. Even though the vegetables provided a less bioavailable source of vitamin A, the plasma retinol response in the vegetable groups did not differ significantly from the response in the low-dose capsule or vitamin A-fortified rice groups who received preformed retinol. Moreover, the use of the current bioconversion factor of 12:1 (12) indicates that the vegetables provided half as much vitamin A as did the low-dose capsule, vitamin A-fortified rice, and goat liver groups, which may explain why the plasma retinol response tended to be lower in the vegetable groups. The amount of vitamin A absorbed from the vegetable sources may have been sufficient to increase tissue concentrations and normalize dark adaptation but not sufficient to increase plasma retinol concentrations. In addition, because hemodilution increases as pregnancy progresses, plasma retinol concentrations tend to decline during pregnancy, especially in malnourished women with low dietary vitamin A intake (1). In addition to slightly increasing retinol concentrations in most of the treatment groups, it is possible that the intervention prevented a decline in plasma retinol concentrations.

Among the treated nightblind women, PTs were responsive to amounts of daily vitamin A intake that are comparable to the amounts of vitamin A obtained from a dietary supply, whereas plasma retinol concentrations were insensitive to these amounts of supplementation. These results call into question an earlier study that, on the basis of the lack of serum response, reported no effect of a food-based approach (31). A similar result was reported for a small group of vitamin A-depleted US men (32). Abnormal dark adaptation improved rapidly (ie, within a few days) in response to supplementation with 150 μ g retinol/d, but that dosage had no effect on plasma retinol concentrations, which increased to the low normal range (0.66–0.73 μ mol/L) only after \approx 2–4 mo of supplementation with higher daily doses (ie, 300–600 μ g/d) of vitamin A. Thus, plasma retinol was much slower to respond than was dark adaptation in these vitamin A-depleted men.

In summary, improvement in dark adaptation and self-reported recovery from nightblindness during pregnancy did not differ significantly among women who received small daily doses of vitamin A from cooked and pureed green leafy vegetables, cooked pureed carrots, cooked goat liver, or retinyl palmitate. Food-based approaches should be considered for treating nightblindness in women of child-bearing age. 

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MJH contributed to the study design, the training of field personnel, study management, laboratory analyses, data analysis, and the writing of the manuscript; PP was the study coordinator at the field site and contributed to the training of field personnel, data collection and data analysis; JMG contributed to study management at the field site, the training of field personnel, and data collection and analysis; JMP conducted the statistical analyses and contributed to the study design, data analysis, and the writing of the manuscript; RKS contributed to the study design and the training of personnel and provided overall management of the study in Nepal; KHB contributed to the study design and management, data analysis, and the writing of the manuscript. None of the authors had any financial or personal interests in the Bill and Melinda Gates Foundation or the Program for Appropriate Technology in Health.

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