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Circulating levels of retinol, tocopherol and carotenoid in Nepali pregnant and postpartum women following long-term β-carotene and vitamin A supplementation

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Objective: To characterize circulating carotenoid and tocopherol levels in Nepali women during pregnancy and post-partum and to determine the effects of β-carotene and vitamin A supplementation on their concentration in serum.
Design: Randomized community supplementation trial.
Setting: The study was carried out from 1994 to 1997 in the Southern, rural plains District of Sarlahi, Nepal.
Subjects: A total of 1431 married women had an ascertained pregnancy, of whom 1186 (83%) provided an analyzable serum sample during pregnancy; 1098 (77%) provided an analyzable 3–4 months post-partum serum sample.
Interventions: Women received a weekly dose of vitamin A (7000 μg RE), β-carotene (42 mg) or placebo before, during and after pregnancy. Serum was analyzed for retinol, α-tocopherol, γ-tocopherol, β-carotene, α-carotene, lycopene, lutein + zeaxanthin, and β-cryptoxanthin concentrations during mid-pregnancy and at ~3 months post-partum.
Results: Compared to placebo, serum retinol, β-carotene, γ-tocopherol, β-cryptoxanthin and lutein + zeaxanthin concentrations were higher among β-carotene recipients during pregnancy and, except for β-cryptoxanthin, at postpartum. In the vitamin A group, serum retinol and β-cryptoxanthin were higher during pregnancy, and retinol and γ-tocopherol higher at postpartum. Lutein + zeaxanthin was the dominant carotenoid, regardless of treatment group, followed by serum β-carotene. Serum lycopene level was lowest, and very low compared to the US population. Serum retinol was higher, and carotenoid and α-tocopherol lower, at postpartum than during pregnancy in all groups.
Conclusions: Pregnant and lactating Nepali women have lower serum carotenoid and tocopherol levels than well-nourished populations. β-carotene supplementation appeared to increase levels of tocopherol and other carotenoids in this population.
Sponsorship: This study was carried out under Cooperative Agreement no DAN 0045-A-5094 and HRN A-00-97-00015-00 between the Office of Health and Nutrition, US Agency for International Development (USAID), Washington, DC and the Center for Human Nutrition, Johns Hopkins School of Public Health, Baltimore, MD, with additional support from Task Force Sight and Life, Basle, Switzerland, NIH grant no RR04060, and the Bill and Melinda Gates Foundation, Seattle, WA. The study was a joint undertaking between the Center for Human Nutrition and the National Society for the Prevention of Blindness, Kathmandu, Nepal.
Descriptors: supplements; β-carotene; vitamin A; Nepali women
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Introduction

In recent years, the consumption of foods high in carotenoid or their purified forms, especially β-carotene, have been of interest in industrialized countries due to possible preventative effects on cancer and cardiovascular diseases (Jha *et al*, 1995; Van Poppel & Goldbohm, 1995; Malone, 1991; Ziegler, 1989). More than 600 chemically different carotenoids have been characterized (Olson, 1990). The six major carotenoids found in human blood are β-carotene, α-carotene, β-crypotoxanthin, lycopene, lutein and zeaxanthin, the latter two being mostly reported combined. In well-nourished Western populations the level of serum lycopene has been reported to be higher than other

carotenoids (Ascherio *et al*, 1992; Forman *et al*, 1996; Nierenberg *et al*, 1997; Canfield *et al*, 1997,1998), possibly due to higher consumption of tomato products, which are high in lycopene. In contrast, circulating levels of lutein have been found to be higher and lycopene lower in women in one rural African population (Malawi); (Lan *et al*, 1999) and in Japan among males (Kitamura *et al*, 1997) compared to healthy American adults. This could be due to higher consumption of foods high in lutein, such as dark green leafy vegetables in these populations. Some carotenoids such as β-carotene, α-carotene, and β-crypotoxanthin have provitamin A activity which, when present in the diet, helps protect undernourished populations from vitamin A deficiency.

In well-nourished groups, high-dose β-carotene supplementation increased circulating levels of β-carotene, α-carotene and lycopene and reduced lutein concentration (Nierenberg *et al*, 1997; Wahlqvist *et al*, 1994; Albanes *et al*, 1992; Micozzi *et al*, 1992; Kostic *et al*, 1995). Large doses of β-carotene have been reported to lower vitamin E concentrations in animals and humans (Xu *et al*, 1992; Mobarhan *et al*, 1994). Others have found both serum retinol and α-tocopherol levels not to change after long-term β-carotene supplementation (Nierenberg *et al*, 1997; Albanes *et al*, 1992; Micozzi *et al*, 1992; Willett *et al*, 1983). There are many more reports of carotenoid interactions, which van den Berg (1999) has reviewed in detail. In undernourished populations, the majority of studies reporting nutritional effects of β-carotene intake, either in dietary or purified form, have focused on changes in serum retinol concentration. Few data exist characterizing the serum carotenoid and tocopherol distributions in undernourished populations (Lan *et al*, 1999) and little information is available on the effects of β-carotene or vitamin A supplementation on tocopherol or other carotenoid concentrations in such groups. If tissue concentrations of these compounds affect health, then it is of interest to investigate the impact of carotenoid supplementation on circulating levels of these analytes. To our knowledge no studies have reported the effects of β-carotene supplementation on serum carotenoid and tocopherol profiles in malnourished populations of pregnant and lactating women.

The present study was carried out: (1) to characterize the circulating levels of major carotenoid and tocopherol in a population of rural, Nepalese women during and shortly after pregnancy; and (2) to determine the impact of long-term, low-dose weekly β-carotene and vitamin A supplementation on circulating maternal levels of retinol, tocopherol and carotenoid. The study formed part of a large randomized, double-masked, placebo-controlled community trial in Nepal that observed routine maternal vitamin A or β-carotene supplementation to reduce mortality related to pregnancy by more than 40% (West *et al*, 1999).

Methods and materials

Field procedures

The study was carried out from 1994 to 1997 in the southern, rural plains district of Sarlahi, Nepal. Details of the intervention trial and effects of supplementation on mortality of women related to pregnancy (West *et al*, 1999; Christian *et al*, 2000), fetal loss and infant mortality have been reported (Katz *et al*, 2000). The protocol was approved by the Nepal Health Research Council in Kathmandu, Nepal and the Joint Committee on Clinical Investigation at the Johns Hopkins School of Medicine, Baltimore, MD.

Briefly, married women of reproductive age were randomized by ward to receive weekly a capsule containing 7000 μg retinol equivalents (RE) as preformed retinyl-palmitate (providing 1000 μg RE/d, an equivalent of ~1 RDA), 42 mg of β-carotene (~7000 μg RE at a 6:1 conversion ratio) or a placebo. All capsules contained a negligible amount of DL α-tocopherol (5 mg), added as an antioxidant preservative. Women were continuously supplemented before, during, and after their pregnancies for a period of $\sim 3\frac{1}{2}$ y. The present study was conducted in a subset of 27 contiguous wards (nine randomized to each type of supplement) of a total of 270 wards that participated in the larger trial. Women were visited at home and dosed weekly, at which time pregnancy status was also monitored by history. Following informed consent, women reporting to be pregnant were enrolled into a clinic-based sub-study at a median gestational age of 4 months. Subjects were transported to a clinic where blood was drawn by venipuncture. Approximately 3 months after delivery, mothers returned to the clinic for a second phlebotomy. A 7 day food frequency questionnaire was administrated to most women ~1 week before blood was drawn during pregnancy and postpartum.

Non-fasting blood was collected into vacutainers that were wrapped in aluminum foil and kept at room temperature for approximately 1–2 h. Tubes were then centrifuged (1500 ***g***) for 20 min. Serum was separated and kept in liquid nitrogen both in the field and during transport to Johns Hopkins University in Baltimore where samples were stored at −80°C until time of analysis.

Chemicals and reagents

All reagents were HPLC grade, purchased from JT Baker (Phillipsburg, NJ). *Trans*-retinol, lycopene, α- and β-carotene, and α- and γ-tocopherol standards were obtained from Sigma Chemical Company (St Louis, MO). F. Khachik (University of Maryland, College Park, MD) kindly provided β-cryptoxanthin, lutein and zeaxanthin standards. Ethyl β-apo-8′-carotenal was purchased from Fluka Company, and used as internal standard.

Laboratory analysis

Serum samples were analyzed for total cholesterol and triglyceride enzymatically using kits purchased from

Sigma Chemical Co. Serum samples were analyzed for simultaneous determination of retinol, α- and γ-tocopherol, lutein + zeaxanthin, lycopene, β-crypotoxanthin and α- and β-carotene by a reverse-phase high-performance liquid chromatography (HPLC) using a modified method of Nomura *et al* (1997). Approximately 200−400 μl of serum was deproteinized with an equal volume of ethanol containing an internal standard (Ethyl β-apo-8′-carotenal) to which 800 μl of hexane was added. The mixture was vortexed and centrifuged. The hexane layer (top part) was removed to a clean test tube. The procedure was repeated on the aqueous solution. The hexane layers were pooled together and dried under nitrogen. The dried residue was dissolved in the mobile phase: dichloromethane (4:1) and then placed in the ultrasonic bath for 1 min, vortexed and centrifuged for 3 min to remove the impurities. A 20−40 μl of sample was injected into a Beckman, System Gold HPLC (Beckman, Columbia, MD) with a Scanning Detector (Module 167) attached to an automated auto-sampler (717Plus AS, Waters Associates, Milford, MA). The column (Spherisorb, ODS2, 3 μ, 150 × 4.6 mm, Alltech Associate) with a similar packing guard column (40 × 4.6 mm) was eluted isocratically with acetonitrile:-dioxane:methanol (0.15 M ammonium acetate added); (83:13:4) and 1% of triethylamine. The detector was set in channel A at wavelength 325 nm to detect retinol, then was changed to 295 nm for tocopherol and 450 nm for detection of lutein + zeaxanthin, lycopene and α- and β-carotene. The channel B was also set at 450 nm for detection of β-cryptoxanthin. The precision and accuracy of the method was checked by serum standard reference material (SRM 968B) which was purchased from the National Institute of Standard and Technology (NIST, Gaithersburg, MD) and multiple serum samples from a serum pool were analyzed daily. All analyses were conducted in the Center for Human Nutrition laboratory at Johns Hopkins, which participates in the NIST round robin sample analysis program that monitors the accuracy of the methods used for this study.

Statistical analysis

Only the first enrolled pregnancy of each recruited woman was eligible for this study. Pre-intervention baseline blood samples were not collected. We consider pregnancy and postpartum serum analyte distributions among placebo recipients to represent the unintervened state against which effects of supplementation are compared. Differences in analyte concentrations across the three supplement groups were first tested by analysis of variance, followed by a *t*-test to compare individual groups. Multiple linear regression analysis was used to estimate mean differences in analyte concentration between groups of women receiving either vitamin A or β-carotene *vs* placebo, adjusted for differences in maternal age, mid-upper arm circumference (MUAC), reported consumption of selected high-carotenoid foods (mango, papaya, orange, tomato and green leafy vegetable), smoking and drinking of alcohol during the week prior to blood draw, season, serum total lipid (cholesterol + trigylceride) concentration, socio-economic status (owning a radio, and reading or writing) and duration of supplementation. Standard errors obtained for β-coefficients were used to test values for statistical significance against a null of zero. The Statistical Analysis System (version 6.12, SAS Institute Inc, Cary, NC) software package was used for data analysis.

Results

A total of 1431 women had an ascertained pregnancy in the substudy area (427 placebo, 530 β-carotene, 474 vitamin A), of whom 1186 (83%) (344 placebo, 450 β-carotene, 392 vitamin A) provided an analyzable serum specimen during pregnancy and 1098 (77%) (316 placebo, 405 β-carotene, 377 vitamin A) provided an analyzable 3−4 month post-partum serum specimen.

Age, weight, height, BMI, MUAC and parity were comparable in all three groups of women at the time of pregnancy and postpartum assessments (Table 1). Anthropometric indicators reflect a stunted, low-weight and wasted population of adult women compared to populations in developed countries (Frisancho, 1981; US Department of Health, Education and Welfare, 1979). Higher percentages of women in the β-carotene group smoked cigarettes, drank alcoholic beverages, owned radios and were literate than either the vitamin A or placebo groups (Table 1). Women had been supplemented, on average, for 12 months prior to pregnancy assessment. Women in the β-carotene group took a smaller percentage of their eligible supplements throughout the study and in the 3 months prior to blood being drawn, compared to women in the vitamin A and placebo groups. Approximately 50% of women during pregnancy and 100% in the postpartum period were breast-feeding (Table 1). Fewer placebo mothers reported consuming ripe papaya and tomato than women in other groups (Table 2).

Compared to placebo recipients, serum retinol during pregnancy was higher among vitamin A (+23%) and, to a lesser extent β-carotene supplemented (+10%) women (Table 3). Pregnant women given β-carotene supplements had significantly higher serum levels of β-carotene (+26%), β-cryptoxanthin (+29%), lutein + zeaxanthin (+10%), and lycopene (+31%) compared to women in the placebo group. Serum α-tocopherol was higher in the β-carotene group compared to either the placebo or vitamin A groups. Vitamin A supplemented women had circulating carotenoid and tocopherol levels during pregnancy that were comparable to placebo recipients, with the exception of β-cryptoxanthin. The total carotenoid level was higher in the β-carotene than other two groups. Total cholesterol (mmol/l; placebo = 4.1 ± 1.3, vitamin A = 3.9 ± 1.1, β-carotene = 4.0 ± 1.2) and triglyceride (mmol/l; placebo = 1.42 ± 0.64, vitamin A = 1.41 ± 0.64, β-carotene = 1.40 ± 0.62) concentrations during pregnancy were not different across treatment groups.

Table 1 Selected characteristics of pregnant (P) and post-partum (PP) Nepali women

	Placebo	*Vitamin A*	*β-Carotene*
Age (y)	24.3 ± 4.9 (332)[a]	23.8 ± 5.3 (380)	24.2 ± 5.5 (448)
Weight (kg)			
P	43.5 ± 5.3 (334)	43.8 ± 5.7 (381)	44.1 ± 5.8 (448)
PP	41.6 ± 5.1 (277)	42.1 ± 5.5 (336)	42.8 ± 5.9 (360)
Height (m)	1.5 ± .05 (334)	1.5 ± .05 (381)	1.5 ± .05 (448)
BMI (kg/m^2)			
P	19.3 ± 1.9 (334)	19.3 ± 2.1 (381)	19.6 ± 2.1 (448)
PP	18.6 ± 1.9 (196)	18.6 ± 2.1 (259)	19.2 ± 2.3 (284)
MUAC (cm)[b]			
P	22.3 ± 1.7 (334)	22.4 ± 2.0 (381)	22.5 ± 1.9 (448)
PP	22.1 ± 1.8 (277)	22.1 ± 1.9 (336)	22.5 ± 2.0 (360)
Parity	2.2 ± 1.9 (332)	2.1 ± 2.0 (373)	2.1 ± 2.0 (444)
Gestational age (weeks)	19.5 ± 6.8 (333)	19.1 ± 6.8 (380)	19.3 ± 6.4 (446)
PP (weeks)	13.2 ± 2.5 (277)	13.2 ± 2.5 (336)	13.5 ± 3.3 (359)
Blood drawing post-dose (day)			
P	1.8 ± 1.4 (252)	2.1 ± 1.7 (239)	2.4 ± 1.9 (288)
PP	1.9 ± 1.1 (197)	2.0 ± 1.6 (224)	2.4 ± 1.7 (241)
Smoking cigarettes in past week (%)			
P	18.6^{A} (328)	21.9^{AB} (370)	25.8^{B} (438)
PP	14.7^{A} (251)	21^{A} (304)	23.5^{B} (324)
Drinking alcohol in past week (%)			
P	1.5^{A} (327)	2.2^{A} (370)	6.6^{B} (437)
PP	1.6^{A} (251)	3.3^{A} (304)	4.0^{B} (324)
Eligible supplements taken (%)			
Entire study[c]			
P	76^{A} (334)	78^{A} (381)	70^{B} (448)
PP	77^{A} (277)	79^{A} (336)	72^{B} (360)
In prior 3 months			
P	81^{A} (334)	80^{A} (381)	74^{B} (448)
PP	80^{A} (277)	81^{A} (336)	73^{B} (359)
Owned radios (%)			
P	29^{A} (314)	33^{A} (361)	38^{B} (420)
PP	28^{A} (270)	29^{A} (320)	37^{B} (344)
Literate[d] (%)			
P	12^{A} (314)	15^{A} (361)	23^{B} (420)
PP	10^{A} (270)	13^{A} (320)	24^{B} (344)
Current breast feeding (%)			
P	48 (240)	49 (252)	46 (296)
PP	100 (251)	99.7 (308)	99 (330)

[a]Mean ± s.d., n = number in parentheses.
[b]Mid-upper arm circumference.
[c]Interval extends from week a woman was enrolled into the trial until the week blood was drawn.
[d]Ability to read or write a letter.
[A,B,C]Means with different superscripts indicate significant differences between treatments at $P < 0.05$.

In the post-partum period, women given vitamin A had a mean serum retinol concentration that was 35% and 20% significantly above that in placebo and β-carotene recipients, respectively (Table 4). β-Carotene supplement use was associated with higher post-partum levels of α-tocopherol (+8%), γ-tocopherol (+13%), α-carotene (+31%, $P = 0.089$), β-carotene (+40%), β-cryptoxanthin (+24%), lutein + zeaxanthin (+16%), lycopene (+92%), and total carotenoid (25%) compared to placebo use. β-Carotene supplemented women also tended to have marginally higher carotenoid and tocopherol concentrations than women taking vitamin A. As during pregnancy, total cholesterol (mmol/l; placebo = 3.6 ± 1.1, vitamin A = 3.55 ± 1.03, β-carotene = 3.55 ± 1.07) and triglyceride (placebo = 0.92 ± 0.47, vitamin A = 0.94 ± 0.51, β-carotene = 0.92 ± 0.51) concentrations were nearly identical across treatment groups during lactation. Except for retinol and γ-tocopherol, serum concentrations of all measured analytes were lower during post-partum than pregnancy periods.

Vitamin A and β-carotene supplement-placebo differences in analyte concentrations were adjusted for imbalance, potentially confounding maternal demographic, nutritional and socioeconomic factors (Table 5). Serum retinol increments during pregnancy and post-partum states remained significantly higher than placebo. Although patterns of differences remained, α-tocopherol and β-cryptoxanthin increments in the β-carotene group were no longer statistically significantly higher among post-partum women. Differences in serum lycopene concentration were also not significantly different after adjustment. All other adjusted supplement−placebo differences remained significant.

Table 2 Percentage of women during pregnancy (P) and post-partum (PP) consuming selected fruits and vegetables (≥ 1 time) in a week preceding blood drawing

	Placebo (PL) *P = (327)*[a] *PP = (250)*	*Vitamin A (VA)* *P = (370)* *PP = (305)*	*β-Carotene (βC)* *P = 438* *PP = 325*
Mango			
P	10	7	9
PP	3	3	6
Papaya			
P	12	13	17
PP	2	8	9
Dark leafy vegetables			
P	55	62	60[b]
PP	58	56	58
Oranges			
P	10[b]	12[b]	11[b]
PP	3	3	4
Tomatoes			
P	16	25	30[b]
PP	7	16	22

[a]*n* = number in parentheses, unless otherwise reported.
[b]*n* for PL = 312; VA = 362; βC = 433–436.

Discussion

Long-term oral vitamin A and β-carotene supplementation of Nepalese women increased gestational and postpartum circulating levels of retinol, compared to placebo controls, in an expected order, direction and magnitude for a chronically malnourished, vitamin A-deficient population. In such populations, supplementation with preformed vitamin A may be expected to markedly raise the serum retinol concentration, whereas β-carotene supplementation will also increase serum retinol, but more slowly and to a lower concentration (Rice *et al*, 1999).

We also observed absolute mean serum concentration increases in β-carotene, α- and γ-tocopherol, lycopene, lutein + zeaxanthin and β-cryptoxanthin among β-carotene recipients compared to other groups. After adjusting for multiple, potential confounders, β-carotene recipient serum levels remained significantly higher than those in the placebo group with respect to serum retinol, β-carotene, γ-tocopherol, β-cryptoxanthin and lutein + zeaxanthin during pregnancy and, except for β-cryptoxanthin, post-

Table 3 Serum retinol, tocopherol and carotenoid concentrations[a] (μmol/l) in Nepalese women at mid-pregnancy following routine, weekly vitamin A or β-carotene supplementation

	Placebo (PL) *(334)*[b]	*Vitamin A (VA)* *(381)*	*β-carotene (βC)* *(448)*	*ANOVA*	P-*values* *VA* vs *PL*	*βC* vs *PL*	*βC* vs *VA*
Retinol	1.0 ± 0.36	1.3 ± 0.36	1.1 ± 0.38	0.0001	0.0001	0.0001	0.0001
α-Tocopherol	15.5 ± 0.49	15.4 ± 0.5[c]	16.2 ± 0.5	0.06	0.81	0.07	0.03
γ-Tocopherol	1.28 ± 0.81	1.25 ± 0.77[c]	1.36 ± 0.83[c]	0.13	0.55	0.20	0.048
α-carotene	0.01 ± 0.02	0.009 ± 0.02	0.011 ± 0.02	0.36	0.68	0.37	0.16
β-carotene	0.15 ± 0.14	0.16 ± 0.15	0.20 ± 0.17	0.0001	0.47	0.0001	0.0001
β-cryptoxanthin	0.06 ± 0.06	0.07 ± 0.07	0.09 ± 0.09	0.0001	0.009	0.0001	0.019
Lutein + zeaxanthin	0.45 ± 0.30	0.48 ± 0.28	0.50 ± 0.31[c]	0.08	0.29	0.029	0.22
Lycopene	0.016 ± 0.032	0.018 ± 0.04	0.023 ± 0.048	0.07	0.30	0.016	0.18
Total carotenoid[d]	0.69 ± 0.44 (333)	0.74 ± 0.42 (379)	0.82 ± 0.49 (444)	0.0001	0.15	0.0001	0.005

[a]Mean ± s.d.
[b]*n* = Number in parentheses, unless it is reported otherwise.
[c]*n* for PL = 333; VA = 372–380; βC = 442–447.
[d]Total carotenoid = β-carotene + α-carotene + β-cryptoxanthin + (lutein + zeaxanthin) + lycopene.

Table 4 Serum retinol, tocopherol and carotenoid levels in Nepali women at 3–4 months post-partum following routine, weekly vitamin A or β-carotene supplementation[a] (μmol/l)

	Placebo (PL) *(277)*[b]	*Vitamin A (VA)* *(336)*	*β-carotene (βC)* *(360)*	*ANOVA*	P-*values* *VA* vs *PL*	*βC* vs *PL*	*βC* vs *VA*
Retinol	0.98 ± 0.45	1.5 ± 0.46	1.2 ± 0.47	0.0001	0.0001	0.0001	0.0001
α-Tocopherol	10.8 ± 3.9	11.1 ± 3.6	11.7 ± 3.8	0.007	0.37	0.0034	0.02
γ-Tocopherol	1.4 ± 0.76	1.5 ± 0.86	1.6 ± 0.87	0.003	0.048	0.0005	0.14
α-carotene	0.006 ± 0.017[c]	0.006 ± 0.019[c]	0.008 ± 0.019[c]	0.23	0.60	0.089	0.25
β-carotene	0.12 ± 0.14[c]	0.12 ± 0.13[c]	0.19 ± 0.18[c]	0.0001	0.72	0.0001	0.0001
β-cryptoxanthin	0.040 ± 0.049[c]	0.045 ± 0.052[c]	0.052 ± 0.056[c]	0.009	0.12	0.0020	0.12
Lutein + zeaxanthin	0.35 ± 0.24	0.35 ± 0.20	0.41 ± 0.26	0.001	0.82	0.0031	0.002
Lycopene	0.0003 ± 0.002[c]	0.001 ± 0.009[c]	0.003 ± 0.024[c]	0.075	0.17	0.0384	0.14
Total carotenoid[d]	0.51 ± 0.36 (202)	0.54 ± 0.32 (232)	0.68 ± 0.42 (271)	0.0001	0.37	0.0001	0.0001

[a]Mean ± s.d.
[b]*n* = number in parentheses, unless it is reported otherwise.
[c]*n* for: PL = 243–266; VA = 303–326; βC = 326–355.
[d]Total carotenoid = β-carotene + α-carotene + β-cryptoxanthin + (lutein + zeaxanthin) + lycopene.

Table 5 Adjusted differences[a] in serum retinol, tocopherol and carotenoid concentrations in pregnant and post partum Nepali women in supplementation *vs* placebo control women

	Retinol	*α-Tocopherol*	*γ-Tocopherol*	*β-Carotene*	*α-Carotene*	*β-Cryptoxanthin*	*Lutein + zeaxanthin*	*Lycopene*
Pregnant	(989)[b]	(983)	(975)	(987)	(989)	(989)	(986)	(987)
β-Carotene	**0.082 ± 0.03**[c]	0.34 ± 0.31	**0.12 ± 0.06**	**0.15 ± 0.012**	0.0017 ± 0.0014	**0.014 ± 0.005**	**0.042 ± 0.021**	0.0028 ± 0.00
Vitamin A	**0.29 ± 0.29**	0.19 ± 0.32	− 0.01 ± 0.07	0.007 ± 0.013	− 0.0009 ± 0.0015	**0.011 ± 0.006**	0.022 ± 0.021	0.0013 ± 0.003
Post-partum	(818)	(818)	(819)	(791)	(767)	(726)	(819)	(744)
β-Carotene	**0.13 ± 0.04**	0.46 ± 0.3	**0.23 ± 0.07**	**0.064 ± 0.13**	0.0017 ± 0.0016	0.0058 ± 0.005	**0.044 ± 0.018**	0.0013 ± 0.00
Vitamin A	**0.5 ± 0.04**	0.13 ± 0.3	**0.18 ± 0.07**	0.003 ± 0.013	0.00075 ± 0.0016	− 0.00065 ± 0.005	0.0088 ± .018	− 0.00021 ± 0.00

[a]Adjusted by mother's age, parity, weeks gestation, weeks post-partum, MUAC, smoking, alcohol consumption, selected food consumption (mango, papaya, orange, dark leafy vegetable, tomato), total lipid (cholesterol + triglyceride), socioeconomic status (SES) and season by linear regression.
[b]n = number in parentheses.
[c]β-Coefficient ± standard error.
Bold values represent β-coefficients that are significantly different from $\beta = 0$, $P < 0.05$.

partum periods. Among vitamin A recipients, analytes for which serum increments over placebo remained statistically significant after adjustment were retinol and β-cryptoxanthin during pregnancy and retinol and γ-tocopherol during post-partum. We conclude that the gestational and postpartum serum concentrations of several carotenoid and tocopherol, some of which have known antioxidant function, were increased in chronically malnourished Nepalese women who were supplemented with 42 mg of β-carotene on a weekly basis. Arithmetically, the supplement was equivalent to 6 mg/day, an amount representing a normal dietary intake, although inefficiencies that may have existed in absorbing and utilizing a single, weekly large bolus of β-carotene were unknown.

These findings contrast with mixed results from trials in well-nourished populations. A recent study by Mayne *et al* (1998) reported that up to 60 months of β-carotene supplementation (50 mg/day) did not affect serum retinol, α-tocopherol, lycopene and lutein + zeaxanthin concentrations. The serum α-carotene level was increased 2-fold after supplementation, but the authors suggested that this might have been due to contamination of capsules with α-carotene (Mayne *et al*, 1998). Wahlqvist *et al* (1994) observed an increase in serum β-carotene, lycopene, and α-carotene following β-carotene supplementation (20 mg/day) for 2 y. Albanes *et al* (1992) also reported that, following 2 months of β-carotene (20 mg/day) supplementation, there was a marked increase in serum β-carotene, α-carotene and β-cryptoxanthin but a significantly reduced lutein concentration compared to a non-supplemented group. Similarly, decreases in lutein concentration were reported by Micozzi *et al* (1992) following β-carotene supplementation. Nierenberg *et al* (1997) observed significant increases in circulating levels of serum β-carotene but no change in retinol, α-tocopherol or other carotenoid concentrations following supplementation with 25 mg/day of β-carotene for 4 y compared to placebo. Willett *et al* (1983) observed no changes in vitamin E level in well-nourished adults after β-carotene supplementation. Xu *et al* (1992), however, observed a significant decrease in plasma tocopherol after 6 months of β-carotene supplementation (15–60 mg/day). Similar results were reported from their animal data (Xu *et al*, 1992) and by others (Mobarhan *et al*, 1994). In adequately nourished individuals given large doses of lipid soluble molecules, such as carotenoid, it has been suggested that there may be competition at the micellar solubilization stage within the intestine that may affect efficiency of absorption (Xu *et*, 1992; Willett *et al*, 1983). Further, Kostic *et al* (1995) have also reported that there might be a competitive effect on carotenoid as well. They observed that the mean area under the curve for plasma lutein was reduced after administration of β-carotene and lutein supplements together. In contrast, competitive or negative effects of β-carotene supplementation on the status of other carotenoid and tocopherol were not evident from distribution in serum in rural Nepalese women.

Lutein + zeaxanthin was the primary circulating carotenoid both during pregnancy and post-partum periods, accounting for 63–69% of all those measured. The circulating lutein + zeaxanthin level in this Nepali population was similar (Ribaya-Mercado *et al*, 1995; Rock *et al*, 1995) or higher (Ascherio *et al*, 1992; Forman *et al*, 1996; Canfield *et al*, 1997,1998; Mayne *et al*, 1998) than reported among American adults. This comparability, between a markedly malnourished population in Nepal and well-nourished groups in the United States, is unexplainable. It could be due to an intake of leafy and green vegetables that is comparable to or higher than in the United States, but dietary data revealed only ~60% of Nepalese women in our study consumed green leaves weekly (Table 2), and only ~16% more than one time per week (West *et al*, 1999). Other carotenoid-rich foods tended to be consumed even less frequently. However, the level of lutein + zeaxanthin in our women was lower than seen among pregnant women in Malawi (Lan *et al*, 1999), suggesting even higher intakes or other unknown factors that influence circulating levels of carotenoids in such rural populations.

Serum β-carotene was the second highest circulating carotenoid, which accounted for 20–28% of all measured carotenoid during pregnancy and post-partum periods. The level of circulating β-carotene in our population was much lower than distributions reported in the well-nourished lactating (Canfield *et al*, 1997,1998) or non-lactating women (Forman *et al*, 1996; Rock *et al*, 1995). This may

not be surprising among controls, given the low intakes of vegetables, fruit and dietary fat (data not shown) that might be expected to enhance carotenoid absorption (Takyi, 1999). However, serum concentration of β-carotene was exceedingly low even among women routinely supplemented with β-carotene, perhaps reflecting combined effects of inefficient absorption of a single, large weekly supplement, efficient bioconversion of absorbed β-carotene to retinol in vitamin A-deficient individuals (van Vliet *et al*, 1996) or rapid utilization of absorbed and intact β-carotene for possible antioxidant (Allard *et al*, 1994) or other metabolic (Moriguchi *et al*, 1996; Prabhala *et al*, 1991) functions.

Serum lycopene was very low in our women, as has been observed in Malawi (Lan *et al*, 1999). During pregnancy serum lycopene only accounted for $\sim$3% of all circulating carotenoid, and comprised less than 0.5% during post-partum. In contrast, lycopene is one of the predominant blood carotenoids in premenopausal women (Forman *et al*, 1996; Canfield *et al*, 1997,1998; Rock *et al*, 1995) and other healthy groups (Ascherio *et al*, 1992; Nierenberg *et al*, 1997) living in the United States. The β-cryptoxanthin level in our pregnant women was similar to those reported in Malawian pregnant women (Lan *et al*, 1999), but lower than seen in American populations (Forman *et al*, 1996; Canfield *et al*, 1997,1998; Rock *et al*, 1995). Circulating α-tocopherol levels were 45–75% lower in our population than in well-nourished lactating women or other healthy American men or women (Ascherio *et al*, 1992; Mayne *et al*, 1998; Ribaya-Mercado *et al*, 1995).

Although serum carotenoid and tocopherol levels remained generally below concentrations observed in well-nourished populations, regular, weekly provision of a dietary β-carotene supplement to women of reproductive age resulted in significant elevations in serum retinol, γ-tocopherol, β-cryptoxanthin and lutein + zeaxanthin. Vitamin A supplementation had a lesser effect, but neither intervention provided evidence of decreasing other carotenoid or tocopherol concentrations, as has been observed in well-nourished populations.

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