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Maternal Vitamin A or β-Carotene Supplementation in Lactating Bangladeshi Women Benefits Mothers and Infants but Does Not Prevent Subclinical Deficiency[1,2,3]

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ABSTRACT The effects of maternal postpartum vitamin A or β-carotene supplementation on maternal and infant serum retinol concentrations, modified relative dose-response (MRDR) ratios and breast milk vitamin A concentrations were assessed during a community-based trial in Matlab, Bangladesh. At 1–3 wk postpartum, women were randomly assigned to receive either (1) a single dose of 200,000 international units [60,000 retinol equivalents (RE)] vitamin A followed by daily placebos ($n = 74$), (2) daily doses of β-carotene [7.8 mg (1300 RE)] ($n = 73$) or (3) daily placebos ($n = 73$) until 9 mo postpartum. Compared to placebos, vitamin A supplementation resulted in lower maternal MRDR ratios (i.e., increased liver stores) and higher milk vitamin A concentrations at 3 mo, but these improvements were not sustained. The β-carotene supplementation acted more slowly, resulting in milk vitamin A concentrations higher than the placebo group only at 9 mo. Irrespective of treatment group, over 50% of women produced milk with low vitamin A concentrations (≤ 1.05 μmol/L or ≤ 0.28 μmol/g fat) throughout the study. Overall, mean maternal serum retinol concentrations were not affected by supplementation. Compared to the placebo group, the mean MRDR ratio of 6-mo-old infants was higher in the vitamin A group. Infants (33%) had serum retinol concentrations <0.70 μmol/L and 88% had MRDR ratios ≥ 0.06. We conclude that while both interventions were beneficial, neither was sufficient to correct the underlying subclinical vitamin A deficiency in these women nor to bring their infants into adequate vitamin A status.   J. Nutr. 129: 356–365, 1999.

KEY WORDS: • humans • lactation • vitamin A • β-carotene • supplementation

The traditional view that preschool age children represent the main population at risk of vitamin A deficiency has been replaced by a growing awareness that subclinical and even clinical vitamin A deficiency also occurs in women of reproductive age and infants less than 6-mo-old (Christian et al. 1998, Humphrey et al. 1992, Katz et al. 1995, Underwood 1994). In recent years, efforts to develop interventions for these population groups have increased. One effective strategy for simultaneously improving the vitamin A status of lactating women and their breastfeeding infants is maternal vitamin A supplementation during the postpartum period. The World Health Organization (WHO) supports this approach and currently recommends that all lactating women living in endemic areas of vitamin A deficiency should be given one dose of 200,000 international units (IU) [60,000 retinol equivalents (RE)][6] vitamin A within 8 wk postpartum. WHO also recommends that supplementation take place as soon as possible after delivery in order to maximize the benefits on maternal vitamin A status, breast-milk vitamin A concentrations and subsequent infant vitamin A status (WHO/UNICEF/IVACG task force 1997).

Previous randomized studies demonstrated the biological efficacy of maternal vitamin A supplementation. In a placebo-controlled trial in Indonesia, a 300,000-IU (90,000 RE) dose administered at 1–3 wk postpartum resulted in improvements

in maternal vitamin A status, the vitamin A content of breast milk and infant vitamin A status (Stoltzfus et al. 1993). Similar findings were reported from a trial in Thailand in which a 300,000-IU (90,000 RE) dose was given within 3 d of delivery (Thanangkul et al. 1974). In Bangladesh, a 200,000-IU (60,000 RE) dose given at delivery also resulted in improvements in maternal vitamin A status and breast-milk vitamin A concentrations, but infant status was not assessed (Roy et al. 1997). In addition, in a nonrandomized study in Bangladesh, a 300,000-IU (90,000 RE) dose given within 3 mo postpartum was found to have a positive impact on infant mortality (de Francisco et al. 1995).

An alternative strategy for improving the vitamin A status of young infants is to supplement them directly, either at birth or during other contacts with the health-care system. In Bangladesh, infants who received immunizations and vitamin A during Expanded Program on Immunization contacts had increased serum retinol concentrations when compared to those who received immunizations and placebos (Mahalanabis et al. 1997, Rahman et al. 1995). However, 10–15% of the infants in these studies developed bulging fontanelle shortly after dosing (Baqui et al. 1995, de Francisco et al. 1993). While the condition was transient and there appears to be no long-term side effects associated with this phenomenon (van Dillen et al. 1996), concerns about this approach to infant dosing remain. In a safety study conducted in Indonesia, a 50,000-IU (15,000 RE) dose of vitamin A given to infants at birth was associated with a 2% excess rate of bulging fontanelle, but this condition was not associated with an increase in intracranial pressure or with increased rates of any other sign or symptom of morbidity (Agoestina et al. 1994). However, utilizing maternal supplementation programs to deliver vitamin A to young infants would prevent this potential problem, as well as benefit mothers.

In contrast to supplementation programs, food-based interventions are promoted as a more sustainable and long-term solution for the problem of vitamin A deficiency. Most food-based programs attempt to increase the consumption of low-cost β-carotene-rich fruits and vegetables, since foods containing preformed vitamin A are generally more expensive. However, very little data exist on the efficacy of β-carotene interventions for improving the vitamin A status of lactating women. While observational studies suggest that women with higher dietary β-carotene intakes produce breast milk with higher vitamin A content (Newman 1993), a recently conducted placebo-controlled trial in Indonesia found that maternal vitamin A status and breast-milk vitamin A concentrations increased in response to β-carotene consumed as synthetic supplements, but not to β-carotene in vegetables (de Pee et al. 1995). A variety of factors affect the efficiency with which increased β-carotene intakes from fruits and vegetables can improve vitamin A status (de Pee and West 1996), and further research is needed to determine the efficacy of β-carotene interventions for improving the vitamin A status of lactating women.

To investigate the efficacy of both maternal vitamin A supplementation as currently recommended by WHO and daily, dietary level synthetic β-carotene supplementation, we conducted a community-based, individually randomized, placebo-controlled trial among lactating women in Matlab, Bangladesh. In this report we describe the main effects of maternal postpartum supplementation on subsequent maternal and infant vitamin A status.

MATERIALS AND METHODS

Study population. This study was conducted in the 70 villages of the International Centre for Diarrhoeal Disease Research Centre for Health and Population Research (ICDDR,B) Maternal Child Health Family Planning Project (MCH-FP project) intervention area located 45 km southeast of the capital city of Dhaka in the Matlab thana of rural Bangladesh. Rice, jute and fish are the main agricultural products of this river delta region. ICDDR,B has maintained demographic surveillance of the population since the 1960s and over the years has provided preventive and curative health services in the community in conjunction with ongoing health research projects (Fauveau 1994).

The existing network of 80 MCH-FP community health workers and logistical support services was utilized for birth detection, capsule delivery, compliance monitoring and motivational activities during the supplementation study. The MCH-FP community health workers routinely visit all households with a married woman of childbearing age twice a month and provide family-planning services, immunizations and treatment or referral to an ICDDR,B clinic for a variety of health problems. Women participating in the supplementation study received these services as usual. The additional study-specific visits at 2 wk, 3, 6 and 9 mo postpartum were conducted by a separate team of trained field workers.

All women registered in the MCH-FP area who delivered a live infant in the service area between June 14–August 29, 1994, were eligible for the trial. After the report of a live birth was received, an enrollment visit was scheduled while infants were in the eligible age range of 7–21 d. Data collected from the MCH-FP records indicated that 368 women delivered live infants during the enrollment period. Women (300) remained eligible for the trial after excluding those who delivered their infants outside of the service area (n = 25), those with infants >21 d-old (n = 35), those whose infants had died (n = 5) and those with severely ill infants (n = 3). Of the 300 eligible families, 48 (16%) refused to participate, and 32 births (11%) were not detected due to the absence of community health workers, a time lag in birth reporting or for other unknown reasons. We successfully recruited 220 (73%) of the eligible women into the study, 218 with single and 2 with twin births.

This study was approved by The Johns Hopkins University School of Hygiene and Public Health Committee on Human Research and the Ethical Review Committee at ICDDR,B. Informed consent was obtained from all study participants in accordance with guidelines at The Johns Hopkins University and ICDDR,B.

Randomization. The trial was individually randomized and double-blinded. Because the cultural practices in the region prohibit some women from leaving their homes during the first few weeks postpartum, women were given a choice of completing the enrollment visit at 2 wk (±1 week) postpartum either at the central Matlab clinic or in their homes. During the enrollment visit, they were assigned a study identification number that randomly allocated them to one of three treatment groups and to a follow-up schedule that determined the location of their follow-up visits at 3, 6 and 9 mo postpartum. During clinic visits, women provided a full milk sample and a blood sample. During home visits, women provided a casual milk sample, but no blood sample (details described below).

Before beginning the study, individual treatment codes and follow-up schedules were assigned to a sequence of identification numbers in blocks of 18 using a random number table (Smith and Morrow 1991). Each block contained all possible combinations of three treatment groups (vitamin A, β-carotene or placebo) and six follow-up schedules. The six follow-up schedules assigned women to complete clinic visits at either 0.5 and 3, 0.5 and 6, 0.5 and 9, 3 and 6, 3 and 9 or 6 and 9 mo postpartum. When they were not assigned to a clinic visit, the women completed their follow-up visits at home. At each visit, half of the women in each of the three treatment groups completed a home visit and the other half completed a clinic visit.

Supplementation. At enrollment, women in the vitamin A group received one 200,000-IU (60,000 RE) dose of retinyl palmitate while women in the β-carotene and placebo groups received placebos. After that, all women took daily capsules until 9 mo postpartum. Women in the β-carotene group received capsules containing 7.8 mg of β-carotene, and women in the vitamin A and placebo groups received placebos. Using a conversion factor of 6 μg of all-trans-β-

carotene: 1 RE, the β-carotene dose was designed to approximate one U.S. recommended dietary allowance of vitamin A for lactating women in the first 6 mo postpartum [National Research Council (U.S.) Subcommittee on the Tenth Edition of the RDAs 1989].

The study capsules were manufactured by Tischon Corporation (Salisbury, MD) and delivered to the field and to study participants coded as type A, B or C. Except for the enrollment capsules, which were distributed individually, the capsules were packaged in groups of 18 in blister pack strips. Although the vitamin A and placebo capsules used at enrollment differed slightly in color, they were individually wrapped in foil before distribution, making direct comparisons between the treatment groups unlikely. The β-carotene and placebo capsules distributed in blister packs were maroon-colored and identical in outward appearance.

At the enrollment visit, women consumed their first capsule under the supervision of a field worker. They were then given one pack of capsules with instructions to take one capsule every morning with their first meal of the day. Over the next 8 mo, the MCH-FP community health workers supplied all study participants with a new pack of capsules every 2 wk. Compliance was monitored through counts from returned capsule packs and spot checks in the field.

Data collection. Serum retinol and the modified relative dose-response test. On days of clinic visits, women came to the central Matlab clinic and received an oral dose of 3,4-didehydroretinyl acetate (8.8 μmol) for the determination of their MRDR ratio (Tanumihardjo et al. 1996). This was immediately followed by a high-fat cookie and tea with lemon. During the waiting period, women were given other snacks low in vitamin A. A blood sample was collected from the antecubital vein 5 h after dosing.

At the 6-mo visit, infants were given an oral dose of 3,4-didehydroretinyl acetate (5.3 μmol) for the determination of their MRDR ratio (Tanumihardjo et al. 1996). Mothers were encouraged to breast feed their infants immediately after dosing and on demand throughout the waiting period. A blood sample was collected from the femoral vein 5 h after dosing.

After collection, the blood samples were slowly expressed into a foil-covered 8-mL glass vial, allowed to clot in the dark at room temperature for ~1 h and then centrifuged. Serum (500 μL aliquots) was stored at −20°C for up to 3 mo at the field site, transported to Dhaka on ice, stored at −70°C, then transported in liquid nitrogen to Baltimore, MD, and stored at −70°C until analysis.

Serum retinol and didehydroretinol concentrations were assayed at The Johns Hopkins University using reversed-phase high performance liquid chromatography (HPLC). Thawed serum (400 μL) was combined with 75 μL of internal standard [retinyl acetate (Sigma Chemical Co., St. Louis, MO) dissolved in ethanol] and 400 μL of ethanol. This mixture was extracted twice with 750 μL of hexane. The hexane layers were then pooled and evaporated completely under nitrogen.

The residue was redissolved in 50 μL of methanol/dichloromethane (4:1, v/v), and 30 μL was injected onto an equilibrated HPLC system by a Waters WISP 710B autosampler (Waters Corp., Milford, MA). A System Gold Beckman Programmable Detector Module 166 (Beckman Instruments, Inc., Columbia, MD) monitored the wavelength at 350 nm. A System Gold Beckman pump with a 110B Solvent Delivery Module (Beckman Instruments, Inc.) delivered the methanol/water (90:10, v/v) mobile phase at a flow rate of 1.0 mL/min for 8 min and then at 1.5 mL/min until 14 min to the Waters Spherical C18 Resolve 15-cm reversed-phase column (Waters Corp.). Peak areas were integrated by the System Gold software (Version 8.0) which also functioned as the system controller. The didehydroretinol and retinol concentrations were determined in ng/mL from standard curves established in the laboratory.

Quality control was monitored using vitamin A reference materials from the National Institute of Standards and Technology (Gaithersburg, MD). Serum obtained from well-nourished adults who had been dosed with 3,4-didehydroretinyl acetate (8.8 μmol) was used to construct a set of quality-control samples. Day-to-day assay performance was assessed by analyzing 1–3 of these quality-control samples along with each batch of study samples. Over the sample analysis period, the within-run and between-run coefficient of variation for the quality-control samples were 4 and 5% for retinol and 5

and 6% for the molar 3,4-didehydroretinol/retinol (MRDR) ratio, respectively.

The serum retinol values were converted from ng/mL to μmol/L for data analysis. In infants, serum retinol concentrations <0.70 μmol/L were used to define low vitamin A status. In both women and infants, an MRDR ratio ≥0.06 was considered indicative of marginal vitamin A status (WHO 1996).

Breast milk vitamin A and fat content. Milk samples were collected from all women at each visit. Samples were collected using two different techniques: "full" collection during clinic visits, or "casual" collection during home visits. For full collection, a trained field worker used a manual breast pump (White River, Laguna, CA) to express the entire contents of one breast which had not been used to feed an infant for ≥2 h. Milk was collected from the left breast except when a breast infection was present or if milk production had stopped. Samples were collected between 1030 and 2115 h with a median collection time of 1145 h. The late-night milk collections occurred when evening clinic visits were scheduled for a few Muslim women in order to accommodate the traditional dawn-to-dusk fasting period associated with Ramadan.

For casual milk collection, mothers manually expressed ~5 mL of milk into a glass collection jar without control over the time since last breastfeeding episode. A milk sample was collected from the breast which had not been used to feed the infant for the longer period of time or rarely from the only breast capable of producing milk. Casual milk samples were collected between 0800–1900 h with a median collection time of 1050 h.

All milk samples were stored in a cooler on ice and protected from light until processing later the same day. For processing, the milk samples were warmed to room temperature and homogenized by gentle swirling. The fat content of each milk sample was determined in triplicate using the creamatocrit method (Lucas et al. 1978). The equation published by Lucas (1978) was used to convert the volume measurement to grams of milk fat per liter of milk. Milk (500 μL aliquots) was stored at −20°C for up to 3 mo at the field site, transported on ice to Dhaka and then stored at −70°C until analysis.

Milk samples were analyzed at ICDDR,B using reversed-phase HPLC. Milk (400 μL aliquots) was thawed, combined with 200 μL of a pyrogallol in methanol solution (100 g/L), 400 μL of internal standard (β-apo-8′-carotenal-methyl-oxime) and 1.0 mL of a 5.35 mol/L solution of potassium hydroxide in a methanol/water mixture (80:20, v/v). The samples were saponified at 4°C for 16–20 h. Following the addition of 2.0 mL of saturated sodium chloride solution, the sample was extracted twice with a hexane/ether (80:20, v/v) mixture, cleaned with water and sodium sulfate, dried under nitrogen and reconstituted with 200 μL of mobile phase. The mobile phase consisted of acetonitrile, dichloromethane, ammonium acetate in water (10 g/L) and triethylamine in a ratio of 89:10:1:0.1 (v/v/v/v). Of the reconstituted sample, 25 μL was injected onto an equilibrated HPLC system with a 5-μm YMC-Pack ODS-AL column (YMC, Wilmington, NC). Retinol and internal standard peaks were detected at 325 and 450 nm, respectively. Day-to-day assay performance was monitored by analyzing two quality-control samples with each batch of study samples. The within- and between-run coefficient of variation for the milk vitamin A content of the quality-control samples were 3 and 6%, respectively.

The vitamin A content of milk was calculated as concentration per volume (μmol/L) and per gram of milk fat (μmol/g). The latter value was obtained by dividing the vitamin A concentration per volume (μmol/L) by the fat concentration (g/L) to obtain μmol/g. According to WHO criteria, values ≤1.05 μmol/L and ≤0.28 μmol/g of fat were considered low (WHO 1996). In the present report, values obtained from casual and full milk samples were combined because analyses of the differences between treatment groups revealed that both of the milk sampling methods yielded similar conclusions about the effects of supplementation.

Anthropometric data. Women and infants were weighed to the nearest 0.1 kg at each visit using a digital scale model 770 (Seca Corp., Columbia, MD). Scales were standardized on a daily basis using a 1-kg weight. The women were weighed wearing lightweight clothing. Infants wearing minimal clothing were weighed while in their mother's arms, and their weight was obtained by subtracting the

TABLE 1

Baseline characteristics of study participants in the β-carotene, placebo and vitamin A groups[1]

	Treatment group					
	β-carotene *n* = 73		Placebo *n* = 73		Vitamin A *n* = 74	
	n	Frequency	*n*	Frequency	*n*	Frequency
		%		%		%
Maternal characteristics						
Age, *y*						
<20	12	16	10	14	11	15
20–29	45	62	44	60	44	59
≥30	16	22	19	26	19	26
Total pregnancies						
1	16	22	18	25	20	27
2–4	47	64	44	60	42	57
≥5	10	14	11	15	12	16
Education, *y*						
0	31	43	34	46	30	40
1–5	20	27	18	24	20	27
≥6	17	23	14	19	20	27
Missing	5	7	8	11	5	7
Religion						
Muslim	58	80	63	86	61	82
Hindu	15	20	10	14	13	18
			Means ± SD			
BMI[2], *kg/m²*		18.9 ± 1.5		19.0 ± 1.9		18.9 ± 1.6
Infant characteristics		*n* = 73		*n* = 74		*n* = 75
		%		%		%
Sex						
Male	38	52	34	46	33	44
Female	35	48	40	54	42	56
			Means ± SD			
Weight, *kg*		3.0 ± 0.5		2.9 ± 0.5		2.8 ± 0.5

[1] Treatment group effects were examined separately for β-carotene vs. placebo and vitamin A vs. placebo. Comparisons of frequencies were tested by χ² test; mean values by *t* test. No significant differences (*P* < 0.05) were found.

[2] Calculated from weight at 0.5 mo and height at 6 mo postpartum. For β-carotene group (*n* = 69); for placebo group (*n* = 70); for vitamin A group (*n* = 71).

mother's weight alone. Maternal height was measured at the 6-mo visit using a locally constructed height measuring device. Maternal body mass index at baseline was calculated as weight (kg)/height (m)² using the weight at 0.5 mo and height at 6 mo postpartum.

Infant dietary assessment. At the 3-, 6- and 9-mo visits, mothers were interviewed about their infants' dietary intake using 24-h recall interviews and open-ended food-frequency questionnaires. Complementary feeding was assessed from the 24-h recall data and defined as giving any type of food or liquid (other than water or breast milk) in any amount to the infants. The vitamin A and β-carotene content of individual foods was assigned using food tables from Bangladesh (Darnton-Hill et al. 1988), and those containing >100 RE/100 g were considered vitamin A-rich foods. At the 6-mo visit, mothers were presented with a selection of locally available infant vitamin syrups and asked to identify the amount and type of any supplemental vitamins their infants had been given.

Demographic and socio-economic data. Baseline data on maternal age, educational level and religious affiliation were obtained from the existing MCH-FP database for all women in the area who delivered live infants during the recruitment period. Data on the total number of reported pregnancies were also obtained for the women enrolled in the study.

Statistical analysis. The sample size for the study was calculated to detect a difference in the proportion of individuals with low vitamin A status between a group who received active supplements (either vitamin A or β-carotene) and the placebo group who did not. Therefore, the data were analyzed by comparing separately the values in the vitamin A to the placebo group and the values in the β-carotene to the placebo group. For categorical variables, comparisons were made using χ² analyses. For continuous variables, comparisons were made using two-sided *t* tests. Because the MRDR ratios were skewed to high values, the data were log transformed to normalize distributions prior to statistical testing, and geometric means are reported. Although *P* values <0.05 are considered as statistically significant, values ≤0.10 are reported to indicate potentially important trends in the data. Statistical analyses were conducted using SPSS 7.5 (SPSS Inc., Chicago, IL).

RESULTS

Baseline characteristics. The baseline characteristics of the 220 women and their infants who participated in the study are shown by treatment group in **Table 1.** The three groups did

TABLE 2

Dietary intake of 6-mo-old infants in the β-carotene, placebo and vitamin A groups[1]

	Treatment group					
	β-carotene (n = 69)		Placebo (n = 70)		Vitamin A (n = 71)	
	n	Frequency	n	Frequency	n	Frequency
		%		%		%
Still breastfeeding[2]						
Yes	69	100	70	100	71	100
Ate complementary food[3] on day prior to interview						
Yes	65	94	64	92	62	87
No	4	6	6	8	9	13
Ever consumed a vitamin A-rich food[4] during life						
Yes	23	33	25	36	25	35
No	46	67	45	64	46	65
Ever consumed supplemental infant vitamins during life						
Yes	6	9	14	20	12	17
No	63	91	56	80	59	83

[1] Comparisons of frequencies were examined separately for β-carotene vs. placebo and vitamin A vs. placebo. No significant differences ($P < 0.05$) were found.
[2] Any amount of breastfeeding.
[3] Any food or liquid other than water or breast milk.
[4] >100 retinol equivalents/100 g food.

not differ in maternal age, gravidity, educational level, religious affiliation or body mass index. Infant weights were also similar at enrollment.

In addition to the 220 enrolled women, 148 other women living in the MCH-FP area delivered live infants during the recruitment period. These women were not enrolled because they delivered their infants outside the MCH-FP service area, because their infants were >21 d old, because they refused to participate, because the births were not detected during the enrollment period, because their infants had died or because their infants were severely ill at the time of potential enrollment. The distributions of age, educational level and religious affiliation did not differ between the enrolled and nonenrolled women (data not shown). The enrolled group contained a slightly, but not significantly, higher proportion of women in the 20–29-y-old age bracket (61%) as compared to the nonenrolled group (52%). Of the 148 nonenrolled cases, 32% (n = 48) were due to refusals, making this the most common reason for nonenrollment. In addition, many of the younger women were ineligible because they delivered their infants outside of the MCH-FP area. A common practice in the area is for a woman to return to her parent's home for the delivery of her first child.

Compliance with supplementation and follow-up rates. Compliance with daily capsule consumption and the completion of follow-up visits was high. Twenty-three different women missed one or more visits resulting in overall follow-up rates of 98, 95 and 92% at 3, 6 and 9 mo, respectively. Among women who completed follow-up visits, the mean compliance rate with daily capsule consumption was ≥95%. In order to deliver the intended intervention, regular capsule consumption was most critical in the β-carotene group and was consistently high. Because the first capsule was administered under supervision, women in the vitamin A group received the full-intended amount of supplemental vitamin A regardless of subsequent compliance.

Infant dietary intake. The pattern of complementary feeding, consumption of vitamin A-rich foods and infant vitamin

syrups in 6-mo-old infants was comparable between treatment groups (**Table 2**). At 6 mo, all of the infants were still breastfeeding, and although 91% were also receiving complementary foods, these were foods generally low in vitamin A content. According to the food frequency data, only 33% of 6-mo-old-infants had ever been fed a vitamin A-rich food in any amount during their life. Although the practice was not recommended by study staff, 15% of the infants (n = 32) had been given supplemental infant vitamin syrups. In 27 of these cases, the mothers reported syrup brands known to contain vitamin A. The estimated vitamin A intake from these syrups ranged from 22,500 to 210,000 IU with a median of 90,000 IU. In the other cases, brand information was not reported (n = 4) or the syrup given did not contain vitamin A (n = 1).

Vitamin A status. *Maternal modified relative dose-response ratios and serum retinol concentrations.* Compared to the placebo group, vitamin A supplementation resulted in significantly improved vitamin A stores in women at 3 mo postpartum, evident by a lower proportion of women with an MRDR ratio ≥0.06 ($P < 0.01$) and a lower mean MRDR ratio ($P < 0.05$) in the vitamin A group (**Table 3**). The vitamin A-supplemented women continued to have better vitamin A status at 6 and 9 mo as assessed by the MRDR test, but the impact of supplementation was less than that observed at 3 mo.

Although not statistically significantly different than the placebo group at 2 wk, the mean MRDR ratio of women was higher in the β-carotene group, and the proportion of women with a ratio indicative of low liver stores (≥0.06) was twice as high. However, this trend was reversed at subsequent time points. At 6 mo the mean MRDR ratio of women in the β-carotene group was lower ($P < 0.05$) than the placebo group.

In the subset of women assessed at 2 wk and 9 mo postpartum, the mean MRDR ratio (95% confidence interval) in the β-carotene group (n = 12) was 0.067 (0.044–0.102) at 2 wk and 0.043 (0.029–0.063) at 9 mo. The values for the placebo group (n = 12) at these time points were 0.033 (0.024–0.046) and 0.049 (0.031–0.077), respectively. At 2 wk women in the

TABLE 3

Maternal modified relative dose-response ratios in the β-carotene, placebo and vitamin A groups by time postpartum[1]

		Treatment group							
		β-carotene			Placebo			Vitamin A	
Time postpartum, *mo*	*n*	Geometric mean (95% CI)[2]	Frequency ≥0.06	*n*	Geometric mean (95% CI)	Frequency ≥0.06	*n*	Geometric mean (95% CI)	Frequency ≥0.06
			%			%			%
0.5	35	0.040 (0.030–0.053)	31	35	0.032 (0.026–0.038)	14	36	0.032 (0.025–0.039)	19
3	36	0.051 (0.040–0.064)	42	35	0.054 (0.043–0.069)	54	34	0.038[2] (0.031–0.047)	18[3]
6	32	0.031[2] (0.024–0.038)	19	36	0.045 (0.037–0.055)	33	35	0.039 (0.031–0.049)	31
9	35	0.039 (0.032–0.048)	26	31	0.052 (0.039–0.069)	42	32	0.044 (0.037–0.052)	28

[1] Treatment group effects were examined separately for β-carotene vs. placebo and vitamin A vs. placebo. Comparisons of mean values were tested by *t* test; frequencies by χ^2 test. CI, confidence interval.
[2] $P < 0.05$ for vitamin A vs. placebo.
[3] $P < 0.01$ for vitamin A vs. placebo.
[4] $P < 0.05$ for β-carotene vs. placebo.

β-carotene group had higher ($P < 0.05$) ratios (i.e., worse liver vitamin A stores) than women in the placebo group. However, when compared to the placebo group, the change in MRDR ratios indicated an improvement ($P < 0.01$) over time in the liver vitamin A stores of women in the β-carotene group.

In contrast, neither β-carotene nor vitamin A supplementation affected the mean maternal serum retinol concentrations (**Table 4**). However, mean concentrations were close to 1.40 μmol/L throughout the study, and low values were infrequent. Out of the 412 serum samples from the 220 women, only 1.5% were <0.70 μmol/L and 18.9% were <1.05 μmol/L. In U.S. adults, serum concentrations ≥1.05 μmol/L are considered indicative of adequate vitamin A status (Pilch 1987). A similar cutoff has not been established for populations in developing countries.

Significant differences in the proportion of women with low-serum retinol concentrations were not identified using the cutoff of 1.05 μmol/L. However, when increased to 1.40 μmol/L, a significant difference in proportions ($P < 0.01$) was detected between the β-carotene and placebo groups at 9 mo postpartum (Table 4), consistent with the continually improving liver stores among the β-carotene group.

Breast milk vitamin A. The vitamin A concentration of breast milk, expressed both as vitamin A per volume and vitamin A per gram of fat, is shown by treatment group in **Table 5.** At baseline, the vitamin A concentration of milk was not statistically different between the three groups. As expected, milk vitamin A content was highest in transitional milk (0.5 mo) and lower thereafter.

The mean milk vitamin A concentration in the vitamin A group, expressed both per volume and per gram milk fat, was significantly higher than in the placebo group at 3 mo ($P < 0.01$), but then declined to placebo group values at 6 and 9 mo. Breast-milk vitamin A concentrations in the β-carotene group were similar to those in the placebo group at 3 and 6 mo, but were significantly higher at 9 mo ($P < 0.05$). The proportion of women with low milk vitamin A concentrations showed similar treatment effects as the mean values. However, this proportion exceeded 50% in each group throughout the entire study, despite the positive effects of supplementation.

TABLE 4

Maternal serum retinol concentrations in the β-carotene, placebo and vitamin A groups by time postpartum[1]

		Treatment group							
		β-carotene			Placebo			Vitamin A	
Time postpartum, *mo*	*n*	Means ± SD	<1.40 μmol/L	*n*	Means ± SD	<1.40 μmol/L	*n*	Means ± SD	<1.40 μmol/L
		μmol/L	%		μmol/L	%		μmol/L	%
0.5	35	1.56 ± 0.71	43	35	1.68 ± 0.53	40	36	1.79 ± 0.60	28
3	36	1.43 ± 0.43	47	35	1.33 ± 0.42	60	34	1.45 ± 0.47	44
6	32	1.57 ± 0.59	38	36	1.52 ± 0.56	53	35	1.47 ± 0.38	46
9	35	1.55 ± 0.47	34[2]	31	1.36 ± 0.45	71	32	1.47 ± 0.46	50

[1] Treatment group effects were examined separately for β-carotene vs. placebo and vitamin A vs. placebo. Comparisons of mean values were tested by *t* test; frequencies by χ^2 test.
[2] $P < 0.01$ for β-carotene vs. placebo.

TABLE 5

Breast milk vitamin A concentrations per volume and per gram fat in the β-carotene, placebo and vitamin A groups by time postpartum[1]

| | | | Treatment group | | | | | | | | |
| | | β-carotene | | | Placebo | | | Vitamin A | | | |
Indicator	Time postpartum, mo	n	Means ± SD	≤1.05 μmol/L	n	Means ± SD	≤1.05 μmol/L	n	Means ± SD	≤1.05 μmol/L
			μmol/L	%		μmol/L	%		μmol/L	%
Vitamin A per volume	0.5	73	1.39 ± 1.19	49	73	1.51 ± 1.08	37	74	1.71 ± 1.34	31
	3	73	0.85 ± 0.55	75	72	0.83 ± 0.43	79	69	1.20 ± 1.00[2]	57[2]
	6	69	0.99 ± 0.62	62	69	0.87 ± 0.61	74	70	0.85 ± 0.53	73
	9	70	1.00 ± 0.58[3]	63[3]	65	0.79 ± 0.44	80	64	0.91 ± 0.68	75

| | | | Treatment group | | | | | | | | |
| | | β-carotene | | | Placebo | | | Vitamin A | | | |
Indicator	Time postpartum, mo	n	Means ± SD	≤0.28 μmol/g	n	Means ± SD	≤0.28 μmol/g	n	Means ± SD	≤0.28 μmol/g
			μmol/g	%		μmol/g	%		μmol/g	%
Vitamin A per gram fat	0.5	73	0.32 ± 0.17	48	73	0.34 ± 0.18	43	74	0.37 ± 0.19	41
	3	73	0.22 ± 0.08	73	72	0.23 ± 0.11	72	69	0.28 ± 0.14[4]	58[5]
	6	69	0.24 ± 0.10	68	69	0.24 ± 0.16	78	70	0.24 ± 0.11	69
	9	69	0.26 ± 0.08[6]	62[3]	65	0.21 ± 0.10	82	64	0.24 ± 0.13	67

[1] Treatment group effects were examined separately for β-carotene vs. placebo and vitamin A vs. placebo. Comparisons of mean values were tested by *t* test; frequencies by χ^2 test.

[2] $P < 0.01$ for vitamin A vs. placebo.

[3] $P < 0.05$ for β-carotene vs. placebo.

[4] $P < 0.05$ for vitamin A vs. placebo.

[5] $P < 0.10$ for vitamin A vs. placebo.

[6] $P < 0.01$ for β-carotene vs. placebo.

Infant serum retinol concentrations and modified relative dose-response ratios. The infant serum retinol concentrations and MRDR ratios from 6-mo-old infants are shown in **Table 6.** The mean serum retinol concentration in the vitamin A group was higher than in the placebo group ($P < 0.06$). The overall proportion of infants with serum retinol levels <0.70 μmol/L was 33%. This proportion was lowest in the vitamin A group and higher in the placebo and β-carotene groups. The serum retinol concentration (means ± SD) was not significantly different between infants who were given supplemental vitamin syrups known to contain vitamin A ($n = 27$) as compared to those ($n = 181$) who were not ($0.82 ± 0.23$ μmol/L vs. $0.80 ± 0.22$ μmol/L).

The mean MRDR ratio was lower in both the vitamin A ($P < 0.01$) and β-carotene groups ($P < 0.07$) than in the placebo group, indicating better liver stores among infants whose mothers received supplements. In addition, the entire distribution of MRDR ratios was shifted toward lower values in these groups. Ratios >0.12 were less common in the vitamin A (33%, $P < 0.01$) and β-carotene groups (39%, $P < 0.05$) as compared to the placebo group (59%). However, regardless of treatment group, more than 84% of all infants had MRDR ratios indicative of low liver stores.

DISCUSSION

The purpose of this study was to measure the effects of postpartum maternal vitamin A or β-carotene supplementa-

tion on maternal and infant vitamin A status. We found that both interventions had beneficial effects, which varied over time as predicted by the nature of the interventions. The one-time 200,000 IU (60,000 RE) dose of vitamin A had an immediate impact on maternal liver stores and breast-milk vitamin A levels which quickly declined as the dose was utilized. In contrast, maternal liver stores built up slowly in the group receiving daily dietary level β-carotene supplements, and the effects of this intervention were most evident during the latter half of the study. Both interventions improved breast-milk vitamin A concentrations and resulted in improved liver vitamin A stores and slightly higher serum retinol concentrations in 6-mo-old infants.

Other investigators (Roy et al. 1997, Stoltzfus et al. 1993, Thanangkul et al. 1974) also reported that a single postpartum dose of 200,000–300,000 IU (60,000–90,000 RE) vitamin A improved maternal vitamin A status and increased breast-milk vitamin A concentrations. In the present study, the benefits of a 200,000-IU (60,000 RE) dose of vitamin A were short-lived. At 9 mo postpartum, more women in both the vitamin A and placebo groups had lower liver stores than when the study began. This suggests that the 200,000-IU (60,000 RE) dose of vitamin A was insufficient to protect women's liver stores from being depleted as a result of lactation.

Similar to a recent study conducted in Indonesia (de Pee et al. 1995), we found that daily, dietary level synthetic β-carotene supplements improved maternal vitamin A status. While the proportion of women in the placebo group with low liver

TABLE 6

Infant serum retinol concentrations and modified relative dose-response (MRDR) ratios in the β-carotene, placebo and vitamin A groups at 6 mo of age[1]

Treatment group	n	Indicator			
		Serum retinol		MRDR ratio	
		Means ± SD	<0.70 μmol/L	Geometric mean (95% CI)[2]	Frequency ≥0.06
		μmol/L	% (n)		% (n)
β-carotene	69	0.80 ± 0.22	41 (28)	0.102 (0.090–0.114)[2]	84 (58)
Placebo	70	0.77 ± 0.21	34 (24)	0.118 (0.106–0.132)	93 (65)
Vitamin A	69	0.84 ± 0.23[3]	25 (17)	0.092 (0.081–0.105)[4]	87 (60)

[1] Treatment group effects were examined separately for β-carotene vs. placebo and vitamin A vs. placebo. Comparisons of mean values were tested by t test; frequencies by χ^2 test. CI, confidence interval.
[2] $P < 0.07$ for β-carotene vs. placebo.
[3] $P < 0.06$ for vitamin A vs. placebo.
[4] $P < 0.01$ for vitamin A vs. placebo.

stores increased from 14 to 42% from the beginning to the end of the study, the proportion in the β-carotene group remained nearly the same (26 and 31%). This suggests that the daily β-carotene dose was able to prevent the depletion of liver stores over time, but not to correct the underlying deficiency. By chance, women in the β-carotene group started the study with poorer vitamin A status than the placebo group. This may have caused the impact of β-carotene supplementation to be underestimated in this study.

At baseline, serum retinol concentrations averaged ~1.70 μmol/L, which may explain the fact that mean serum retinol did not increase with either type of supplementation. Serum retinol concentrations are homeostatically controlled over a wide range of total body stores, and as vitamin A status improves, serum retinol becomes less responsive to interventions (Olson 1984). However, at 9 mo postpartum significantly fewer women in the β-carotene group had values <1.40 μmol/L than in the placebo group. Thus, β-carotene supplementation was effective in improving the serum retinol concentrations of women with the lowest initial vitamin A status.

Breast-milk vitamin A concentrations responded quickly to vitamin A supplementation and more slowly to β-carotene supplementation. Previous studies of vitamin A supplementation found a more prolonged impact on breast-milk vitamin A concentrations. In Indonesia (Stoltzfus et al. 1993) and Thailand (Thanangkul et al. 1974), a higher dose of vitamin A [300,000 IU (60,000 RE)] was effective in maintaining higher breast-milk vitamin A concentrations among supplemented women for 8 and 9 mo, respectively. In urban Bangladeshi women who received a 200,000-IU (60,000 RE) dose at delivery, significantly higher breast-milk vitamin A concentrations were observed at 6 mo postpartum (Roy et al. 1997). In the present study, women receiving β-carotene supplements produced breast milk with increasingly higher vitamin A concentrations from 3 to 9 mo, but the concentration was significantly different from the placebo group only at 9 mo.

Similar to other investigators working in Bangladesh, we found that breast milk was the most important dietary source of vitamin A for infants (Brown et al. 1982, Zeitlin et al. 1992). In Bangladesh nearly all infants are exclusively breast fed during the first few months of life, and some continue partial breastfeeding past 2 y of age (Huffman et al. 1980). Although nearly all 6-mo-old infants received complementary foods in the present study, these were foods low in vitamin A. A low proportion of infants (13%) consumed additional vitamin A from supplemental vitamin syrups. However, at 6 mo the status of these infants was similar to the rest of the infants in the study. We attribute the observed improvements in infant vitamin A status to increased breast-milk vitamin A concentrations.

Infant vitamin A status followed the trends in breast-milk vitamin A concentrations prior to 6 mo postpartum. The best status was observed among infants in the vitamin A group, followed by those in the β-carotene and placebo groups. Previous epidemiological studies and empirical calculations suggest that while breast-milk vitamin A levels in the range observed in this study (~1 μmol/L) are sufficient to meet basal needs and avoid clinical deficiency among infants less than 6-mo-old, they are inadequate to build up significant liver reserves of vitamin A (Underwood 1994). Our data support these observations. We found that although liver stores were improved, 85% of the infants of supplemented mothers still had MRDR ratios indicative of low liver stores.

Other recent studies in Bangladesh found high proportions of infants with subclinical vitamin A deficiency. Of 40 apparently healthy breast-fed infants 5–7 mo of age, 75% had relative dose-response values indicative of marginal vitamin A status, and 60% had serum retinol concentrations <0.70 μmol/L (Wahed et al. 1997). In another study, 56% of the breast-fed infants who had received up to three doses of 50,000 IU (15,000 RE) vitamin A still had serum retinol concentrations <0.70 μmol/L at 25 wk of age (Mahalanabis et al. 1997).

In spite of the beneficial effects of maternal supplementation, subclinical vitamin A deficiency remained prevalent in this population. The prevalence of subclinical deficiency among individuals in the supplemented groups ranged from 25% using maternal MRDR ratios to 75% using low breast-milk vitamin A content, to 85% using infant MRDR ratios. Because these indicators measure different biological processes, the estimates differ. However, the pattern is consistent, and the problem would be classified as moderate-to-severe using any of these indicators according to WHO guidelines (WHO 1996).

Issues with β-carotene supplementation. The conversion of β-carotene to vitamin A is affected by a wide variety of factors. Absorption and conversion are unfavorably influenced

by low levels of dietary fat, the presence of infections, fevers and parasitic infestation (Burri 1997). We did not measure or control the amount of dietary fat consumed along with the β-carotene supplements. Taking the capsules with a high-fat meal might have enhanced the absorption of β-carotene and improved the response to the intervention.

Helminth infections have been shown to adversely affect the absorption of nutrients in children (Jalal 1991) and may have decreased β-carotene absorption among the women in this study. Although population-based studies of helminth infections in Matlab have not been conducted, a clinic-based study of individuals seeking treatment for diarrhea found that in 20–49-y-old women, 69% were infected with *Ascaris lumbricoides*, 31% with *Trichuris trichiura* and 33% with hookworms (Hossain et al. 1981). Women were not treated for helminth infections as part of this study protocol. At 9 mo postpartum, 25% reported they had taken some kind of medication in the past year to treat kirmi (the local term for worms). Systematically treating women for helminth infections might have improved their response to the β-carotene supplementation.

The β-carotene supplementation might have affected serum and breast-milk β-carotene concentrations, but this was not measured in the present study. In Indonesia, synthetic β-carotene supplements given to lactating women resulted in increased serum β-carotene concentrations (de Pee et al. 1995). Data from a recent trial conducted in Nepal in which women of reproductive age received weekly supplements of synthetic β-carotene or vitamin A also suggest that β-carotene may have positive impacts on maternal health aside from those attributed to its provitamin A activity (West, Jr. et al. 1997). Thus, although β-carotene acted more slowly than vitamin A to improve maternal and infant vitamin A status in the present study, it might have had other positive effects that were not measured.

Vitamin A postpartum dosage levels. The currently recommended 200,000-IU (60,000 RE) postpartum dose of vitamin A should allow a healthy woman to maintain her liver reserves while producing breast milk with normal vitamin A concentrations for 60 d. This calculation assumes: good initial vitamin A status; good health; dietary intake adequate to meet basal needs for vitamin A; additional requirement of 500 RE/day due to lactation [National Research Council (U.S.) Subcommittee on the Tenth Edition of the RDAs 1989]; 50% retention of the supplemental dose (Kusin et al. 1974, Pereira and Begum 1973) and 100% utilization of the retained dose for breast-milk production. Few, if any, of these assumptions are met by women in developing countries. In these settings women often have inadequate dietary intakes of vitamin A, and subclinical deficiency is common. Supplemental vitamin A given to subclinically deficient women may be utilized first for maternal needs, rather than for breast-milk production. Thus, the amount of vitamin A from a single 200,000-IU (60,000 RE) dose that remains available for transfer into breast milk may be much less than is required to produce milk with even moderate levels of vitamin A for more than 1–2 mo.

We conclude that, as a long-term strategy, β-carotene supplementation is efficacious for improving the vitamin A status of lactating women. To benefit breastfeeding infants by 6 mo of age, maternal β-carotene supplementation needs to begin during pregnancy or earlier. Our data suggest that including β-carotene in prenatal supplements could increase breast-milk vitamin A concentrations and subsequent infant vitamin A status. Because previous studies suggest that synthetic supplements tend to overestimate the impact of food-based interventions (de Pee and West 1996), we recommend

that dietary interventions which aim to improve breast-milk vitamin A concentrations by increasing maternal intakes of β-carotene should be directed toward pregnant as well as lactating women or to all women of reproductive age.

We also conclude that among populations where subclinical vitamin A deficiency is prevalent, the currently recommended postpartum dose of 200,000-IU (60,000 RE) vitamin A for lactating women is simply not enough. In this study, a single 200,000-IU (60,000 RE) dose did not completely correct the subclinical vitamin A deficiency found among women at the beginning of lactation. During lactation, the dose did not maintain maternal vitamin A status for more than a few months or improve breast-milk vitamin A concentrations to levels capable of building adequate liver vitamin A stores in their 6-mo-old breastfeeding infants. Previous studies using larger doses demonstrated a larger and more prolonged impact of supplementation on maternal and infant vitamin A status with no evidence of adverse effects. The current recommendation should be reviewed and a higher dosage considered. A combination program to provide postpartum vitamin A supplements and prenatal or continuing supplements that contain β-carotene may also be feasible in some settings. In addition, improving dietary vitamin A intakes among all women of reproductive age should be a priority public-health measure. Years of experience with vitamin A supplementation programs for preschool age children clearly teach us that supplementation programs alone cannot completely solve the problem of vitamin A deficiency (UNICEF 1998).

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