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Short-term β-carotene supplementation of lactating mothers consuming diets low in vitamin A

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We have previously shown that β-carotene supplementation of the diets of healthy U.S. mothers increases serum and milk β-carotene concentrations. Building on these results, we investigated the possibility that β-carotene supplementation could enhance the vitamin A status of mothers and their nursing infants. Three 30-mg doses of β-carotene were administered on 3 consecutive days to 44 lactating mothers who had vitamin-A–poor diets. Concentrations of maternal serum and milk carotenoids and retinol were evaluated at baseline and after 2 and 3 days of supplementation. Infant serum carotenoids and retinol were measured at baseline and 2 days following maternal supplementation. β-Carotene supplementation markedly elevated maternal serum and milk β-carotene concentrations (nine- and sevenfold, respectively) and resulted in smaller, transient increases of α-carotene, lycopene, and β-cryptoxanthin concentrations in maternal serum. Maternal serum and milk retinol were unchanged in response to the treatment. In contrast, maternal β-carotene supplementation significantly increased infant serum retinol ($P \leq 0.001$) and β-carotene concentrations remained unchanged. These results imply that breast milk β-carotene can supply retinol for the nursing infant. Further research is needed to identify the site of bioconversion of milk-derived β-carotene to retinol and to describe the factors that regulate this process. (J. Nutr. Biochem. 10:532–538, 1999) © *Elsevier Science Inc. 1999. All rights reserved.*

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Introduction

Vitamin A supplementation reduces both morbidity and mortality of children in developing countries[1–3] and vitamin A supplementation of lactating mothers improves the vitamin A status of nursing infants.[4] Unfortunately, vitamin A intervention programs alone have not provided a sustainable solution to childhood vitamin A deficiency in developing countries.[5] Therefore, we and others have suggested that provitamin A carotenoids should be investigated as potential sources of additional vitamin A.[6,7]

We previously reported that a single β-carotene supplement substantially increases β-carotene in the maternal serum and milk of healthy mothers.[8] Other studies have shown that serum and milk retinol concentrations of lactating women with low levels of vitamin A[4,9] are increased by retinol supplementation. In addition, consumption of β-carotene-rich foods increases serum retinol concentrations of children.[10] However, prior to this study, neither the response of mothers with low levels of vitamin A to β-carotene supplementation nor the potential of breast milk carotenoids as a source of vitamin A for the nursing infant had been systematically investigated. This study shows that short-term maternal β-carotene supplementation rapidly and markedly increases maternal serum and milk β-carotene concentrations and significantly increases serum retinol of nursing infants.

Subjects and methods

Materials

Unless otherwise stated, all chemicals were technical grade or better and were obtained from Aldrich (Milwaukee, WI USA) or

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Sigma Chemical Co. (St. Louis, MO USA). Solvents for chromatography were high performance liquid chromatography (HPLC) grade and were obtained from Burdick Jackson (Muskegon, MI USA). Ethanol was obtained from Quantam Chemical Corp., USI Division (Tuscola, IL USA).

Subjects

Forty-four lactating mothers and their nursing infants (ages 1–24 months) were recruited from Via Cristina, a community organized around a health clinic in the marginal barrios of Tegucigalpa, Honduras. Participants were recruited from the local clinic at Via Cristina in collaboration with health care professionals at the Universidad Nacional Autonóma de Honduras (Tegucigalpa, Honduras). All mothers lived within walking distance of the clinic. Prior to sample collection, mothers provided informed consent in accordance with regulations of the University of Arizona Human Subjects Committee. Mothers who smoked, were ill, had infants with chronic illnesses, or whose infants were less than 1 month old were excluded from the study.

The field team consisted of two local phlebotomists, six fourth-year medical students, one physician from the Universidad Nacional Autonóma de Honduras, and three of the authors (LC, RK, and DT). Immediately after their arrival in the clinic on the first day, medical students obtained health histories for mothers and infants and a 24-hour dietary recall for mothers. Gravidity, parity, days of lactation, and age of mothers were self-reported. Age and birth weight of infants were reported by the mothers. Current weights of infants were determined using a Salter scale and recumbent length was measured as described by UNICEF.[11]

The next day, and on each study day thereafter, blood samples were collected from the mothers, and immediately thereafter, β-carotene beadlets (30-mg capsules, Hoffmann-La Roche Inc., Nutley, NJ USA) were administered with a breakfast that provided approximately 8 g of fat. The meal consisted of corn tortillas, black beans, crema (a local sour cream product), and 250 mL whole milk. On days one and four, 5 to 10 mL of midstream milk was obtained by the mothers at mid-morning using manual expression with supervision from the physician or a medical student. Because these mothers nursed on demand, it was not possible to obtain full breast expressions. Blood samples were obtained from infants on days 1 and 4 only. To minimize stress to the infant, only one attempt was made to obtain their blood. While in the clinic, one of our field team lectured the mothers in Spanish on topics of hygiene and nutrition, and mothers were provided with gifts for their participation. For completion of the entire study, mothers received certificates and gifts equivalent in value to $30 (US). Forty-three mothers and 31 infants completed all phases of the study; 13 infants were lost to the study because of illness, because mothers refused permission to obtain blood on both days, or because we were unable to obtain sufficient blood on both days to perform a reliable analysis. Of the 31 infants completing the study, 11 were males and 20 were females.

Samples

Serum. Blood was maintained protected from light at ambient temperature in the field and transported to the laboratory at Hospital Escuela, Universidad Nacional Autonóma in Tegucigalpa. Serum was prepared immediately on arrival in the laboratory by centrifugation (5 minutes, 600×g). Serum was portioned into 1 to 2 mL samples and stored at −20°C. Milk samples were stored protected from light in coolers maintained at −4°C in the field, transported to the laboratory, and stored at −20°C. Frozen milk and serum samples were packed in antibacterial icepacks (Microban) and hand-carried by air to Arizona by one of the research staff at the conclusion of the study. Immediately upon arrival in Tucson, samples were delivered to the laboratory and stored at −70°C until they were analyzed. For quality control, blood was drawn from one of the authors (LC) in the field on each day that blood was drawn from the mothers. Carotenoid and retinol concentrations of samples drawn in the field were compared with those of the same investigator from blood drawn in Tucson. Serum samples were precipitated with ethanol to remove protein and lipids extracted from the supernatant with hexane as previously described.[12–14]

Milk. Analysis of milk retinol and carotenoids has been described previously.[8] Briefly, for analysis of retinol, 1 mL of milk was hydrolyzed for 16 hours (overnight) at 25°C with 50% KOH (w/w) in 5 mL ethanol. Because this treatment results in oxidation of β-carotene,[13] for analysis of carotenoids, samples were hydrolyzed separately as follows. Milk (4 mL) was saponified essentially as described for retinol [3 mL 50% KOH (w/w) in 5 mL ethanol at 25°C] but the incubation time was limited to 30 minutes. Following saponification, β-carotene was extracted with hexane as previously described.[14]

β-Carotene capsules. To verify the concentration of β-carotene in the capsules (Hoffmann La Roche), contents of three randomly selected capsules were removed and weighed. One-tenth of the total content was reserved for analysis. Samples were suspended in double-distilled water, extracted exhaustively with methylene chloride, dried over sodium sulfate, evaporated to dryness under nitrogen, re-suspended in HPLC mobile phase, analyzed, and quantitated as described below. β-Carotene concentrations in the supplement were verified by HPLC to be 90% of the stated dosage or greater. No other carotenoids were detected.

HPLC analysis

Hexane extracts were evaporated to dryness under nitrogen and re-suspended in 150 μL of the mobile phase [methanol:tetrahydrofuran (90:10 v/v) containing 0.25 g/L butylated hydroxytoluene]. The extract (50 μL of the 150 μL sample) was injected onto a YMC (Morris Plains, NJ USA) reversed phase C_{18} column using an IBM auto sampler (Model LC/9050 SE). Samples were eluted isocratically at a flow rate of 1.7 mL/min using a Waters model 510 pump (Waters Associates, Milford, MA USA). The HPLC effluent was detected with a Milton Roy programmable detector (SM 4000) and controlled by a Maxima 820 Chromatography Workstation (Waters Associates). Carotenoids were detected at 450 nm and retinol at 325 nm. Automated integration of retinol and each carotenoid on the chromatograms was verified manually by the technician. Where baseline resolution could not be achieved, tangent skimming analyses were performed to remove bias. Limits of detection for the method, using a 1 mL blood sample, were 0.005 μmol/L and 0.020 μmol/L for retinol and carotenoids, respectively. For a 4-mL milk sample, limits of detection were 0.002 μmol/L and 0.005 μmol/L for retinol and carotenoids, respectively. Limits of detection are defined as an HPLC peak or integral with a signal to noise ratio of 3 or greater.

Milk lipids

Lipid content of the milk was determined as percent of total volume by "creamatocrit" assay.[15]

Quantitation

The HPLC was calibrated at the beginning of the study using standard curves constructed from authentic β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and retinol [National Institutes of Standards and Technology (NIST)] and the same

Table 1 Anthropometric and biochemical characteristics of subjects on first day of study

	Mean ± SD	Range	*n*
Mothers			
Age (yr)	23.7 ± 6.4	15–46	44
Gravidity	3.1 ± 1.9	1–9	44
Parity	2.9 ± 1.8	1–9	44
Milk lipid (g/L)	69.0 ± 31.0	15.0–141.0	44
Infants*			
Age (mos)	9.5 ± 6.7	1–24	44
Length (cm)	64.5 ± 13.7	28.3–81.3	42
Weight (kg)	8.1 ± 2.0	3.7–23.0	44
Birth weight (kg)	3.3 ± 0.65	2.3–5.6	40

*23 females, 21 males.

curves were used for standardization throughout the study. Concentrations of standards and analytical technique were verified quarterly for all analytes in the NIST "round robin" assay. Serum and milk pools were constructed at the beginning of the study from a population of well-nourished healthy mothers in metropolitan Tucson ($n = 7$) and analyzed daily with each sample batch. To construct the pool, freshly collected samples were combined and mixed thoroughly and multiple 1 to 2 mL samples for milk or serum were removed and frozen at −70°C until they could be analyzed. Because the HPLC method used in this study did not provide baseline resolution of lutein and zeaxanthin, the sum of their concentrations is reported as a single value. Recovery of carotenoids and retinol from both serum and milk was estimated as previously described.[14]

Dietary analyses

A 24-hour dietary recall questionnaire was administered to the women by the field research team. Interviewers were instructed in use of the instrument by the field physician. Diets of the mothers contained very few foods high in carotenoids, and at the time the study was done, individual carotenoids had not yet been quantitated in Honduran foods. Therefore, because our objective was to describe the dietary pattern of the study population rather than to quantitate consumption of individual carotenoids, a qualitative analysis of the dietary data was performed. Serving sizes were recorded in volumes or as weight.

Statistical analysis

Statistical calculations were performed with Microsoft Excel 5.0 (Microsoft Corp., Redmond, WA USA) or STATA (Version 6.0, Stata Corporation, College Station, TX USA). Variables that were analyzed were anthropometrics, dietary intake, retinol, and five carotenoids. Retinol and carotenoids were assayed in milk and in serum of mothers and infants. Standard descriptive statistics were performed for each variable and significant relationships between variables were defined at a probability level less than 0.01 using paired, two-tailed *t*-tests, regression analyses, or analysis of variance.

Results

Anthropometrics

Data pertinent to the study are shown in *Table 1*. Infants were evenly divided by gender (23 females and 21 males). Only two infants were exclusively breastfed. Three infants were below the third percentile for height-for-age and weight-for-age. These infants were referred to the health center for evaluation and were excluded from the study. The remainder were between the fifth and fiftieth percentiles for height and weight.

Table 2 Maternal dietary intake of foods high in vitamin A*

Food	Number of servings†						
	0	1	2	3	4	5	6
Fruits and vegetables‡	17	18	6	2	0	0	1
Dairy products§	24	11	7	1	1	0	0
Meat, eggs, fish‖	8	21	14	1	0	0	0
Sugar**	9	23	10	1	1	0	0

*$n = 44$, day 1 of study.
†Previous 24-hour intake reported by mothers.
‡Carrots, watermelon, oranges; one piece contains approximately 100 g.
§Crema, milk, ice cream, cheese. Serving sizes were 1 tbsp (15 mL) "Crema," 1 cup milk (approximately 225 mL), 1 cup ice cream (approximately 150 g), 1 oz (approximately 28 g) cheese.
‖Primarily chicken and eggs; 100 g meat, one egg.
**1 tsp (approximately 5 g) refined sugar or one soft drink.

Dietary analyses

A summary of foods containing significant concentrations of vitamin A or provitamin A carotenoids reported by mothers using a 24-hour recall instrument is shown in *Table 2*. Because the Honduran sugar supply has been fortified since 1993 with retinol (6.6 mg/kg),[16] sugar and soft drinks were included as vitamin A-containing foods. Thirty-nine percent of the women reported intake of no fruits and vegetables high in β-carotene and 80% reported one serving or less. Similarly, 55% reported no intake of dairy products and 80% reported one serving or less. In contrast, 82% reported consuming one or more servings of meat.

Serum carotenoids

Levels of serum cartenoids for mothers and infants are reported in *Table 3*.

Mothers. Serum β-carotene concentrations of mothers increased 9.6-fold in response to two 30-mg doses of β-carotene. Maternal serum β-carotene reached maximal concentrations after two doses (day 3) and were not further increased by the additional 30-mg dose (day 4). Following the second 30-mg dose, there were small but significant increases in concentrations of maternal serum α-carotene (1.4-fold, $P < 0.0001$), lycopene (1.5-fold, $P < 0.0001$), and β-cryptoxanthin (1.3-fold, $P < 0.001$). Of these, however, only β-carotene concentrations remained significantly increased by day 4. Lutein/zeaxanthin concentrations were not significantly increased ($P < 0.34$). Of the carotenoids we measured in serum of mothers, initial concentrations of lycopene were the highest (0.134 μmol/L) and α-carotene the lowest (0.067 μmol/L).

Infants. Because initial concentrations of infant serum carotenoids were not significantly different for males and females ($P < 0.22$ and $P < 0.50$, respectively), males and females were treated as a single group. Initial concentrations

Table 3 Effect of β-carotene supplementation on retinol and major carotenoids of serum and milk

	β-Carotene*	α-Carotene*	β-Cryptoxanthin*	Lycopene*	Lutein/zeaxanthin*	Retinol*
Serum						
Mothers	$n = 43$	$n = 42^{\S}$	$n = 42^{\S}$	$n = 43$	$n = 42^{\S}$	$n = 43$
Day 1	$0.092 \pm 0.05^{\ddagger}$	$0.067 \pm 0.04^{\ddagger}$	$0.071 \pm 0.032^{\ddagger}$	$0.134 \pm 0.07^{\ddagger}$	0.113 ± 0.03	1.57 ± 0.38
Day 3	$0.88 \pm 0.40^{\dagger\ddagger}$	$0.093 \pm 0.04^{\dagger\ddagger}$	$0.090 \pm 0.04^{\dagger\ddagger}$	$0.205 \pm 0.10^{\dagger\ddagger}$	0.120 ± 0.03	1.57 ± 0.39
Day 4	$0.88 \pm 0.38^{\dagger\ddagger}$	$0.080 \pm 0.44^{\ddagger}$	$0.075 \pm 0.27^{\ddagger}$	$0.135 \pm 0.07^{\ddagger}$	0.113 ± 0.03	1.64 ± 0.34
Infants	$n = 25^{**}$	$n = 20^{\dagger\dagger}$	$n = 26^{\|}$	$n = 21^{\S\S}$	$n = 31$	$n = 31$
Day 1	0.059 ± 0.03	0.03 ± 0.12	0.041 ± 0.03	0.063 ± 0.06	0.07 ± 0.04	0.88 ± 0.28
Day 4	0.062 ± 0.03	0.03 ± 0.01	0.045 ± 0.03	0.072 ± 0.07	0.08 ± 0.04	$1.02 \pm 0.25^{\dagger}$
Milk (uncorrected)	$n = 29^{\dagger\dagger}$	$n = 28^{\ddagger\ddagger}$	$n = 29^{\dagger\dagger}$	$n = 29^{\dagger\dagger}$	$n = 29^{\dagger\dagger}$	$n = 36$
Day 1	0.017 ± 0.01	0.006 ± 0.004	0.010 ± 0.01	0.013 ± 0.01	0.023 ± 0.01	1.73 ± 1.08
Day 4	$0.099 \pm 0.09^{\dagger}$	0.009 ± 0.01	$0.014 \pm 0.01^{\dagger}$	0.020 ± 0.02	0.018 ± 0.01	1.68 ± 0.96
Milk (relative to fat)						
	$n = 29^{\dagger\dagger}$	$n = 28^{\ddagger\ddagger}$	$n = 29^{\dagger\dagger}$	$n = 29^{\dagger\dagger}$	$n = 29^{\dagger\dagger}$	$n = 36$
Day 1	0.25 ± 0.14	0.087 ± 0.067	0.142 ± 0.093	0.175 ± 0.085	0.330 ± 0.017	24.79 ± 12.8
Day 4	$1.51 \pm 1.27^{\dagger}$	0.130 ± 0.117	0.197 ± 0.163	0.304 ± 0.339	0.305 ± 0.017	27.79 ± 12.7

*Mean concentrations, μmol/L ± SD for serum and uncorrected milk, nmol/g lipid ± SD for milk relative to fat.
†Means are significantly different from day 1, $P < 0.01$ using a paired, two-tailed "*t*" test.
‡Means are significantly different ($P < 0.01$) using repeated measures analysis of variance.
Concentrations were below our levels of detection for $1^{\S}$, $5^{\|}$, 6^{**}, $7^{\dagger\dagger}$, $8^{\ddagger\ddagger}$ or $10^{\S\S}$ subjects on at least one day of the study.

of major carotenoids in infant serum ranged from approximately one half (α-carotene and lycopene) to two thirds (β-carotene) those in maternal serum. Concentrations of β-carotene, α-carotene, β-cryptoxanthin, and lycopene were below our levels of detection on at least one sampling day for 6, 10, 5, and 10 infants, respectively. Neither β-carotene nor the other major carotenoids measured in infant serum were significantly changed by maternal β-carotene supplementation. Neither initial infant serum β-carotene concentrations nor changes in serum β-carotene were related to the age of the infant.

Milk carotenoids

Of the 43 mothers who completed the study, 36 provided sufficient milk for analysis on both days 1 and 4. Because full breast expressions could not be obtained, to control for changes in lipid concentrations over the nursing episode,[14] results in *Table 3* are presented both as uncorrected concentrations (μmol/L) and relative to fat concentrations (nmol/g lipid). Milk β-carotene concentrations were increased 5.8-fold following supplementation. Changes in lutein/zeaxanthin and lycopene and α-carotene were insignificant ($P < 0.08$, 0.09, and 0.02, respectively). However, there were small but statistically significant increases in β-cryptoxanthin concentrations ($P \leq 0.002$). Changes in milk carotenoids were the same when expressed as simple concentrations or relative to fat, demonstrating the uniformity in fat content of the samples.

Lutein/zeaxanthin concentrations (0.023 μmol/L) were the highest of the carotenoids we measured in milk. Initial concentrations of milk carotenoids ranged from approximately one fifth (β-carotene and lutein/zeaxanthin) to one tenth (α-carotene and lycopene) their concentrations in serum. Decreases in milk carotenoid concentrations over the lactation period were not significant ($r = 0.22$, $P < 0.21$).

Serum retinol

Changes in maternal serum retinol (*Table 3*) following supplementation were not statistically significant. However, infant serum retinol concentrations were significantly increased following maternal β-carotene supplementation (0.14 ± 0.03 μmol/L, $P < 0.001$). Initial infant serum retinol concentrations ranged from 0.5 to 1.2 μmol/L, with 25% of infants exhibiting serum retinol concentrations of less than 0.7 μmol/L. As was the case for carotenoids, initial concentrations of retinol were not significantly different for male and female infants ($P < 0.5$).

Milk retinol

Milk retinol was not significantly changed by β-carotene supplementation. Milk retinol concentrations decreased with duration of lactation over 1 to 24 months ($r = 0.25$, $P < 0.0001$). Initial concentrations of mothers' milk retinol and β-carotene were strongly related ($r = 0.75$, $P < 0.0001$).

Discussion

Initial serum β-carotene concentrations of women in this study were less than one-fourth the recommended serum "threshold concentration" (0.4 μmol/L) for maintenance of healthy adults[18] and were comparable to those of U.S. subjects consuming carotenoid depleted diets for more than 60 days[17] (approximately 10% those of U.S. lactating mothers).[6,8,19] As is shown in *Table 2*, provitamin A food sources were extremely low, and the majority of vitamin A in the diet was from animal or fortified sources.

Two 30-mg doses of β-carotene increased maternal serum β-carotene concentrations almost 10-fold. Serum concentrations following supplementation (0.88 μmol/L) were comparable to those of healthy unsupplemented Tucson women[6,8] and increases were comparable to or greater

than those of U.S. subjects following prolonged high-dose β-carotene supplementation.[20–22] A third supplement resulted in no further increases.

Following the second β-carotene supplementation (day 3), there were small but significant mean increases in maternal serum α-carotene, lycopene, and β-cryptoxanthin concentrations (*Table 3*). In contrast, in our earlier studies of the same five carotenoids in Tucson women, only β-carotene concentrations were significantly increased. Artificial inflation of peaks due to oxidation of the β-carotene supplement to degradation products that co-elute on HPLC with other carotenoids[23] cannot be ruled out. However, because we rejected HPLC peaks with no baseline resolution or with a signal to noise ratio of less than 3, this explanation is unlikely. Similarly, because analysis of supplements revealed only β-carotene, contamination of the supplement with other carotenoids appears unlikely.

The interactions of carotenoids during their absorption and transport is poorly understood and the data are conflicting. Both inhibition and stimulation by individual carotenoids on absorption of other carotenoids have been reported.[20,24–28] Unfortunately, however, the varying experimental designs preclude a direct comparison of the results. Moreover, the present study differs from the available literature in that it is the first report of the effects of β-carotene supplementation on lactating women and their infants consuming diets low in vitamin A. Thus we can offer no clear explanation of the data. Because of their extremely low serum initial carotenoid concentrations, trace quantities of these carotenoids in the meals we furnished may be responsible for their increased concentrations in serum. In addition, the increased absorption of other carotenoids we observed may reflect increased efficiencies of absorption and metabolism of carotenoids in these women and infants due to their low vitamin A status. This possibility warrants further investigation.

Initial milk β-carotene concentrations were approximately 20% those of mothers studied in Tucson[6,8] or Philadelphia[19] and were markedly increased (approximately sevenfold) after three 30-mg β-carotene supplements. There were also small, but significant increases in β-cryptoxanthin ($P < 0.002$). Possible reasons for increases in concentrations of other carotenoids following β-carotene supplementation were discussed above. Particularly because corn tortillas were furnished with every meal, it is likely that these small increases in milk β-cryptoxanthin reflect increased dietary intake of corn products during the study. Changes in concentrations of the other milk carotenoids we measured were insignificant. In agreement with results of others,[19] decreases in concentrations of β-carotene with duration of lactation were not significant.

Average initial serum retinol concentrations of lactating mothers (*Table 3*) were approximately 80% those of Tucson women, and like those in Tucson women, these concentrations were not significantly increased by β-carotene supplementation.[6,8] Milk retinol concentrations were also similar to those of Tucson mothers (approximately 75%) and also were unaffected by β-carotene supplementation. Serum retinol concentrations of Indonesian lactating women[4,29,30] were 25 to 40% lower and milk retinol concentrations approximately 25% higher[4] than those in the present study, and both serum and milk were increased following high-dose retinol supplementation. In addition, supplementation of lactating Indonesian women with a β-carotene-enriched wafer (3.5 mg/day for 12 weeks) significantly increased their serum retinol concentrations.[30] In both of these studies, however, β-carotene supplementation was continued over a longer period. Thus, the available data suggest that response to supplementation depends on the initial vitamin A status of the individual as well as the duration of supplementation. As previously reported,[30,32,33] and in contrast to carotenoid concentrations, milk retinol concentrations in the present study decreased with duration of lactation.

Most studies of serum carotenoids in children have been of older children. Serum β-carotene concentrations of infants in this study were approximately half those of Guatemalan preschoolers,[34] approximately one quarter those of 3 to 9 year olds in neighboring Belize,[35] and 10 to 15% those of healthy Japanese[36] and French[37] children. In 6 of the 31 infants completing our study, β-carotene concentrations were below our levels of detection on at least 1 day of the study. Therefore, these children have limited stores of retinol and extremely low provitamin A carotenoids. Because retinol stores are rapidly depleted during acute infection,[38] the possibility that low carotenoid stores can exacerbate the risk for infection in children should be further investigated.

In spite of their remarkably low initial concentrations, and contrary to results in mothers, maternal β-carotene supplementation did not result in increased concentrations of β-carotene or other carotenoids in infant serum. In contrast, infant serum retinol was significantly increased by maternal β-carotene supplementation (0.14 ± 0.03 μmol/L, $P < 0.001$). Although suggestive, our data do not allow us to determine whether this increase could be accounted for by increases in maternal carotenoids. Evidence for β-carotene in breast milk as an effective source of retinol for the nursing infant will require detailed kinetic studies that employ tracer methodology.

Approximately 25% of the infants in our study had serum retinol concentrations of less than 0.7 μmol/L. In agreement with this data, the Honduran Ministry of Health recently reported that approximately 30% of Honduran infants have serum vitamin A concentrations in this (vitamin A-low) range. Unfortunately, except in the case of extreme deficiency (concentrations < 0.35 μmol/L), serum retinol concentrations are an imprecise measurement of vitamin A status. Elimination of the lowest concentrations by supplementation has been proposed as a more reliable criterion of vitamin A deficiency in a population.[25] By this criterion, 25 of the 31 infants were vitamin A deficient. Furthermore, for the infants having serum retinol concentrations of less than 0.70 μmol/L, the average increase was 0.27 μmol/L, more than three times the group mean. Thus, these children are potentially at increased risk for infection due to limited vitamin A and essentially depleted provitamin A stores as discussed above.

Total milk lipid concentrations were comparable to those of well-nourished mothers.[6,8,39] However, the milk β-carotene concentrations measured here were higher relative to serum β-carotene concentrations than those in the Tucson study. The 1:3 ratio of β-carotene concentrations in milk to

serum in this study compared with the ratio of 1:10 in Tucson mothers[14] suggests that maternal serum β-carotene is preferentially exported to milk at the expense of maternal stores when milk β-carotene is low.

In summary, short-term β-carotene supplementation of mothers consuming diets low in vitamin A significantly increased maternal serum and milk β-carotene and serum retinol of their nursing infants. These results are the first direct demonstration of increased infant serum retinol in response to maternal β-carotene supplementation and provide the basis for further studies to investigate the process of maternal β-carotene absorption, metabolism, and transport to infant serum retinol.

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