Kinetics of the response of milk and serum β-carotene to daily β-carotene supplementation in healthy, lactating women

Louise M Canfield, Anna R Giuliano, Eleanor M Neilson, Beverly M Blashil, Ellen J Graver, and Hui H Yap

ABSTRACT Changes in concentrations of milk and serum carotenoids, retinol, and α-tocopherol of five healthy, well-nourished, lactating women were measured over a 28-d supplementation trial with 30 mg β-carotene and for 4 wk thereafter. β-Carotene supplementation increased mean β-carotene concentrations in milk and serum 6.4- and 7.4-fold, respectively. Concentrations of other major carotenoids, retinol, and α-tocopherol did not change substantially in either milk or serum. Uptake of β-carotene into both serum and milk followed apparent first-order kinetics, occurring more rapidly into serum (t½ = 5.5 d) than into milk (t½ = 9 d). After supplementation, milk and serum β-carotene concentrations decayed slowly, reaching approximately twofold initial concentrations by 4 wk. Kinetics of uptake and decay in milk β-carotene concentrations paralleled those in serum. The data show that short-term supplementation of healthy, lactating mothers with purified β-carotene at approximately fivefold the average daily dietary intake substantially increased milk and serum β-carotene concentrations while not interfering with concentrations of other carotenoids, retinol, or α-tocopherol in milk or serum. Thus, an increased intake of β-carotene by healthy, lactating women increases the supply of milk β-carotene available to their breast-feeding infants. Am J Clin Nutr 1998;67:276–83.

KEY WORDS β-Carotene, lycopene, canthaxanthin, lutein, retinol, carotenoids, vitamin E, breast-feeding, women

INTRODUCTION Carotenoids provide a major source of vitamin A in breast milk in developing countries and may contribute to the immunoprotective effect of breast milk. Because the average daily dietary intake of vitamin A (retinol plus β-carotene) by unsupplemented lactating women in these countries [660 retinol equivalents (RE)/d] is less than half that of women in developed countries (1540 RE/d) and less than the recommended safe amount for lactating women (850 RE/d) (1), these studies are of particular importance in developing countries where preformed vitamin A is not consistently available in the mother’s diet.

To evaluate the potential of breast milk β-carotene as a source of β-carotene or provitamin A for infants, the maternal factors regulating milk β-carotene metabolism in healthy, well-nourished individuals must first be established. Among the most important of these relations is that of milk and serum carotenoids. The increase in serum β-carotene concentrations in healthy adults and children in response to both dietary intake and supplementation with β-carotene is well documented (2–5); however, before our studies, nothing was known about the effects of maternal β-carotene on breast milk carotenoids and related lipids or of the relation between the concentrations of carotenoids in milk and those in serum.

Our previous studies showed that a single 60-mg β-carotene supplement increases β-carotene concentrations in both serum and milk of healthy mothers without significantly affecting other carotenoids, retinol, or α-tocopherol. A threefold increase in serum and milk β-carotene concentrations was achieved in response to a single 60-mg supplement, a twofold increase was sustained for > 1 wk, and initial serum and milk concentrations were correlated with maternal dietary intake (6). The present studies complement and extend these studies to the investigation of the kinetics of the response of milk and serum carotenoids, vitamin E, and retinol of healthy, lactating mothers supplemented daily with β-carotene. Analysis of the effects of diet and other maternal factors affecting β-carotene concentrations in serum and milk is currently in progress in our laboratory.

SUBJECTS AND METHODS

Materials

Unless otherwise stated, all chemicals were technical grade or better and were obtained from Aldrich Chemical (Milwaukee) or Sigma Chemical Co (St Louis). Solvents for chromatography were HPLC grade and were obtained from Burdick Jackson (Muskegon, MI). Ethanol was obtained from Quantam Chemical Corp, USI Division (Tuscola, IL).

Subjects

Subjects were five healthy, exclusively breast-feeding mothers from middle- to upper-middle-class neighborhoods in the Tucson, AZ, metropolitan area. Mothers were recruited from well-baby...

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2 Supported by grant RO1-HD-26715 from the National Institutes of Health. Ameda Egnell Co (Cary, IL) provided the electric breast pumps and Hoffmann-La Roche Inc (Nutley, NJ) supplied the β-carotene supplements.

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clinics at local hospitals and by word of mouth among health care professionals at the University of Arizona Medical Center. Mothers were >1 mo postpartum, were between the ages of 23 and 36 y, were of normal height and weight, had no chronic diseases, were not taking medication or steroid contraceptives routinely, had children with healthy growth patterns, and were nonsmokers.

Mothers received monetary compensation and were provided information on lactation and infant care in return for participation in the study. Mothers were given a “baby book” containing information on lactation management, nutrition, and infant care, and infant growth charts. A 24-h “lactation hotline” was provided for the mothers for the duration of the study. All subjects completed the study, but data collected from one mother who developed an infection midway through the study were not included. Before sample collection, subjects signed informed consent forms in accordance with regulations of the University of Arizona Human Subjects Committee.

Anthropometric measurements

Anthropometric and biochemical characteristics of the mothers are shown in Table 1. Gravidity, parity, days of lactation, ages and heights of mothers, prepregnancy weights, and weights at parturition were self-reported. Weights at the beginning of the study were determined in the homes of the subjects with a digital gravimetric balance (Black and Decker, Shelton, CT). Body fat was estimated with a single bioelectrical impedance measurement in the homes of the subjects with a body composition analyzer system (model BIA-103; RJL Systems, Inc, Detroit). The skin was prepared with alcohol before placement of electrodes. Two electrodes were placed on each of the hands and feet, for a total of eight access points. Four sets of readings were taken by using the following combinations of electrode placement: right-right, left-right, right-left, and left-left. The lowest resistance and reactance values were selected by the technician for calculation and analysis of body composition as described previously (7).

Administration of supplements

Purified β-carotene in capsules (Hoffmann-La Roche Inc, Nutley, NJ) was taken daily for 28 d with breakfast, which included 170 g (6 oz) high-fat yogurt (Yoplait, Minneapolis). β-Carotene was extracted from the capsules as described previously (9). Serum samples were hydrolyzed for 16 h (overnight) at 25 °C. Because this treatment results in oxidation of some carotenoids (8), carotenoids were hydrolyzed separately. Milk (4 mL) was saponified essentially as described for retinol [3 mL 50% KOH (wt:wt) in 5 mL ethanol, samples were hydrolyzed for 16 h (overnight) at 25 °C. This treatment results in oxidation of some carotenoids (8), carotenoids were hydrolyzed separately. Milk (4 mL) was saponified essentially as described for retinol [3 mL 50% KOH (by wt) in 5 mL ethanol at 25 °C] but the incubation time was limited to 0.5 h. After saponification, carotenoids were extracted with hexane as described previously (9). Serum samples were precipitated with ethanol to remove protein, and lipids were extracted from the supernate with hexane as described previously (7).

β-Carotene capsules

To verify the concentration of β-carotene in the capsules, contents of the capsules were removed and weighed. Two-tenths of the total content was reserved for analysis. Samples were dissolved in double-distilled water, extracted exhaustively with methylene chloride, dried over sodium sulfate, evaporated to dryness under N₂, resuspended in HPLC mobile phase, and stored at –70 °C until analyzed. HPLC analysis

Hexane extracts were evaporated to dryness under N₂ and resuspended in 150 µL of the mobile phase [methanol:tetrahydrofuran (90:10, by vol) containing 0.25 g butylated hydroxytoluene/L]. The extract (50 µL of the 150-µL sample) was injected onto a YMC (Morris Plains, NJ) reversed-phase C₁₈ column with an IBM autosampler (model LC/9050 SE, Wilmington, DE). Samples were eluted isocratically at a flow rate of 1.7 mL/min with a model 510 pump (Waters Associates, Milford, MA). The HPLC effluent was detected with a programmable detector (model SM 4000; Milton Roy Co, Rochester, NY) and controlled by a Maxima 820 Chromatography Workstation (Waters Associates). Carotenoids were detected at 450 nm, retinol at 325 nm, and α-tocopherol at 300 nm. Automated integration of each carotenoid, vitamin A, and α-tocopherol peak on all chromatograms was verified manually by the HPLC operator. Where baseline resolution could not be achieved, tangent skimming

Collection of milk samples

Mothers were trained in their homes by the field staff on the use of the electric breast pumps (Ameda Egnell Co, Cary, IL) and proper use was monitored periodically. Milk samples were collected by the mothers in their homes. Thirteen samples were collected by each mother over an 8-wk period (days –1, 0, 1, 2, 4, and 6 and once weekly for 7 wk thereafter). To ensure that residual milk did not contaminate breast milk samples, the baby breast-fed from the breast to be sampled 2-3 h before collection, and the breast was then completely emptied by using the breast pump. Mothers used the same breast for collection of all samples throughout the study. The complete contents of one breast were collected with an electric breast pump under subdued lighting into light-protected, sterile polypropylene containers or glass bottles. Two midafternoon milk samples were collected on each collection day, following the normal feeding schedule of the infant. To avoid trapping lipid in protein aggregates caused by freezing and thawing, samples were maintained covered in household refrigerators (4 °C) until collected on the following morning by the field research team. On arrival in the laboratory, samples were immediately warmed to 37 °C, mixed by stirring, and portioned into 4–8-mL samples for storage at –70 °C until analyzed.

Collection of serum

The field research team collected fasting serum samples from the mothers immediately before administration of β-carotene supplements with the morning meal. Twelve blood samples were obtained from each mother as described for milk collections except that blood was obtained on days 0, 1, 2, 4, and 7 and once weekly for 7 wk thereafter. Blood was maintained at ambient temperature (≤2 h) and transported to the laboratory; serum was prepared by centrifugation at 600 × g for 5 min at 25 °C. Serum samples were portioned into 1–2-mL samples and stored at –70°C until analyzed.

Sample preparation

For analysis of milk α-tocopherol and retinol, samples (1 mL) were diluted 1:3 with deionized, double-distilled water and mixed gently. After addition of 3 mL 50% KOH (wt:wt) in 5 mL ethanol, samples were hydrolyzed for 16 h (overnight) at 25 °C, which demulsifies the lipid extraction and results in oxidation of some carotenoids (8). Carotenoids were hydrolyzed separately. Milk (4 mL) was saponified essentially as described for retinol [3 mL 50% KOH (by wt) in 5 mL ethanol at 25 °C] but the incubation time was limited to 0.5 h. After saponification, carotenoids were extracted with hexane as described previously (9). Serum samples were precipitated with ethanol to remove protein, and lipids were extracted from the supernate with hexane as described previously (10).
analyses were performed to remove bias. The limit of detection was defined as an integral producing a signal-to-noise ratio \( \geq 3 \).

**Milk lipids**

The lipid content of the milk was determined as a percentage of total volume by the “creamatocrit” assay as described previously (11).

**Quantitation**

The HPLC was calibrated at the outset of the study by using standard curves constructed from authentic \( \beta \)-carotene, \( \alpha \)-carotene, \( \beta \)-cryptoxanthin, lycopene, lutein, retinol, and \( \alpha \)-tocopherol (National Institutes of Standards and Technology) and the same curves were used for standardization throughout the study. Concentrations of standards and analytic techniques for all analytes were verified quarterly with the National Institutes of Standards and Technology “round robin” assay. Serum and milk pools were constructed at the beginning of the study from the same population and multiple 4-mL or 1-mL aliquots for milk and serum, respectively, were removed and frozen at –70°C until analyzed. Technically, were removed and frozen at –70°C until analyzed. Technically variability for the serum pool for \( \beta \)-carotene (n = 44) and retinol (n = 25) was <10%: 0.473 ± 0.47 \( \mu \)mol/L (CV: 9.9%) and 1.78 ± 0.12 \( \mu \)mol/L (CV: 6.9%), respectively. CVs for the milk pool for \( \beta \)-carotene and retinol were 16.8% (23.6 ± 4.0 nmol/mL) and 9.7% (1.29 ± 0.13 nmol/mL), respectively. All samples were extracted and analyzed in duplicate. Because the HPLC method used in this study did not provide baseline resolution of lutein/zeaxanthin, the sum of their concentrations is reported as a single value. Recovery of carotenoids and retinol from both serum and milk was estimated to be essentially complete by exhaustive extraction (9).

**Dietary analyses**

Usual maternal intakes of vitamin A, carotenoids, fat, and fiber were estimated by three 24-h dietary intake records completed on 2 nonconsecutive weekdays and 1 weekend day immediately before the study. To ensure accurate reporting of foods consumed and portion sizes, subjects participated in one-on-one training sessions in their homes taught by the field research team. Subjects were instructed in careful recording of their food intake for the 3-d dietary intake records. Emphasis was placed on accurate measurement of food portions and documentation of recipes, brand names, and methods of food preparation.

The 3-d dietary intake records were reviewed for completeness by the field research team and the Nutrition Core Unit staff, and missing information and clarification of food descriptions were obtained by contacting respondents by telephone. A 25% sample of records was chosen randomly for recoding and reentry by a second technician. Intakes of vitamin A were estimated by using the US Department of Agriculture’s Continuing Survey of Food Intakes by Individuals 1985–1986 (CSFII-86; USDA, Washington, DC) database. Carotenoid intake was assessed by using a revised database that we developed using recently published laboratory determinations (12, 13). A matching program was used that attaches the NUTRITIONIST III (14) food code and gram weight output to the database. This matching program generated a summary of total nutrients for each day of intake for each person.

**Data analysis**

Descriptive statistics and correlations were performed by using EXCEL 5.0 (Microsoft Corp, Redmond, WA). Linear regression analyses were performed by using SAS 6.1 software (15). Half-times of increase of \( \beta \)-carotene in serum and milk were determined by plotting the natural logarithm (ln) of the difference between maximum concentrations and concentrations at each collection point versus time.

To facilitate comparisons in kinetic studies, concentrations of all carotenoids were normalized to the mean as follows. Concentrations at days 0 and 1 or days –1 and 0 for serum and milk (nmol/g lipid), respectively, were averaged to provide each individual’s baseline concentration. Individual baseline concentrations were then averaged to provide the group mean. A normalization factor was next determined by expressing each individual baseline concentration relative to the group mean. All subsequent concentrations were multiplied by the appropriate normalization factor to provide concentrations over the 8-wk period.

**RESULTS**

Mothers participating in this study at entry were well established in lactation and were consuming normal diets (Table 1). Study participants had been lactating an average of 279 d and were producing a mean milk volume of 62.9 mL per breast-feeding episode. The mean dietary intakes of \( \alpha \)-tocopherol, retinol, and total energy (kJ/d) at baseline were greater than or equal to the recommended dietary allowance for all study participants (16). Percentage body fat (26.2 ± 7.1%) was within the normal range for women in this age group (28.2 ± 7.4 y).

Initial serum and breast milk retinol, \( \alpha \)-tocopherol, and carotenoid concentrations are presented in Table 2. As reported earlier (9), both intra- and interindividual variability were significant in milk carotenoids. Therefore, to more accurately assess baseline serum concentrations, the mean of two consecutive fast-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Anthropometric and biochemical characteristics of subjects ( ^1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>28.2 ± 7.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.9 ± 4.5</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.2 ± 1.3</td>
</tr>
<tr>
<td>Parity</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.5 ± 5.2</td>
</tr>
<tr>
<td>At parturition</td>
<td>73.5 ± 7.7</td>
</tr>
<tr>
<td>Day 1 of study</td>
<td>62.2 ± 9.5</td>
</tr>
<tr>
<td>Duration of lactation (d)</td>
<td>279 ± 9.3</td>
</tr>
<tr>
<td>Average milk volume per breast-feeding episode (ml) ( ^2 )</td>
<td>62.9 ± 12.9</td>
</tr>
<tr>
<td>Milk lipid (g/L)</td>
<td>65.0 ± 7.0</td>
</tr>
<tr>
<td>Body fat (% by wt)</td>
<td>26.2 ± 7.1</td>
</tr>
<tr>
<td>Dietary ( \alpha )-tocopherol (mg/d)</td>
<td>69.4 ± 56.4</td>
</tr>
<tr>
<td>Dietary retinol (RE/d)</td>
<td>2543.8 ± 792.2</td>
</tr>
<tr>
<td>Dietary ( \beta )-carotene (( \mu )g/d)</td>
<td>4034.3 ± 3501.4</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>9785.1 ± 788.7</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2338.7 ± 188.5</td>
</tr>
</tbody>
</table>

\( ^1 \) x ± SD; \( n = 5 \). RE, retinol equivalents.

\( ^2 \) Group mean of five mothers. Milk volume for each of the mothers was determined by averaging 26–36 full expressions of a single breast collected twice in the afternoon each collection day over the period of the study.
ing serum samples per individual was calculated. Daily milk retinol, α-tocopherol, and carotenoid concentrations were estimated from the average of two midafternoon samples by using methods described previously (6, 9). Concentrations of retinol, α-tocopherol, and carotenoids are expressed relative to lipid concentrations to minimize variation in the data (6, 9). Concentrations of α-tocopherol and retinol in milk were similar to those reported previously by us and others (9, 17–20). Concentrations of milk carotenoids were similar to those reported earlier by us (9, 18) and =10% of those reported by investigators using methods that did not differentiate among the carotenoids (18, 20).

β-Carotene concentrations in breast milk increased dramatically after supplementation with 30 mg β-carotene/d for 28 d (Figure 1). For clarity, initial concentrations are presented both corrected and uncorrected for lipid (Table 2). Increases in milk β-carotene of individual mothers ranged from <75 to >400 nmol/L (<1 to >6 nmol/g lipid) with increases ranging from 5.1- to 8.8-fold (mean: 6.4-fold). Concentrations of milk β-carotene rose sharply during the first week of supplementation and continued to increase over the 28-d supplementation period. When the ln of changes in concentrations was plotted relative to time, a straight line was observed (r = 0.99), indicative of first-order kinetics, with a half-time of increase of 9 d (Figure 1, inset). When supplementation was discontinued, milk concentrations decreased slowly, remaining elevated about twofold over initial concentrations 1 mo after supplementation. Mean increases in milk concentrations were weakly and inversely related to initial concentrations (r = –0.21). However, the two subjects with the highest initial baseline β-carotene concentrations had the highest concentrations 4 wk after supplementation.

As shown in Figure 2, there was substantial variation in concentrations of milk β-cryptoxanthin and lycopene. Mean concentrations of these two carotenoids appeared to rise over the period of the study, but because of the large inter- and intraindividual variation, the changes were not significant (P > 0.1). Breast milk concentrations of lutein/zeaxanthin and α-carotene were not changed significantly by β-carotene supplementation.

Analysis of retinol and α-tocopherol in milk requires separate, extractions and HPLC analyses from those used for analysis of breast milk carotenoids (see Methods). Because the study participants were replete in retinol and α-tocopherol, no significant changes in these analytes were expected as a result of the intervention. Therefore, breast milk retinol and α-tocopherol concentrations were analyzed in only two mothers over 6 wk. Milk retinol and α-tocopherol concentrations for these two mothers are presented in Figure 3. Milk retinol concentrations varied

![FIGURE 1. Increase in milk β-carotene concentrations in five mothers supplemented with 30 mg β-carotene/d for 28 d. Each data point is the mean (±SEM) of two midafternoon samples for each mother, analyzed in duplicate. Calculations of nmol β-carotene/L relative to lipid are described in the text. The trend line is described by the equation y = 1.0 × 10^{-3}x^3 + 1.0 × 10^{-4}x^2 – 0.015 × 10^{-3}x + 0.47x + 1.3 (r = 0.97). The first-order kinetics of β-carotene uptake (inset) were determined from the natural logarithm (ln) of the differences between the maximum β-carotene concentration and concentrations at each collection point versus time.]

![FIGURE 2. Concentrations of breast milk lutein/zeaxanthin, β-cryptoxanthin, lycopene, and α-carotene for 8 wk in five mothers supplemented with 30 mg β-carotene/d for 28 d. Each data point is the mean of two midafternoon milk samples for each mother, analyzed in duplicate. Methods for converting to nanomolar concentrations of individual carotenoids are described in the text. The dotted lines indicate mean (±SEM) milk β-carotene concentrations. The solid lines are trend lines calculated from the average data: A, y = 1.4 × 10^{-3}x + 0.49 (r = 0.39); B, y = 8.5 × 10^{-4}x + 0.42 (r = 0.80); C, y = 7.0 × 10^{-3}x + 0.91 (r = 0.50); and D, y = 2.0 × 10^{-4}x + 0.27 (r = 0.11).]

### Table 2

**Initial serum and milk concentrations of major carotenoids, retinol, and α-tocopherol**

<table>
<thead>
<tr>
<th>Component</th>
<th>Serum (μmol/L)</th>
<th>Milk (nmol/L nmol/g lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein/zeaxanthin</td>
<td>0.196 ± 0.013</td>
<td>22.3 ± 3.3 0.356 ± 0.057</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.188 ± 0.008</td>
<td>26.2 ± 3.6 0.389 ± 0.047</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.844 ± 0.045</td>
<td>47.6 ± 5.5 0.722 ± 0.081</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.176 ± 0.038</td>
<td>13.3 ± 3.7 0.205 ± 0.049</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.713 ± 0.099</td>
<td>66.4 ± 19.2 1.003 ± 0.204</td>
</tr>
<tr>
<td>Retinol</td>
<td>1.72 ± 0.10</td>
<td>2240 ± 0.27 34 ± 3.0</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>30.20 ± 0.28</td>
<td>5800 ± 900  NA</td>
</tr>
</tbody>
</table>

1. Mean ± SE of two samples from pre-intervention days.
2. n = 6 unless otherwise indicated.
3. n = 3.
4. Not applicable (see Results).
substantially over the period of the study; however, in these two subjects concentrations did not increase in response to β-carotene supplementation. Because milk α-tocopherol concentrations have not been shown to be related to milk lipid concentrations (6, 17), concentrations of α-tocopherol are presented uncorrected for lipids. Over the period of the study, milk α-tocopherol concentrations tended to decline in subject 1 whereas α-tocopherol concentrations in the milk of the other mother (subject 2) were unchanged. Changes in α-tocopherol and retinol concentrations were unrelated in these two subjects.

Increases in serum β-carotene concentrations in response to supplementation are shown in Figure 4. Similar to the rise in breast milk β-carotene, serum concentrations increased from 3.5- to 11.1-fold (average 7.4-fold). When the ln of the increase in concentrations was plotted relative to time, a straight line indicative of first-order kinetics with a half-time of 5.5 d was obtained. When supplementation was discontinued, serum concentrations decreased slowly, remaining elevated 1.8-fold relative to initial concentrations 1 mo after supplementation. Beta-carotene increased 4.1 ± 0.6 nmol/L (range: 3.35–4.83 nmol/L) and increases in serum β-carotene concentrations were inversely correlated with initial concentrations (r = −0.35).

The effect of daily supplementation with β-carotene on serum lycopene, β-cryptoxanthin, lutein/zeaxanthin, and α-carotene is shown in Figure 5. Mean concentrations of lutein and cryptoxanthin increased slightly and concentrations of lycopene and α-carotene decreased over the period of the study. None of these changes was significant (P > 0.1).

Serum retinol concentrations fluctuated over the period of the study, but final concentrations were not significantly different from initial concentrations (Figure 6, P > 0.1). Similarly, serum α-tocopherol concentrations decreased slightly over the study period; however, because of large intra- and interindividual variations, the initial and final concentrations were not significantly different (Figure 6, P > 0.1).

Changes in milk and serum β-carotene concentrations are compared in Figure 7. For logistical reasons, serum collections preceded those of milk by ~8 h. Therefore, our protocol did not allow us to determine whether β-carotene appeared in milk before 8 h postsupplementation. However, increases in breast milk β-carotene paralleled those in serum with a lag time not greater than the difference in collection times (~8 h).

DISCUSSION

Daily supplementation with 30 mg β-carotene resulted in a significant increase in both serum and milk β-carotene in this population of healthy, well-nourished mothers. This dose of β-carotene has frequently been used in clinical studies (4, 21, 22) and is approximately fivefold the average daily US intake of β-carotene (23). The mechanism of uptake of carotenoids into the mammary gland is not understood. However, kinetic analysis of the data is consistent with first-order kinetics with a half-time of 5.5 d.

FIGURE 6. Increase in serum β-carotene for 8 wk in five mothers supplemented with 30 mg β-carotene/d for 28 d. The trend line calculated from the group mean is defined by the equation $y = 10^{1.0 \times 10^{-4} x^3 - 1.49 \times 10^{-2} x^2 + 0.44 x + 0.81}$ ($r = 0.98$). The first-order plot of uptake of β-carotene into serum (inset) was constructed by plotting the natural logarithm (ln) of the differences between maximum β-carotene concentrations and concentrations at each collection point versus time.

Intestinal absorption of β-carotene and other carotenoids is not completely understood and is affected by a variety of factors...
including diet, dose, individual variation, and physiologic state (5). The ability of individual carotenoids to influence the absorption of other carotenoids is also poorly understood and may differ among individuals. The currently available data, though incomplete, appear to support the hypothesis that at doses similar to those used in the present study, β-carotene does not significantly impair the absorption of other carotenoids from the diet (9, 24, 25). Fluctuations in milk β-cryptoxanthin paralleled those in serum and may reflect variations in dietary intake. In contrast, milk lycopene concentrations do not mirror those in serum lycopene. As discussed previously (9, 10), because of the large individual variability in milk and serum concentrations, chemical lability, and fluctuations in dietary intake, large sample sizes will be required to quantitatively interpret small changes in serum and milk concentrations of carotenoids.

Evidence that ingestion of other carotenoids affects circulating β-carotene concentrations is equivocal. Supplementation of ferrets with a single large dose (10 mg/kg) of canthaxanthin or lycopene inhibited the absorption of β-carotene by 50% and 25%, respectively (5). Conversely, in humans, when β-carotene was ingested during three 5-d study periods concurrently with canthaxanthin, the serum response to canthaxanthin was depressed ~40% in two subjects relative to the response to canthaxanthin alone (26), but canthaxanthin ingestion had no effect on serum β-carotene. A complex interaction was reported in humans between β-carotene and lutein at single doses of 15 mg. β-Carotene reduced the plasma lutein response by 40% compared with lutein treatment alone, but lutein administration alone either enhanced or suppressed the β-carotene response, depending on the subject (27). Thus, the effects of ingestion of other carotenoids on β-carotene absorption cannot be predicted on the basis of existing data.

Changes in serum β-carotene concentrations varied from 3.5- to 11-fold (mean: 7.4-fold) among subjects in response to supplementation with 30 mg β-carotene for 28 d. Uptake into serum followed apparent first-order kinetics and was approximately twice as rapid as uptake into milk, with a half-time of 5.5 d. Kinetics of β-carotene decay in serum and milk were similar and both remained elevated about twofold higher than initial concentrations by 30 d postsupplementation. Thus, β-carotene stores were maintained to a similar extent in serum and in milk in these healthy, well-nourished mothers. It remains to be seen whether maternal serum β-carotene stores are mobilized to milk in undernourished mothers. These studies are currently in progress in our laboratory.

We and others (2, 6, 28, 29) have reported that the rank order of serum and milk β-carotene concentrations is maintained in response to β-carotene supplementation; ie, subjects who begin the study with the highest β-carotene concentrations maintain the highest concentrations throughout the study. In contrast, in this study, initial and final β-carotene concentrations showed a weak inverse association in serum (r = –0.35) and in milk (r = < –0.21). We have no satisfactory explanation for these different results. However, the usual diet of our subjects was high in fruit and vegetables containing β-carotene. Therefore, their body stores may have been essentially saturated before supplementation so that further intake had little effect on serum and milk concentrations. Consistent with this hypothesis, the two mothers with the highest baseline milk β-carotene concentrations had the highest concentrations at the conclusion of the study. The relation of carotenoid stores to serum and milk response to carotenoid intake remains to be elucidated and should be tested in a large number of subjects.

Increases in milk and serum β-carotene concentrations were similar (6.4- and 7.4-fold higher than baseline concentrations, respectively) and the pattern of changes in concentrations of...
order process of uptake of these are approximate only, by 24 h were unable to document the initial rate of uptake from serum which is dependent only on serum milk serum and milk sample collections were spaced tors. (5) and taken up into the mammary alveolar cell by LDL recep-
tors. As found in other studies (6, 17, 18), retinol concentrations were similar in serum and milk. Serum retinol concentrations are not sensitive to diet in healthy individuals (30). Therefore, as expected, serum and milk retinol concentrations of healthy mothers were unaffected by β-carotene supplementation.

In the two mothers we examined, there was no significant effect of β-carotene supplementation on concentrations of α-tocopherol in milk (Figure 3). The effect of β-carotene supplementation on serum α-tocopherol concentrations is controversial. Supplementation with β-carotene in various trials has resulted in no effect (31), small increases (32), or small decreases (33, 34) in plasma α-tocopherol concentrations. However, the preponderance of evidence favors the hypothesis that β-carotene does not interfere with vitamin E absorption (5). As shown in Figure 6, interindividual variation in serum α-tocopherol concentrations may explain these different results, at least in part. These variations may be expected to be particularly important when sample sizes are small. In agreement with others (17), we observed no relation of milk α-tocopherol concentrations to concentrations of other milk lipids (Table 2 and Figure 3).

Collectively, our data show that supplementation of healthy, well-nourished mothers with β-carotene substantially increased serum and milk β-carotene concentrations while not interfering with concentrations of other carotenoids, retinol, or α-tocopherol in serum or milk. Therefore, increasing maternal supplies of β-carotene by fortification, supplementation, or dietary means should be a viable mechanism for increasing milk β-carotene intake in infants. It is not known whether metabolism of β-carotene to retinol occurs in the mammary gland. However, these data show that consumption of β-carotene by lactating mothers will result in an increased supply of β-carotene in their milk. This may be of particular importance in developing countries, where carotenoids in breast milk provide a major source of provitamin A for infants (1). Furthermore, although no changes in serum or milk retinol were expected in response to β-carotene supplementation in this population, it remains to be seen whether β-carotene supplementation can enhance the retinol status of mothers and infants in developing countries. These studies are currently under way in our laboratory.

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