β-Carotene in breast milk and serum is increased after a single β-carotene dose

Louise M Canfield, Anna R Giuliano, Eleanor M Neilson, Hiu H Yap, Ellen J Graver, Haiyan A Cui, and Beverly M Blashill

ABSTRACT Normal lactating mothers were administered a single dose of 60 or 210 mg β-carotene and changes in serum and milk retinol, α-tocopherol, and carotenoids were monitored for 8 d. Average serum β-carotene concentrations increased 4.1- and 4.0-fold after the 60- and 210-mg doses, respectively. Milk β-carotene concentrations increased 4.1- and 3.0-fold after the 60- and 210-mg doses, respectively. Maximum serum concentrations were reached 24 h after both supplements, although concentrations of milk β-carotene continued to rise for 2–3 d. After 8 d, both serum and milk β-carotene concentrations remained about twofold higher than baseline concentrations. Increases in serum or milk β-carotene concentrations were not dose-dependent. Initial serum and milk concentrations of β-carotene predicted increases after supplementation, and increases in serum β-carotene concentrations predicted those in milk. Concentrations of milk carotenoids were less than one-tenth their respective concentrations in serum. Lutein, β-cryptoxanthin, lycopene, α-carotene, retinol, and α-tocopherol concentrations in serum or milk did not change significantly after β-carotene supplementation. Retinol esters account for most of the retinol equivalents in the milk of well-nourished mothers. Initial and maximum concentrations of β-carotene in serum and milk were strongly correlated for individual mothers. Collectively, the data showed that a single 60-mg supplement of β-carotene sustained elevated β-carotene concentrations in serum and milk for > 1 wk in normal mothers but did not affect concentrations of other major carotenoids, retinol, or α-tocopherol. Am J Clin Nutr 1997;66:52–61.

KEY WORDS β-Carotene, carotenoids, lycopene, cryptoxanthin, lutein, retinol, vitamin A, α-tocopherol, vitamin E, human milk, breast-feeding, absorption

INTRODUCTION Dietary β-carotene is converted in vivo to vitamin A (1) and is a potential source of vitamin A for infants in breast milk. In addition, β-carotene may confer long-term protection against chronic disease and contribute to the immunoprotective effect of human milk (2, 3). Although well-nourished mothers provide sufficient retinol in their milk to support their infant’s needs, the average daily dietary intake of vitamin A (retinol plus β-carotene) by unsupplemented lactating women in developing 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 intake for lactating women in the United States (850 RE/d) (4). Although it has long been recognized that these mothers and their infants would benefit from an additional source of vitamin A (5), dietary sources of preformed vitamin A are relatively expensive and the logistics of vitamin A supplementation are complex. In addition, because it is difficult to identify and exclude women who are pregnant in developing countries, retinol supplementation is problematic because of its potential toxicity to the fetus in the first trimester. In contrast, β-carotene is inexpensive, is widely available in common foods, and has no known toxicity in this population.

Based on these considerations, we have begun a comprehensive study of the effects of β-carotene supplementation of lactating mothers and their infants and report here the results of the first of these studies. The effects of β-carotene supplementation on serum β-carotene concentrations in adults and children, as well as the relation of dietary intake with serum carotenoid concentrations, have been studied in detail (6–14). In addition, we previously identified and quantitated the major carotenoids in human milk (15); however, the effects of β-carotene supplementation on carotenoids in milk have not been reported. Therefore, before initiating supplementation trials in poorly nourished mothers, it was necessary to determine the effects of β-carotene supplementation in normal lactating mothers as a point of reference. We report changes in maternal milk and serum concentrations of the major carotenoids, retinol, and α-tocopherol for 8 d after a single 60- or 210-mg dose of β-carotene.

SUBJECTS AND METHODS

Materials

Unless otherwise stated, all chemicals were technical grade or better and were obtained from Aldrich Chemical (Milwaukee, WI). 1 From the Department of Biochemistry, University of Arizona, Tucson; the Arizona Disease Prevention Center, Tucson; and the Arizona Cancer Center, Tucson.

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

3 Address reprint requests to LM Canfield, Department of Biochemistry, University of Arizona, Tucson, AZ 85721. E-mail: canfield@ccit.arizona.edu.

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Subjects

Subjects were healthy, well-nourished, lactating mothers from middle- to upper-middle-class neighborhoods in the Tucson, AZ, metropolitan area. Mothers were recruited from well-baby clinics at local hospitals and by word-of-mouth among health care professionals at the University of Arizona Medical Center. Mothers were aged 23–36 y, had no chronic diseases, did not take any medication or steroid contraceptives routinely, did not smoke, and had children with normal growth patterns. With the exception of one 11-mo-old infant, who was breast-fed five to seven times per day, all infants were ≤ 6 mo of age and were not receiving formula or supplemental foods.

All subjects signed informed consent forms in accordance with regulations of the University of Arizona Human Subjects Committee. 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, infant care, and infant growth charts. A 24-h "lactation hotline" was provided for the mothers for the duration of the study. All mothers completed the study.

Administration of supplements

Purified β-carotene in capsules (Hoffmann-La Roche Inc, Nutley, NJ) was administered with breakfast, which included ≈170 g (6 oz) full-fat yogurt (Yoplait, Minneapolis). Six subjects received 60 mg (group 1) and six received 210 mg (group 2) β-carotene on day 1 of the study. Doses were selected to approximate vitamin A doses typically administered in field studies. Either a single 60-mg dose administered weekly for 10 wk or a 210-mg dose given weekly for 3 wk would approximate 300 000 IU vitamin A, a dosage typically administered for vitamin A deficiency (5, 16–18). In addition, 60 mg is near the maximum amount in the typical Western diet (5–6 mg/d) over the period of testing (3, 9), and 210 mg approximates a daily intake of 20–30 mg/d during the test period. These dosages are commonly administered in clinical trials (7, 8, 19). Supplements were administered with yogurt by the field team immediately after obtaining the first serum sample (day 1). β-Carotene was extracted from the capsules as described below and concentrations were determined by HPLC to be ≥ 90% of the stated dose.

Anthropometric measurements

Gravimetry, parity, 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 & Decker, Shelton, CT). Body fat was estimated by 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 the 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 (20).

Dietary analyses

Maternal intakes of vitamin A, carotenoids, fat, and fiber were estimated by three 24-h dietary-intake records completed on 2 consecutive weekdays and 1 weekend day, which were selected randomly during the study. To ensure accurate reporting of foods consumed and portion sizes, subjects participated in individualized training sessions in their homes, taught by the field research team. Subjects were instructed to carefully record 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 randomly chosen for recoding and reentry by a second technician. Intakes of vitamin A, fat, and fiber were estimated by using the US Department of Agriculture (USDA) Continuing Survey of Food Intakes II-86 database (USDA, Washington, DC). Carotenoid intake was assessed by using a revised database, which we developed using recently published laboratory determinations (21, 22). A matching program that attaches the Nutritionist III (23) food code and gram-weight output to the database was used. This matching program generated a summary of total nutrients for each day of intake for each person.

Blood collection and serum preparation

Fasting blood was collected on days 0, 1, 2, 4, 7, and 9 from the mothers at their homes before breakfast. Blood was collected by antecubital puncture into sterile (no additive), red-toppered, Monject blood-collection tubes (Sherwood Medical, St Louis). Blood samples were held at room temperature for 30 min to 1 h after collection. Serum was obtained by centrifugation (1000 × g) in a clinical centrifuge at room temperature; 0.25-mL samples were stored at −70 °C until analyzed.

Milk collections

Before the study began, mothers were instructed by the field research team in the use of the electric pumps (Ameda Egnell, Cary, IL) and were provided with detailed written instructions for their use. Milk samples were then collected on days −1, 0, 1, 2, 4, 7, and 9 by the mothers using the electric breast pumps in their homes under subdued lighting. The complete contents of one breast were collected into sterile polypropylene containers or glass bottles. To ensure that residual hind milk did not contaminate breast milk samples, the infant fed from the breast to be sampled 2–3 h before collection, the breast was completely emptied with the breast pump, and the volume of milk was measured. Mothers used the same breast for collection of all samples throughout the study. Separate milk samples were collected by mothers at two midafternoon feedings as described previously (15). The mother’s normal feeding schedule was
maintained, and for this reason times of collection varied slightly among individuals. After collection, samples were stored in the subjects' homes in the freezer compartments of household refrigerators (< 0 °C) until they could be collected, usually on the following morning by the field research team. On arrival at the laboratory, samples were immediately warmed to 37 °C, mixed by stirring, portioned into 1–4-mL samples, and stored at −70 °C until analyzed.

Sample analysis

Samples were analyzed as soon as possible after collection, and, when possible, samples collected on a given day (eg, day 1) were analyzed in the same batch. Typically, 12 serum samples or 8 milk samples were extracted and analyzed by HPLC on a single day. All samples were analyzed in duplicate; duplicates were extracted and analyzed on separate days.

Milk retinol and α-tocopherol

Samples (1 mL) were diluted 1:3 with deionized, double-distilled water (Millipore, Bedford, MA) and mixed gently. After the addition of 3 mL 50% KOH (wt:wt) and 5 mL ethanol, samples were hydrolyzed with shaking in a water bath overnight at 25 °C. Hydrolyzed samples were then extracted three times with hexane, evaporated to dryness under nitrogen, and resuspended in the HPLC mobile phase.

Milk carotenoids

Undiluted milk samples (4 mL) were saponified as described previously (15) by addition of 3 mL 50% KOH (wt:wt) in 5 mL ethanol for 0.5 h at 25 °C. Hydrolyzed samples were then extracted twice with hexane, evaporated to dryness under nitrogen, and resuspended in the HPLC mobile phase.

Serum retinol, carotenoids, and α-tocopherol

Serum was prepared from blood samples (0.25 mL) by precipitation with 0.025 mL ethanol containing 0.25 g butylated hydroxytoluene/L. The supernate was extracted twice with hexane as described previously to obtain retinol, carotenoids, and α-tocopherol (15).

β-Carotene capsules

Contents of the capsules were removed and weighed, and one-tenth of the total was reserved for analysis. Samples were dissolved in double-distilled water, extracted exhaustively with methylene chloride, dried over sodium sulfate, evaporated to dryness under nitrogen, and resuspended in the HPLC mobile phase.

HPLC analysis

Samples that had been evaporated to dryness with nitrogen were resuspended in 0.25 mL of the HPLC mobile phase [methanol:terahydrofuran, (90:10, by vol) containing 0.25 g/L butylated hydroxytoluene]. Samples were injected by using a 50-μL loop on an IBM autosampler (model LC/9050 SE; IBM, Wilmington, DE) onto a YMC (Morris Plains, NJ) reversed-phase C-18 column. Samples were eluted isocratically in the HPLC mobile phase at a flow rate of 1.7 mL/min with a model 510 pump (Waters Associates, Milford, MA), a Milton Roy programmable detector (model SM 4000; Rochester, NY), and a Maxima 810 version 3.02 system controller (Waters Associates) (15). Carotenoids, α-tocopherol, and retinol were quantitated at 452, 325, and 300 nm, respectively.

Analysis of milk lipids and quantitation

The lipid content of the milk was estimated by the "crematocrit" assay as described previously (15, 24). The recovery of carotenoids and retinol from both serum and milk was estimated to be essentially complete by exhaustive extraction (15). The HPLC was calibrated with a standard curve constructed from authentic standards. This curve was used throughout the study and was verified by using standards from the National Institute of Standards and Technology (NIST; Gaithersburg, MD). Serum and milk pools were constructed at the beginning of the study from the same population of mothers and were analyzed with each sample batch. The CVs for the serum β-carotene (n = 44) and retinol (n = 25) were < 10%: 0.473 ± 0.47 μmol/L (CV: 9.9%) and 1.78 ± 0.12 μmol/L (CV: 6.9%), respectively. The CVs for the milk pool for β-carotene and retinol were 16.8% (23.6 ± 4.0 nmol/mL) and 9.7% (1.29 ± 0.13 nmol/L), respectively. 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. The analytic technique for analysis of serum carotenoids and retinol was verified quarterly in the NIST Round Robin Assay.

Statistical analyses

Descriptive statistics and correlations were performed with EXCEL 5.0 (Microsoft Corp, Redmond, WA). Linear-regression analyses were performed by using SAS software (SAS Institute, Cary, NC) (25).

RESULTS

Maternal characteristics pertinent to the study are shown in Table 1. Mothers were ± 36 y of age and had no more than two children. Weight, height, weight gain during pregnancy, body fat measurements (26), milk volumes, and lipid composition were in the normal range for this population (27). Daily intake of β-carotene and the other carotenoids was extremely

TABLE 1

Anthropometric and biochemical characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>28.7 ± 3.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.4 ± 3.9</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.6 ± 1.6</td>
</tr>
<tr>
<td>Parity</td>
<td>1.8 ± 1.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Prepregnancy</td>
<td>60.5 ± 10.4</td>
</tr>
<tr>
<td>At parturition</td>
<td>76.3 ± 10.7</td>
</tr>
<tr>
<td>D 1 of study</td>
<td>62.7 ± 11.2</td>
</tr>
<tr>
<td>Days of lactation</td>
<td>176.3 ± 101.4</td>
</tr>
<tr>
<td>Average milk volume per breast-feeding (mL)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>52.0 ± 22.8</td>
</tr>
<tr>
<td>Milk lipid (g/L)</td>
<td>59 ± 17</td>
</tr>
<tr>
<td>Body fat (% by wt)</td>
<td>23.9 ± 7.7</td>
</tr>
</tbody>
</table>

<sup>2</sup> Average of the full expression of a single breast five to seven times over 24 h as described in Subjects and Methods.
variable, but during the study mothers received ≥ 100% of recommended dietary allowances for all nutrients surveyed (Table 2).

Initial serum and milk concentrations of carotenoids are shown in Table 3 and those of retinol and α-tocopherol are shown in Table 4. To estimate initial concentrations of carotenoids, retinol, and α-tocopherol in serum, two blood samples were obtained—one on the day before the supplement was administered (day 0) and one immediately before the supplement was administered with the morning meal (day 1). Samples were analyzed separately and results averaged to provide initial concentrations. Twenty-four-hour milk concentrations were estimated as the mean of two midafternoon samples on 2 successive days immediately before the day on which the supplements were administered (day −1 and day 0, respectively). Each set of samples was analyzed separately and then averaged to provide a baseline value. Because milk retinol is present primarily as retinyl esters (28), milk was hydrolyzed overnight to free retinol before being analyzed.

When expressed as simple concentrations (nmol/L), mean initial concentrations of carotenoids, retinol, and α-tocopherol in milk of mothers receiving 60 mg β-carotene (group 1) were substantially lower than those receiving 210 mg β-carotene (group 2). However, when expressed relative to milk lipid, differences in carotenoids and retinol between the groups were not significant (Table 3). In contrast with the effects of milk lipid on retinol and carotenoid concentrations, differences in milk α-tocopherol concentrations between the two groups were not resolved by normalizing to lipid. Lipid concentrations were similar to those reported for others for mothers at 16 wk lactation (55 g/L) (27, 29).

Responses of serum carotenoid, retinol, and α-tocopherol concentrations to 60 or 210 mg β-carotene supplementation are shown in Figure 1. Of the carotenoids measured, only β-carotene increased significantly after supplementation (panels A and B). Although rank order was maintained during the study (panels C and D), substantial individual variation occurred in serum β-carotene responses. Except for two mothers in group 2 (2-4 and 2-6), the maximum increase in serum β-carotene was reached by 24 h after supplementation. The maximum increase in serum β-carotene was calculated for each mother and then averaged to give the group mean. The mean increase in response to a single 210-mg dose of β-carotene (fourfold, or 1.2 μmol/L) was similar to that in response to a single 60-mg dose (3.1-fold or 1.1 μmol/L). On the assumption that there was an approximate blood volume of 5 L (30), the mean recovery of the 60- and 210-mg doses in maternal serum of mothers (calculated at the maximum concentrations) was inversely related to the dose (5.1% and 1.5%, respectively). In both groups 1 and 2, mean serum β-carotene remained elevated over initial concentrations for 8 d after supplementation (1.6- and 1.8-fold, respectively), although in two mothers (1-2 and 1-6) concentrations had nearly returned to baseline by the end of the study. Serum retinol and α-tocopherol concentrations were not significantly different from initial concentrations in response to supplementation with either dose (panels E and F). No subject reported yellowing of skin or other adverse reactions.

Responses of milk carotenoids, retinol, and α-tocopherol to a single 60- or 210-mg β-carotene supplement are shown in Figure 2. Because milk samples were collected in the afternoon, there was an approximate 8-h lag between the collection of serum and milk samples (Figure 3). Individual variability was greater for β-carotene in milk than in serum. Increases were significant for β-carotene only. Similar to serum, maximum milk β-carotene concentrations were about four times initial concentrations. In contrast with serum, however, uptake into milk continued to increase until almost 80 h (3 d) after the supplement had been given. There was no apparent difference in the rate of decline of milk β-carotene concentrations after the 210-mg dose compared with the 60-mg dose (Figures 2 and 3). Eight days after supplementation with 60 or 210 mg β-carotene, mean milk β-carotene concentrations remained elevated relative to initial concentrations: 1.9-fold and 1.6-fold, respectively. As was the case for serum, rank order was maintained; in mothers whose milk β-carotene concentration was only slightly elevated in response to the supplementation (subjects 1-4, 1-6, 2-1, and 2-2), milk concentrations had nearly returned to baseline by the end of the study. All of these mothers began the study with below average serum and milk β-carotene concentrations. On the assumption that there was an average daily milk production of 0.75 L (24), the maximum fractions of the 60- and 210-mg doses reflected as an increase in milk were 0.08% and 0.02%, respectively, <20% of the increase in serum.

Initial and maximum concentrations of β-carotene in serum (panel A) and milk (panel B) were strongly correlated for individual mothers (Figure 4). When changes in serum β-carotene concentrations were compared with changes in milk β-carotene concentrations, increases in milk and serum β-carotene concentrations were weakly correlated (γ = 0.9x + 0.47, \( R^2 = 0.16 \)). In addition, because the slopes and intercepts of the regression lines for subjects receiving the 60- or 210-mg doses were not significantly different, changes in milk β-carotene concentrations were not dose-dependent.

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TABLE 2
Dietary intake of macro- and micronutrients

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Estimated daily intake †</th>
<th>RDA †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>11,704.1 ± 3183.3</td>
<td>11,340</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>95 ± 26</td>
<td>65</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>98 ± 28</td>
<td>NA</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>37 ± 13</td>
<td>NA</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>32 ± 6</td>
<td>30-40</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>26 ± 13</td>
<td>NA</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>2334.0 ± 750.3</td>
<td>1300</td>
</tr>
<tr>
<td>β-Carotene (µg)</td>
<td>5078 ± 2473</td>
<td>~3500†</td>
</tr>
<tr>
<td>α-Carotene (µg)</td>
<td>1303 ± 762</td>
<td>NA</td>
</tr>
<tr>
<td>Lutein (µg)</td>
<td>2754 ± 2600</td>
<td>NA</td>
</tr>
<tr>
<td>Lycopene (µg)</td>
<td>4551 ± 3447</td>
<td>NA</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>161 ± 57</td>
<td>95</td>
</tr>
<tr>
<td>α-Tocopherol (mg)</td>
<td>16 ± 7</td>
<td>12</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>484 ± 276</td>
<td>280</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>23 ± 15</td>
<td>15</td>
</tr>
</tbody>
</table>

† x ± SD. Average of three self-reported dietary records randomly obtained over the period of the study on 2 weekdays and 1 weekend day.

‡ Recommended dietary allowance (26). NA, not available.

† The daily intake that is correlated with a protective effect against cancer and atherosclerosis (12). No RDA exists.
TABLE 3
Initial serum and milk concentrations of carotenoids

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Serum³</th>
<th>Milk⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td>0.29 ± 0.01</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.18 ± 0.04</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.58 ± 0.03</td>
<td>0.76 ± 0.01</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.25 ± 0.01</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.75 ± 0.2</td>
<td>0.64 ± 0.2</td>
</tr>
</tbody>
</table>

¹ ± SE; n = 6. Group 1 received a 60-mg β-carotene supplement and group 2 a 210-mg supplement.
² Average of two samples, one collected 24 h before and the other collected immediately before administration of the supplement.
³ Average of two sampling periods on 2 successive days immediately before administration of the supplement.
⁴ Significantly different from group 1, P < 0.05.

DISCUSSION

To our knowledge this is the first report of the effects of β-carotene supplementation on concentrations of carotenoids, retinol, and α-tocopherol in human milk. As reported previously (15), the β-carotene concentrations in milk that we quantitated were much lower, approximately one-tenth of those reported by investigators using methods that did not differentiate among the carotenoids (typically absorbance at ~450 nm) (31, 32). As shown in Table 3, β-carotene accounts for only ~35% of the carotenoids that we quantitated in milk. In addition, as discussed previously (15), other carotenoids not eluted in our HPLC system as well as other related compounds contribute significantly to the absorbance at 450 nm in milk. Therefore, quantitation of β-carotene in milk requires HPLC or other methods that can differentiate the various carotenoids from each other and from other species in milk.

Collectively, the provitamin A carotenoids (β-carotene, α-carotene, and cryptoxanthin) account for more than half of the major milk carotenoids (Table 3). Thus, earlier concerns that human milk provides primarily provitamin A carotenoids (16) appear to be unfounded, at least in well-nourished US populations.

As reported previously (15), because carotenoids are enriched in the lipid fraction of milk, inconsistencies in collection techniques, particularly of hind milk, will result in spurious variations in carotenoid concentrations. As shown in Table 3, lutein/zeaxanthin and lycopene were significantly different between groups 1 and 2 when expressed as micromolar concentrations. However, when concentrations in nmol/L were divided by g lipid/L to give nmol/g lipid, differences were not significant between the two groups. Samples in group 2 were the first collected, and mothers were inexperienced with milk-collection techniques. When mothers were reconstituted in collection techniques, individual variation in subsequent samples decreased. This observation emphasizes the importance of proper sample collection for interpretation of milk composition data as discussed previously by others (29). Retinol concentrations were less related to lipid concentrations than were carotenoids (Table 4). Retinol is stable to lengthy hydrolysis procedures whereas carotenoids are not. Therefore, because of the shorter time of hydrolysis, unhydrolyzed lipid in the samples prepared for analysis of carotenoids contributes to the variability in the assay (33). As reported previously (34), milk α-tocopherol and lipid concentrations showed no apparent relation.

Given the substantial intra-individual variability in milk lipids, our average of 59 ± 17 g/L is probably not significantly different from that of Ferris et al (55 g/L at 16 wk) (35). However, milk lipid concentrations in our study were slightly higher than those reported in some other studies (36). Several factors may have contributed to this difference, including fluctuating dietary intakes, variable milk-collection techniques, technical variability in lipid analyses, and different stages of lactation. Although it was assumed previously that no changes in lipid concentration occur after 3 mo, this assumption now appears to be controversial. Clark et al (37) and Ferris et al (35) reported increases from 3.9% and 4.0% at 2 wk to 5.2% and 5.5% at 16 wk, respectively, in well-nourished US mothers. Similarly, Underwood et al (38) reported an increase from 2.8% at 6 wk to 4.6% at 24 mo in Pakistani women. Therefore, because most studies of milk lipid concentrations have been

TABLE 4
Initial serum and milk concentrations of α-tocopherol and retinol

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Serum³⁴</th>
<th>Milk⁵⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>28.94 ± 0.68</td>
<td>29.81 ± 2.51</td>
</tr>
<tr>
<td>Retinol</td>
<td>2.01 ± 0.15</td>
<td>1.80 ± 0.09</td>
</tr>
</tbody>
</table>

³ ± SE; group 1 received a 60-mg β-carotene supplement and group 2 a 210-mg supplement.
⁴ Average of two samples, one collected 24 h before and the other collected immediately before administration of the supplement.
⁵ Average of two sampling periods on 2 successive days immediately before administration of the supplement.
⁶ n = 6.
⁷ n = 3.
conducted at 16 wk or earlier, the longer average period of lactation of the mothers in this study (25 wk) may explain, at least in part, the somewhat higher lipid concentrations that we measured.

Serum \( \beta \)-carotene concentrations were higher in our subjects (0.64–0.75 \( \mu \)mol/L; Table 3) than in normal, nonlactating women in the same age group (0.4–0.6 \( \mu \)mol/L) (6, 9–12, 27, 39–41). Although the effects of lactation on mobilization of carotenoids from tissue stores cannot be ruled out, it is likely that the elevated serum carotenoids in our subjects were attributable to a higher than average dietary intake of foods enriched in vitamin A and provitamin A compounds. As shown in Table 2, the daily intake of \( \beta \)-carotene and the other carotenoids by our subjects was higher than that for normal adults consuming Western diets (3, 9). However, the ratio of serum \( \beta \)-carotene to reported \( \beta \)-carotene intake (\( \approx 10\% \)) was similar to that based on data collected from a different population (9) and is consistent with the high consumption of \( \beta \)-carotene reported by our subjects.

Normative values for vitamin A and carotenoids in the milk of US mothers are not available, and the sample size is insufficient to estimate precisely the milk retinol concentrations of this population. However, milk retinol concentrations were similar to those reported previously in mature milk of normal well-nourished mothers (4, 32). As noted earlier (15), retinol concentrations were approximately the same in serum and milk, whereas milk \( \alpha \)-tocopherol and carotenoid concentrations were an order of magnitude lower. This finding suggests a different mechanism for transfer of \( \alpha \)-tocopherol and the carotenoids from serum to milk than for retinol. On the basis of the results of animal studies, it has been proposed that retinol is delivered to the mammary gland on retinol binding protein and taken into the cell via a receptor-mediated mechanism (42, 43).

In contrast with retinol, carotenoids and vitamin E are concen-
trated in plasma lipoproteins (43, 44) and therefore presumably are delivered via a lipoprotein receptor; however, the mechanism by which carotenoids are transferred into the mammary gland is not understood.

Serum and milk β-carotene concentrations for individual mothers increased three- to fourfold after supplementation (Figures 1 and 2). The increase in serum β-carotene concentrations 24 h after a single 60-mg dose was comparable with that reported by others for adults consuming a single 30- to 50-mg β-carotene supplement (7, 8, 11). The maximum increase in serum β-carotene was reached by the second sampling period postsupplementation. By 4 d after supplementation with either 60 or 210 mg β-carotene, both serum and milk β-carotene concentrations remained significantly elevated (Figures 2 and 3) and after 8 d had declined only to about twofold initial concentrations. Therefore, a single 60-mg β-carotene supplement will maintain elevated serum β-carotene for 1 wk in normal lactating mothers, and supplementation every 3–4 d should maintain maximum concentrations. Increases in milk β-carotene concentrations paralleled those in serum for both groups (Figure 3). Increases in serum and milk were correlated in both group 1 ($r = 0.32$) and group 2

**FIGURE 2.** Response of milk carotenoids, retinol, and α-tocopherol in lactating women to a single 60-mg (group 1; $n = 6$) or 210-mg (group 2; $n = 6$ except for panel F, for which $n = 3$) dose of β-carotene. Panels A, B, E, and F give means ± SEs; panels C and D give individual responses. Each data point represents the average of estimated 24-h concentrations, determined from afternoon collections on 2 separate days as described in Methods. Arrows indicate the day of supplementation. Data for subject 2-4 were collected on days 1–4 and extrapolated from days 4–9. Panels A and B: ■, β-carotene; ●, lycopene; ○, lutein/zeaxanthin; □, α-carotene; ○, β-cryptoxanthin. Panel C: ⊳, 1–1; ■, 1–2; ▲, 1–3; □, 1–4; ○, 2–4; ●, 2–6. Panel D: ⊳, 2–1; ■, 2–2; ▲, 2–3; □, 2–4; ○, 2–5; ●, 2–6. Panels E and F: ■, α-tocopherol; ◇, retinol.
A

FIGURE 3. Kinetics of β-carotene uptake into serum (µmol/L) and milk (nmol/g lipid) after a single 60-mg (A) or 210-mg (B) dose of β-carotene.

(r = 0.32) and were described by the equation y = 0.9x, where x represents increases in serum concentrations. β-Carotene concentrations remained at or near maximum concentrations for ≈4 d in both serum and milk and declined to about twofold initial concentrations after 8 d. The rate of decline in serum β-carotene concentrations was slightly faster after the 60-mg dose. These data suggest that a more sustained elevation of serum concentrations may be achieved with high doses; however, larger sample sizes are required to confirm this hypothesis. The rate of decline in β-carotene concentrations was not significantly different between the two doses.

In agreement with our earlier studies of β-carotene absorption in children after a single dose of 15 or 30 mg β-carotene (19), increases in serum β-carotene were not proportional to dose (Figures 1 and 3). Thus, β-carotene doses > 60 mg are unlikely to result in substantially increased initial serum or milk concentrations in well-nourished lactating mothers, and, on the basis of our earlier studies and those of others (45), 30 mg may be near the optimal dose. Consistent with the lack of a dose-response effect, recovery of the 60- and 210-mg doses in the serum of mothers was inversely related to the dose (5.1% and 1.5%, respectively). These results agree with those of earlier studies (46), which showed that absorption of β-carotene decreases with increasing doses. The increases in milk β-carotene concentrations after the 60- and 210-mg doses were < 20% of those in serum, in agreement with the ≈20-fold lower concentrations in milk than in serum.

Mothers who entered the study with lower β-carotene concentrations in serum, milk, or both had respectively lower concentrations after supplementation (Figures 1, 2, and 4). Thus, as reported previously by us and others (10, 12, 47), rank order of subjects was maintained for β-carotene concentrations. This finding suggests an individual “set point” not strictly dependent on dose, which regulates the amount of β-carotene absorbed into serum, and is consistent with the observation that many individuals have a minimal response to β-carotene supplementation regardless of previous dietary intake (48).

As reported by others (48), a single β-carotene supplement at either dose (60 or 210 mg) did not affect concentrations of the other major serum or milk carotenoids derived from the diet. However, when pharmacologic doses of β-carotene and lutein (~15 mg each) were administered simultaneously, there were significant interactions between the two carotenoids (49). In addition, rats given high doses of canthaxanthin absorbed less β-carotene (46). Taken together, these results support the hypothesis that carotenoids are absorbed differently when administered in physiologic rather than in pharmacologic doses (50).

In agreement with others (8), we observed no effect on serum retinol or α-tocopherol concentrations in response to a single dose of β-carotene.

In summary, supplementation of mothers with a single 60-mg dose of β-carotene substantially increased the amount of β-carotene in the serum and milk of well-nourished lactating mothers by 2–3 d. At doses of 60 and 210 mg, neither the serum nor milk β-carotene response was dose-dependent. A
single 60-mg dose of β-carotene substantially elevated both serum and milk β-carotene concentrations for 3–4 d and the 210-mg dose produced a similar increase.

β-Carotene supplementation did not affect concentrations of other major carotenoids, α-tocopherol, or retinol in serum or milk. Substantial individual variation occurred in serum and milk carotenoid concentrations, both initially and in response to supplementation, with the greater variation in milk carotenoids. Initial serum and milk β-carotene concentrations predicted changes in β-carotene concentrations after supplementation. Using linear-regression analysis, we derived a simple equation that may be useful to predict changes in milk β-carotene concentrations relative to incremental changes in serum.

As shown in Table 3, concentrations of the provitamin A carotenoids (β-carotene, β-cryptoxanthin, and α-carotene) were significantly lower than those of milk retinol in well-nourished mothers. Therefore, in this population, carotenoids furnished only a fraction of the vitamin A supply for the infant. However, for mothers consuming a typical Western diet, as much as half the total vitamin A intake may be from dietary carotenoids (46). Therefore, the contribution of dietary carotenoids to milk and serum retinol concentrations is nutritionally significant even in well-nourished populations and becomes even more important in populations in whom the diet is largely vegetarian. Our findings that β-carotene supplementation did not affect milk retinol concentrations is consistent with the observation that milk retinol concentrations are not increased by supplementation with retinol in well-nourished populations (4). However, before we conducted this study it was not known whether β-carotene could be substantially increased in the milk of normal women by supplementation. Because β-carotene provides a source of vitamin A when needed (46, 51), it is now of great interest to determine whether mothers consuming a diet low in vitamin A have increased serum or milk retinol concentrations or both after supplementation with β-carotene. If so, provitamin A carotenoids have the potential to improve significantly the retinol status of these mothers and their infants. Studies are underway in our laboratory to test this hypothesis.

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