Serum Carotenoid Depletion Follows First-Order Kinetics in Healthy Adult Women Fed Naturally Low Carotenoid Diets

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ABSTRACT Dietary intakes of carotenoids are highly variable in human populations as are serum carotenoid concentrations. However, there are few controlled data relating carotenoid intake to concentration. Most of the data that are available are from measurements of the absorption and decay of large pharmacologic doses of carotenoids, and are therefore of unknown physiologic relevance. Our objective was to determine the half-life (t1/2) of the most abundant carotenoids in blood serum from healthy adult women living under controlled conditions. As part of two carotenoid isotopic studies, we measured serum concentrations of β-carotene, α-carotene, lutein, zeaxanthin, β-cryptoxanthin and lycopene in 19 healthy young adult women that were fed controlled low carotenoid diets for ~10 wk. All other nutrients (vitamins A, E and C) were provided at 100–150% of the 1989 U.S. recommended dietary allowance levels. Exercise and activities were controlled throughout the studies to simulate usual activity patterns. Carotenoid concentrations were measured by reversed-phase HPLC. Serum carotenoid concentration decreases during depletion followed first-order kinetics. The half-lives determined in decreasing order were as follows: lutein (76 d) > α-carotene (45 d) = β-cryptoxanthin (39 d) = zeaxanthin (38 d) = β-carotene (37 d) > lycopene (26 d). Half-lives were unrelated to physical or demographic characteristics such as body mass, body fat, racial background or age in these relatively homogeneous groups. Carotenoids decreased by similar first-order mechanisms, although the rates differed for individual carotenoids. J. Nutr. 131: 2096–2100, 2001.

KEY WORDS: • carotenoid • depletion • half-life • humans • metabolism

Carotenoids are fat-soluble pigments found in fruits and vegetables (1–4). There are over 700 known carotenoids, but only a few are in human tissues in easily measurable quantities. The major carotenoids in human blood include β-carotene, lycopene, lutein, α-carotene, β-cryptoxanthin and zeaxanthin. These carotenoids share many characteristics, including extensive conjugated double-bond systems, bright color and their role as singlet oxygen quenchers. However, they differ in structure, hue and their ability to form vitamin A (1–4).

Carotenoids have a variety of functions that may be of importance to human health. β-Carotene, β-cryptoxanthin and α-carotene can form vitamin A in the human body (1–5). In fact, most of the people of the world depend on carotenoids as the major source of this essential nutrient. Second, many carotenoids such as β-carotene and lycopene appear to be physiologically important antioxidants (6–8). The antioxidant defense system is crucial to human health because oxidative damage has been implicated in the etiology of cancer (9–11), arteriosclerosis (12,13) and degenerative diseases such as cataract (14,15). Third, several carotenoids, especially β-carotene, have been associated with immunologic activity in many, but not all studies (16–19). Fourth, several carotenoids appear to have unique functions. β-Carotene is seques-
tered in the corpus luteum of many animals, where it appears to influence reproductive function (20). Lutein and zeaxanthin are concentrated in the macular pigments of the eye, where they form a pigmented spot that might be related to visual acuity (21).

Studies of carotenoid depletion in general, and determining the rate of carotenoid depletion in particular, are important for identifying critical carotenoid functions. Currently, we do not know whether the functions of carotenoids are essential to human health. Although many studies suggest that carotenoids are useful for preserving life and health, we do not know whether they have specific roles in human health preservation or whether they could be replaced by a variety of other nutrients. For example, other antioxidants such as vitamins C and E might be able to replace the antioxidant functions of β-carotene and lycopene. One reason for this uncertainty is that most human experimental studies have been done by feeding carotenoid supplements in pharmacologic dosages to individuals at increased risk for disease (smokers, former smokers, asbestos workers), typically individuals that are well fed (22–24). When these studies show no beneficial effects and possibly harmful effects, it may seem that the carotenoids have no independent, important function related to human health.
However, carotenoids might have important physiologic functions that are not observed because the system is already saturated with the carotenoid of interest. Carotenoid depletion studies should provide a clearer picture of whether carotenoids have independent functions that are useful to human health.

Human nutrition studies are expensive and potentially harmful to the subjects studied. Therefore, we should do them as efficiently and effectively as possible. To conduct carotenoid depletion studies efficiently, it is necessary to know the time course of carotenoid depletion. In this paper, we present data from two carefully controlled carotenoid depletion studies in adult women and report the half-lives (t1/2) of the six major carotenoids in human serum (β-carotene, α-carotene, lutein, lycopene, zeaxanthin and β-cryptoxanthin). We also investigated the influence of physical and demographic characteristics (such as age, body weight and race) on carotenoid t1/2.

### MATERIALS AND METHODS

#### Study design and subjects.
Details of the study protocols and diets have been reported elsewhere (7,17,19,25–29). Study 1 was a simple carotenoid depletion study in which all subjects followed the same diet plan shown in Table 1 (7,17,25,26). Nine 18- to 42-y-old healthy women with a body mass of 60.5 ± 3.3 kg and body fat of 29.5 ± 6.5 g/100 g lived at the metabolic research unit of the USDA, Western Human Nutrition Research Center (WHNRC) in the summer of 1992 (Table 1). They were fed a 4-d rotational diet of natural foods that was supplemented to contain 100–150% of the United States recommended dietary allowances (RDA; (30)) for all established nutrients, but was low in carotenoids (β-carotene, a- and β-cryptoxanthin). We also investigated the influence of physical and demographic characteristics (such as age, body weight and race) on carotenoid t1/2.

#### Study 2.
Study 2 differed from study 1 in three important aspects. First, the marginal carotenoid diet used in study 2 provided about twice as much β-carotene as the low carotenoid diet used in study 1 (0.15 vs. 0.07 mg/d). Second, it was a double-blind, placebo-controlled study (19,27–29) in 10 healthy women (Table 1). All subjects were fed a marginal carotenoid diet throughout. The control group (n = 5) was supplemented with 0.5 mg β-carotene for a total of 0.65 mg/d (Dry Carotene Beadlets, lot 014240, Roche Diagnostics) for the first 21 d (for a total of 0.65 mg/d). Third, all subjects received an oral bolus of 20 mg β-carotene-d8 (Cambridge Isotope, Boston, MA) with breakfast on d 1 of the study to investigate the metabolic behavior of β-carotene (Table 1; (27–29)). Because the large bolus of β-carotene-d8 prevented serum α- and β-carotene concentrations from declining, we were unable to determine the half-lives of α- or β-carotene in study 2. Subjects in study 2 were 23–43 y old, had a body weight of 70.1 ± 14.8 kg and body fat of 33.7 ± 8.2 g/100 g. They lived on the WHNRC metabolic unit during the winter of 1994 (see Table 1). The women were fed a 6-d rotational diet supplemented with nutrients as in study 1. The diet contained 55% of energy as carbohydrate, 14% as protein and 33% as fat. The P/S ratio was 0.8.

During each study, food intakes and activity patterns for each participant were recorded for 8 d and used to calculate energy amounts that maintained body weights. We made diet composition estimates using the nutrient database compiled from the tape versions of revised USDA Handbook number 8 sections 1–15 (31).

The subjects participated in a controlled nonsedentary exercise program with activity level chosen to avoid significant changes in body composition or oxygen consumption capacity throughout the study. Body weights were measured in the same clothing each day just after awakening and voiding. We estimated fat-free body mass by total body electrical conductivity. Oxygen consumption (VO2 resting) was measured using an automated collection system 2900 Metabolic CART (SensorMedics, Anaheim, CA). Maximal oxygen consumption (VO2 max) was predicted using the Astrand-Rhyming Bicycle test (32). The Human Subjects Review Committees of the USDA and the University of California, Davis, approved the protocols for each study. All subjects gave their informed consent for all procedures.

#### Serum analysis.
Blood was collected from fasting subjects in random order between 0700 and 0815 h on each collection day. Blood was protected from light with aluminum foil, put in an ice bucket and processed within 3 h. Serum was stored at −70°C until shipment to the Centers for Disease Control and Prevention or use. Serum carotenoid concentrations were measured repeatedly during each study. Carotenoids and vitamin A were measured twice during the baseline period in study 1 and six times during depletion (Table 1). Carotenoid concentrations were measured twice during the baseline period in study 1 and six times during depletion (Table 1). Carotenoid concentrations were measured twice during the baseline period in study 1 and six times during depletion (Table 1). Carotenoid concentrations were measured twice during the baseline period in study 1 and six times during depletion (Table 1).

**TABLE 1**

<table>
<thead>
<tr>
<th>Study period</th>
<th>Group</th>
<th>Study days</th>
<th>Blood draw days</th>
<th>Supplemented β-carotene, mg/d</th>
<th>Total dietary carotenoids, mg/d</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Baseline</td>
<td>1–4</td>
<td>1, 3</td>
<td>1.5</td>
<td>1.57</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Depletion</td>
<td>5–72</td>
<td>29, 36, 43, 51, 64, 71</td>
<td>0</td>
<td>0.07</td>
<td>9</td>
</tr>
<tr>
<td>Study 2</td>
<td>First</td>
<td>1–60</td>
<td>2, 3, 9, 23</td>
<td>0</td>
<td>0.15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>1–60</td>
<td>37, 46, 60</td>
<td>0.5</td>
<td>0.65</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>61–81</td>
<td>61, 62, 67, 74, 81</td>
<td>0.5</td>
<td>0.65</td>
<td>10</td>
</tr>
</tbody>
</table>

**Abbreviations used:** P/S, polyunsaturated/saturated fat; RDA, recommended dietary allowances; t1/2, half-life; VO2 resting, resting oxygen consumption; VO2 max, maximal oxygen consumption; WHNRC, Western Human Nutrition Research Center.
Differences of with SAS version 6.03 (Statistical Analysis System, Cary, NC). Presented as means in t1/2 between different carotenoids were evaluated using a paired t test. Differences in t1/2 between different carotenoids were evaluated using a paired t test.

Correlations for serum carotenoids were also constructed (Table 2). Correlations between serum carotenoid half-lives and demographic and physical characteristics (age, race, body weight, percentage of body fat, lean body mass and VO2 max) were calculated with SAS version 6.03 (Statistical Analysis System, Cary, NC). Differences of P < 0.05 were considered significant. Results are presented as means ± SEM.

RESULTS

Body weights, body composition (fat-free mass, percentage of fat) and oxygen consumption (VO2 resting and VO2 max) did not change significantly during these studies. Neither did serum concentrations of vitamin A, which remained in the normal range throughout both studies. Total cholesterol and triglycerides did not differ between studies or groups. Although there were small decreases with time for cholesterol and triglycerides in both studies (~10%), this trend was probably not physiologically important (data not shown). Serum vitamin A concentrations in study 1 did not change, but varied from 1.6 ± 0.2 to 1.7 ± 0.2 μmol/L. Serum vitamin A concentrations in study 2 also did not change, but varied from 1.8 ± 0.2 to 2.2 ± 0.2 μmol/L. Demographic and physical characteristics of subjects participating in study 1 vs. study 2 did not differ, although each study used different subjects.

Demographic and physiologic characteristics (age, ethnic background, body weight, percentage of fat and percentage of lean body mass) did not influence carotenoid kinetics. Cholesterol and triglyceride concentrations did not correlate with carotenoid concentrations or with carotenoid half-lives. Lycopene and lutein concentrations correlated inversely with maximal oxygen consumption initially (r > -0.65, P = 0.04). However, these correlations were not significant at the end of the studies. No other carotenoid concentrations were correlated with oxygen consumption at any time.

Figure 1 shows the decrease in serum carotenoid concentrations for subjects in studies 1 (upper panel) and 2 (lower panel). Not surprisingly, serum carotenoid concentrations decreased significantly during carotenoid depletion. Changes in individual carotenoid concentrations correlated strongly (r > 0.85, P < 0.01) with initial carotenoid concentration for all carotenoids measured. Initial and final β-carotene concentrations also correlated strongly with vitamin A status (estimated by stable isotope dilution) in study 1 (r = 0.80, P = 0.008). Vitamin A status was not estimated by stable isotope dilution in study 2. No other carotenoid concentrations correlated with vitamin A status.

The decreased concentration in one carotenoid generally correlated with decreased concentrations in other carotenoids (Table 2). For example, the decrease in lycopene in study 1 was highly correlated with the decrease in β-carotene, and with cryptoxanthin in both study 1 (r = 0.865) and study 2 (r = 0.659). Not surprisingly, lutein and zeaxanthin decreases were also highly correlated with one another (Table 2).

Four main points emerge from the present studies. First, the decline in serum concentrations of all carotenoids occurred slowly and followed apparent first-order kinetics. Second, kinetic data from studies 1 and 2 were in agreement. The half-lives for lycopene, β-cryptoxanthin, and lutein + zeaxanthin in study 1 were comparable to those of study 2 (Fig. 1, Table 3). Third, the t1/2 for several individual carotenoids differed. Half-lives in decreasing order were as follows: lutein (76 d) > α-carotene (45 d) = β-cryptoxanthin (39 d) = zeaxanthin (38 d) = β-carotene (37 d) > lycopene (26 d) (Table 3). Fourth, changes in serum carotenoid concentrations were not influenced significantly by age, race or body composition (height, weight, fat-free mass, and percentage of fat) in either study (data not shown). However, it should be noted that

### TABLE 2

**Correlation matrices for serum carotenoid concentrations measured in healthy adult women fed otherwise nutritionally adequate low carotenoid diets**

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-Carotene</td>
<td>Lycopene</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.866</td>
<td>1.000</td>
</tr>
<tr>
<td>Lutein + Zeaxanthin</td>
<td>0.685</td>
<td>0.897</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.862</td>
<td>0.772</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lycopene</td>
<td>Lutein</td>
</tr>
<tr>
<td>Lycopene</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Lutein</td>
<td>0.542</td>
<td>1.000</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>0.547</td>
<td>0.842</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.669</td>
<td>—</td>
</tr>
</tbody>
</table>

1 All correlation coefficients are significant, P < 0.0001.
also possible that the differences in half-lives are real. It would not be very surprising if vitamin C depletion increased the rate of carotenoid depletion because vitamin C and carotenoids share antioxidant functions in the body. It is also possible that men metabolize carotenoids faster than women.

Race and ethnicity, and the relatively small variations of age, body weight and body composition, and oxygen consumption did not appear to have a substantial or consistent influence on the half-lives of carotenoids. Similarly, the relatively small differences in serum concentrations of triglycerides, total fat, total protein and cholesterol also did not correlate consistently with the rate or extent of carotenoid depletion (data not shown). Our results are consistent with some previous studies (36,37), but not others (38–40). Again, the differences between our results and previous studies may be due to methodological differences or to real differences in subject groups. The women in our studies were all healthy, young to middle-aged, normal weight women whose serum concentrations of triglycerides, fat, cholesterol and protein remained in a relatively narrow normal range (data not shown). Our study does not rule out the possibility that larger differences in age, body composition or blood chemistries influence carotenoid depletion rates significantly. However, it is possible that demographic and physiologic characteristics affect the kinetics of carotenoid absorption only, and do not influence carotenoid depletion half-lives.

Lycopene concentrations decreased faster than other carotenoids (Table 3). This may be related to its function. Lycopene appears to be a physiologically important antioxidant, the most powerful of the major carotenoid antioxidants (41). However, differences in depletion rates between different carotenoid species were relatively minor. The carotenoid with the fastest metabolism decreased only two to three times as fast as the carotenoid with the slowest metabolism. Furthermore, all carotenoids showed first-order kinetic curve structures, and thus appeared to decrease by similar mechanisms. This suggests that specific carotenoid properties (such as relative antioxidant activity or the ability to form vitamin A) might have only moderate importance for determining carotenoid depletion rates.

**FIGURE 1** Concentrations of serum carotenoids in healthy adult women fed carotenoid depletion diets in studies 1 (upper panel) and 2 (lower panel). Values are means ± SEM, n = 9 or 10.

**DISCUSSION**

There is almost no information on the kinetics of carotenoid depletion in humans. Only a single report of carotenoid concentrations during carotenoid depletion has been published; that study measured plasma kinetics in young men fed a diet low in both vitamin C and carotenoids (35). Rock et al. (35) showed similar nonlinear declines in carotenoid concentrations, but reported half-lives of <12 d for β-carotene, α-carotene and β-cryptoxanthin, between 12 and 33 d for lycopene, and between 33 and 61 d for zeaxanthin/lutein. Thus, their half-lives for lycopene, lutein, and zeaxanthin were similar to ours, but they estimated shorter half-lives for β-carotene, α-carotene and β-cryptoxanthin.

There are two possible reasons for these differences in reported rates, i.e., methodological artifacts and real kinetic differences. Methodological artifacts are possible because the carotenoid measurements of Rock et al. were added to a study that was designed primarily to measure the effects of vitamin C deficiency. In contrast, our studies were designed to investigate carotenoid depletion kinetics, and all other nutrients were provided at U.S. RDA levels. We also were able to make measurements at more appropriate time points. However, it is that the ranges of these variables were relatively small and did not change throughout the studies.

**TABLE 3**

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene</td>
<td>37 ± 5b</td>
<td>Not measured³</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>45 ± 7ab</td>
<td>Not measured³</td>
</tr>
<tr>
<td>Lycopene</td>
<td>27 ± 2c</td>
<td>26 ± 2c</td>
</tr>
<tr>
<td>Lutein</td>
<td>—4</td>
<td>76 ± 17a</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>—4</td>
<td>38 ± 7b</td>
</tr>
<tr>
<td>Lutein + Zeaxanthin</td>
<td>66 ± 10a</td>
<td>67 ± 15a</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>40 ± 5b</td>
<td>39 ± 4b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM.
2 Within each experiment, means not sharing a superscript letter differ, P < 0.01.
3 Subjects in study 2 received an oral dose of 37 μmol β-carotene-d₈ on d 1 of that study. This dose precluded measurement of the half-lives of β-carotene and α-carotene in study 2.

4 Lutein and zeaxanthin comigrated in the method used for study 1, and the time for that single peak is shown as Lutein + Zeaxanthin.
Our results show that all carotenoids are depleted significantly and substantially within weeks when women are fed low carotenoid diets. These transient carotenoid depletions could have serious consequences because carotenoid depletion increases indices of oxidative damage (7,25–28), possibly increasing the risk of cancer and heart disease. Our results should provide the groundwork for further studies of carotenoid depletion kinetics and for assessing the potential physiologic importance of transient carotenoid depletion.

ACKNOWLEDGMENTS

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LITERATURE CITED