Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture

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ABSTRACT Plasma carotenoid concentrations were determined by HPLC in 11 individuals consuming low-carotenoid diets and after taking a carotenoid supplement. Subjects first consumed low-carotenoid diets for 2 wk, then supplemented these diets daily with 8.5 mg β-carotene, 3.5 mg α-carotene, and 0.5 mg lycopene, from natural sources for 4 wk. Serum cholesterol, triglycerides, and lipoproteins were determined before and after supplementation. After 2 wk on the low-carotenoid diet, plasma concentrations of the three carotenoids fell to \( \approx 60\% \) of baseline values. One week after supplementation, α- and β-carotene concentrations returned to baseline and by the end of the supplementation period they were significantly higher than baseline values (\( P < 0.05 \)). Lycopene concentrations increased only slightly. Serum lipids did not change significantly. Overall, plasma concentrations of these carotenoids reflect the amount provided by the supplement. This is the first study reporting increments of serum carotenoids, other than β-carotene, after supplementation. Am J Clin Nutr 1994;59:896–9.

KEY WORDS Carotenoids, β-carotene, α-carotene, lycopene, plasma concentrations, supplementation, blood lipids

Introduction

Carotenoids are the most numerous and widespread group of pigments in nature. They play a crucial role as electron-transport agents in photosynthesis and also protect plants from the damaging action of singlet oxygen and other oxygen radicals (1). Carotenoids are also indispensable cellular components in both animals and humans. Experimental studies indicate that they enhance the immune system (2), inhibit mutagenesis in bacterial systems (3), reduce chemically induced neoplasia (4–6), protect tissues from ultraviolet (UV) light–related damage (7, 8), and prevent malignant transformation of cell cultures (9). Epidemiological studies of the relationship between diet and cancer show that consumption of large amounts of fruits and vegetables lowers the risk of developing many types of cancer (10). A protective effect of carotenoids has been proposed to explain these findings (11–13). Recent studies measuring plasma carotenoid concentrations by HPLC techniques tend to confirm this hypothesis. Both prospective (14, 15) and case-control studies (16) show that plasma concentrations of carotenoids are associated with a lower risk of certain epithelial cancers. Clinical trials are underway to test whether supplementation with purified β-carotene may reduce future cancer rates. Purified β-carotene raises plasma concentrations of β-carotene; however, only carotenoid-containing foods increase concentrations of other individual carotenoids (17). The plasma response to supplements containing β-carotene along with other carotenoids has not been established. In this study we determined changes in plasma β-carotene, α-carotene, and lycopene in response to a daily supplement of carotenoids from a carotenoid mixture in free-living volunteers on a low-carotenoid diet.

Subjects and methods

Eleven healthy men and women volunteers between 22 and 52 y of age participated in this study. They had no history of chronic disease, were not taking medication, and did not smoke. They were within 10% of ideal body weight for their size and had no particular dietary pattern (eg, vegetarianism). Those volunteers who had been taking β-carotene–containing supplements were advised to stop doing so 2 wk before the study began. All subjects were evaluated (weight, blood pressure, pulse, and temperature) at a local clinic at the beginning of the study, 2 wk later, and weekly thereafter. The participants entered the study after signing an appropriate informed-consent form. The study was conducted in accord with the Helsinki Declaration of 1975 as revised in 1983.

The study lasted 6 wk. During this time the volunteers ate a self-selected diet low in carotenoids. Before starting the study the volunteers received detailed instructions about the dietary modifications they had to make. They were given a list of fruits and vegetables to avoid and those that they could eat (Table 1). We estimated that the recommended diet would provide < 0.4 mg β-carotene and α-carotene and no lycopene. They were told to follow their usual diet in every other respect. After 2 wk on this low-carotenoid diet, they began taking six capsules daily of a fruit and vegetable concentrate that provided a total dose of 8.5 mg β-carotene, 3.5 mg α-carotene, and 0.5 mg lycopene. The ingredients providing most of the carotenoids in the mixture were carrot oil, red bell pepper oil, spinach, and tomato concentrate. These were made into a paste with additional olive oil and encapsulated by using 4 mg \( d\alpha\)-tocopherol as an antioxidant. Each capsule made negligible contributions of energy (24 kJ), fiber, and other nutrients to the volunteer’s daily diet. Supplementation

1 From the Neo-Life Company of America, Fremont, CA.
2 Supported by the Neo-Life Company of America, Fremont, CA.
3 Address reprint requests to A Carugh, Neo-Life Company of America, PO Box 5012, 3500 Gateway Boulevard, Fremont, CA 94537-5012.

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### Table 1
List of foods that participants could or could not consume during the study period

<table>
<thead>
<tr>
<th>Fruits and juices to avoid</th>
<th>Fruits that can be eaten</th>
<th>Vegetables to avoid</th>
<th>Vegetables that can be eaten</th>
<th>Other foods to avoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricots</td>
<td>Apples</td>
<td>Asparagus tips</td>
<td>Artichokes</td>
<td>Spaghetti sauce</td>
</tr>
<tr>
<td>Cantaloupes</td>
<td>Pineapples</td>
<td>Broccoli</td>
<td>Avocados</td>
<td>Pesto sauce</td>
</tr>
<tr>
<td>Canned fruit salad</td>
<td>Bananas</td>
<td>Brussels sprouts</td>
<td>Beans</td>
<td>Pizza</td>
</tr>
<tr>
<td>Cherries</td>
<td>Raisins</td>
<td>Bok choy</td>
<td>Bean sprouts</td>
<td>Chili</td>
</tr>
<tr>
<td>Pink grapefruits</td>
<td>Blackberries</td>
<td>Carrots</td>
<td>Bects</td>
<td>Hot chili oil</td>
</tr>
<tr>
<td>Guavas</td>
<td>Rhubarb</td>
<td>Dandelion leaves</td>
<td>Cabbages (red, white, and savoy)</td>
<td>Deep-orange cheeses</td>
</tr>
<tr>
<td>Mandarin oranges</td>
<td>Blueberries</td>
<td>Green beans</td>
<td>Celery</td>
<td></td>
</tr>
<tr>
<td>Mangoes</td>
<td>Raspberries</td>
<td>Greens (collard, turnip, mustard, and red and green leaf lettuce)</td>
<td>White corn</td>
<td></td>
</tr>
<tr>
<td>Nectarines</td>
<td>Boysenberries</td>
<td></td>
<td>Cucumbers</td>
<td></td>
</tr>
<tr>
<td>Oranges</td>
<td>Cranberries</td>
<td>Mixed frozen vegetables</td>
<td>iceberg lettuce</td>
<td></td>
</tr>
<tr>
<td>Papayas</td>
<td>Currants</td>
<td>Peas</td>
<td>Mushrooms</td>
<td></td>
</tr>
<tr>
<td>Passion fruits</td>
<td>Dates</td>
<td>Red peppers</td>
<td>Olives</td>
<td></td>
</tr>
<tr>
<td>Peaches</td>
<td>Figs</td>
<td>Hot peppers</td>
<td>Onions</td>
<td></td>
</tr>
<tr>
<td>Plantains</td>
<td>Gooseberries</td>
<td>Pimentos</td>
<td>Green peppers</td>
<td></td>
</tr>
<tr>
<td>Plums</td>
<td>Grapes (all types)</td>
<td>Canned pumpkin</td>
<td>Potatoes</td>
<td></td>
</tr>
<tr>
<td>Prunes</td>
<td>Yellow grapefruits</td>
<td>Squash (all orange/yellow types)</td>
<td>Zucchini squash</td>
<td></td>
</tr>
<tr>
<td>Strawberries</td>
<td>Honeydew melons</td>
<td>Spinach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tangerines</td>
<td>Kiwi fruits</td>
<td>Sweet potatoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watermelons</td>
<td>Lemons</td>
<td>Swiss chard</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pears</td>
<td>Tomatoes, all tomato products</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

continued for the next 4 wk. Subjects were instructed to take three capsules with lunch and three capsules with dinner. In addition, the volunteers received 200 mg vitamin C, 275 mg retinol, 1200 µg cholecalciferol, 10 µg vitamin D, and 10 mg magnesium. The volunteers were given the supplements daily for the entire study period. The supplements were taken 30 min before and 30 min after being drawn. Plasma samples were coded, wrapped in aluminum foil, and stored at −20 °C. At the completion of the study, the frozen samples were transported to SRI International (Menlo Park, CA) for analysis. Beta-carotene, α-carotene, and lycopene were analyzed by using the HPLC method of Biehl et al (19). The HPLC system used was model 1084B (Hewlett Packard, Kennett Square, PA). The detector was set at 464 nm and the mobile phase was 80:10:10 (by volume), acetonitrile:methylene chloride:methanol. The column used was a 4.6 mm × 25 cm Ultrasphere ODS (5 µm; Beckman Instruments, Inc, Fullerton, CA). Crystalline α-carotene, β-carotene, and lycopene (Sigma, St Louis) were used as standards, and echinencine (Hoffmann-La Roche, La Grange, NJ) was used as the internal standard. The researcher conducting carotenoid analyses was not aware of the dietary treatment.

**Statistical analyses**

Paired t tests were performed to evaluate differences in changes in serum lipid indexes before and after supplementation (20). Differences associated with $P < 0.05$ were regarded as statistically significant. A one-factor analysis of variance (ANOVA) with repeated measures was performed to evaluate differences in changes in plasma carotenoids between the sampling periods (19). When statistical differences were found, we followed the analysis with a Tukey Studentized range test with a procedure-wise error rate of 5% (19). Correlation coefficients ($r$) were calculated to describe the relationship between plasma concentrations of the different carotenoids throughout the study.

**Results**

Overall compliance with the guidelines of the study appeared to be high even though the participants were free-living. Unscheduled interviews revealed that participants were adhering to their diets and that supplements were being taken. Two partici-
pants missed one of their scheduled blood-collection appointments and five blood samples (week 5) had to be discarded because they had not been properly handled.

Table 2 shows age, body mass index (BMI), and serum lipids of the volunteers at the beginning and end of the supplementation period. BMI and blood lipid indexes were within normal ranges for both men and women. There were no significant changes due to supplementation.

Plasma carotenoid concentrations at baseline, before supplementation, and after supplementation are shown in Table 3. There was a large interindividual variation in plasma concentrations of β-carotene, α-carotene, and lycopene throughout the study. For example, at baseline, β-carotene concentrations ranged from 2008 to 136 nmol/L. After 2 wk on the low-carotenoid diet, plasma concentrations of all three carotenoids fell drastically. Mean β-carotene concentrations fell to 58% of baseline values; α-carotene and lycopene concentrations both fell to 59% of baseline values. Only the changes for lycopene were significant.

After 1 wk of supplementation the values for β-carotene and α-carotene were 109% and 130% of baseline, respectively. The concentrations kept increasing so that by the end of the study they were 168% and 216% of baseline, respectively. It is interesting to note that those individuals who had the lowest β- and α-carotene concentrations at baseline did not experience as large increments in plasma concentrations after supplementation as did those who started the study with high carotenoid concentrations. Despite uniform carotenoid intake during the supplementation period, interindividual variability of β- and α-carotene plasma concentrations at the end of the study was as large as at baseline. Plasma concentrations of these two carotenoids were closely correlated at baseline (r = 0.85) and at the end of supplementation (r = 0.93).

Mean plasma lycopene concentrations increased only slightly during the supplementation period. By the end of the study they were still significantly lower than at baseline. Interindividual variability in lycopene concentrations was smaller than for β- and α-carotene. Plasma lycopene concentrations were poorly correlated with plasma concentrations of other carotenoids, both at baseline and at the end of the supplementation period (r = 0.03 and r = 0.13, respectively, for β-carotene and 0.16 and 0.13, respectively, for α-carotene).

**Discussion**

This study shows that carotenoid intake can be assessed by measuring plasma concentrations of specific carotenoids. Plasma concentrations of β-carotene, α-carotene, and lycopene decreased to ≈60% of baseline values after the 2 wk of the low-carotenoid diet. Concentrations of β-carotene and α-carotene in the same individuals increased when the supplements were taken. Similar declines were shown by Rock et al (21) in individuals consuming a controlled diet providing < 0.4 mg total carotenoids. Because the volunteers were in charge of their diets, we could not calculate the exact amount of carotenoids they consumed. However, if participants followed our dietary instructions, we estimate that they were receiving < 0.4 mg β- and α-carotene from their diets. This estimate is consistent with the data.

Using HPLC methods investigators have reported mean plasma concentrations of β-carotene ranging from 290 to 1060 nmol/L in healthy individuals (19, 21–25). Reported mean α-carotene concentrations range from 80 to 120 nmol/L and lycopene concentrations from 550 to 1000 nmol/L (19, 21, 23, 25). Participants of our study had baseline plasma concentrations of β-carotene and α-carotene higher than those previously observed. One explanation for the higher plasma concentrations reported in this study could be that the volunteers participating in this study are health-conscious individuals. On the 1-d recall questionnaire taken before the study, 9 of the 11 participants consumed ≤ five servings of fruits and vegetables on that day. Before volunteering for the study, all of the participants were familiar with the terms β-carotene and carotenoids and they were aware of research associating ample consumption of dark green and yellow and orange fruits and vegetables with a lower risk of cancer. Our study also showed that mean plasma concentrations of lycopene were only slightly higher than those of β-carotene. Previous studies conducted on male volunteers have shown lycopene concentrations twice as high as β-carotene concentrations when individuals select their own diet (17, 21). This finding is another indication that our participants were consuming higher amounts of β- and α-carotene–rich foods than were participants of other studies.

There was a wide interindividual variability in plasma concentrations of the three carotenoids measured. Large interindividual variability in carotenoid plasma concentrations have been previously reported (22, 24). It is interesting to see that even when a diet designed to be uniform in carotenoid content is consumed, the variability in carotenoid concentrations was maintained, which indicates that the amount of carotenoids in the diet is not the only determinant of carotenoid plasma concentrations. The two individuals with the lowest β- and α-carotene concentrations at baseline responded only slightly to carotenoid supplementation. This could
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have been due to physiological differences leading to reduced bioavailability or to a coincidental lack of compliance. Previous studies have shown that certain individuals have low carotenoid plasma concentrations regardless of the diet (24).

There are different explanations as to why we did not see a significant difference in lycopene concentrations before and after supplementation. It is possible that the participants’ diets before the study had much higher amounts of lycopene than provided by the supplement. Lycopene supplementation, begun after 2 wk on a diet practically devoid of lycopene, could have just prevented further decline in plasma lycopene concentrations. Rock et al (21) showed, in subjects fed a low-carotenoid diet, that lycopene concentrations declined progressively over a 62-d period. In their study, lycopene plasma concentrations fell nearly as much from the third to the fifth week on the low-carotenoid diet as during the first 2 wk on this diet. The investigators suggest that lycopene may have a longer plasma half-life than do β- and α-carotene. Another explanation is that the supplement intake was simply too low to show significant increments in plasma concentrations. Micoczi et al (17) showed a significant mean maximum increment of 34 nmol/L from baseline after feeding 12 mg lycopene/d (from 180 mg tomato juice), for 6 wk to men consuming a low-carotenoid diet. At baseline the subjects had been on a self-selected diet providing lycopene. In our study mean lycopene plasma concentrations increased by 44 nmol/L from presupplementation concentrations. At this time the volunteers had been on a low-carotenoid diet providing no lycopene for 2 wk. Again, the difference was not significant.

The results of our study are consistent with others that show that carotenoid intake has a rapid and important influence on plasma concentrations. This finding indicates that consistent consumption of carotenoid-rich foods is necessary to sustain carotenoid plasma concentrations.

This is the first study to show plasma α-carotene response to a supplement. One of the main ingredients in the supplement is carrot oil. Micoczi et al (17) showed that 29 mg β-carotene and 9 mg α-carotene from 272 g carrots led to a mean maximum plasma increase of 1438 and 971 nmol/L, respectively. Because much greater increments were achieved with purified β-carotene, they suggest that supplementation of the diet with purified β-carotene may be more effective than major alterations to the human diet. However, the purified β-carotene supplements do not result in increments of other carotenoids found in foods. In our study we achieved a mean maximum plasma increase of 949 and 479 nmol/L from a supplement containing 8.5 mg β-carotene and 3.5 mg α-carotene, respectively. Our study thus provides an alternative to purified β-carotene supplementation. This is important because there are other carotenoids present in fruits and vegetables besides β-carotene, which may play a role in cancer prevention.

We acknowledge Arthur Furst and John Miller for continuous discussions and encouragement, Gordon Raskin (Washington Clinic, Fremont, CA) for coordinating collection of blood samples and clinical evaluation of the participants, and Chozo Mitoma and RG Gordon (SRI International, Menlo Park, CA), who conducted the analysis of the carotenoids in the plasma and in the supplements.

References