Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture

Arianna Carughi and Fred G Hooper

Reprinted from THE AMERICAN JOURNAL OF CLINCAL NUTRITION
April 1994 Volume 59 Number 4

Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture[1–3]

Arianna Carughi and Fred G Hooper

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 ≈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*-α-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 Carughi, Neo-Life Company of America, PO Box 5012, 3500 Gateway Boulevard, Fremont, CA 94537-5012.

Received January 14, 1993.

Accepted for publication October 11, 1993.

Am J Clin Nutr 1994;59:896–9. Printed in USA.

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

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

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 *d*-α-tocopherol, and a multivitamin-mineral supplement each day. The vitamin and mineral composition of the daily supplement was as follows: retinol, 1200 μg; cholecalciferol, 10 μg; *d*-α-tocopherol, 10 mg; ascorbic acid, 90 mg; folic acid, 0.4 mg; thiamin, 10 mg; riboflavin, 10 mg; niacin, 50 mg; pyridoxamine, 10 mg; cobalamin, 10 μg; pantothenic acid, 12 mg; iodine, 100 μg; iron, 25 mg; magnesium 35 mg; manganese, 10 mg; potassium, 10 mg; and copper, 2 mg.

A 1-d dietary-recall questionnaire was filled out by the volunteers before the study. To evaluate compliance with the diet throughout the study, we conducted unscheduled interviews with each volunteer during the supplementation period, and asked them to describe what they had eaten the previous day and to tell us, the following day, the number of capsules that they had left.

Blood samples after an overnight fast were collected at the beginning of the study (baseline), after 2 wk on the low-carotenoid diet, and weekly during the supplementation period. Fasting blood samples to determine values for high-density-lipoprotein (HDL) cholesterol, low-density-lipoprotein (LDL) cholesterol, very-low-density-lipoprotein (VLDL) cholesterol, total cholesterol, and triglycerides were collected at the beginning and at the end of the supplementation period. Serum cholesterol and triglycerides were measured by enzymatic methods with the Synchron CK Systems models CX4CE and CX7 (Beckman Instruments, Brea, CA). HDL cholesterol was measured by using the enzymatic assay after precipitation of VLDL and LDL with magnesium (2+) and phototungstate (HDL Cholesterol Reagent, Beckman). Serum concentrations of LDL cholesterol and VLDL cholesterol were calculated from total cholesterol, HDL-cholesterol, and triglyceride concentrations (18).

Blood for carotenoid analyses was collected into heparinized plastic syringes between 0730 and 1000. All samples were protected from light and centrifuged at 2260 $\times$ *g* at 12 °C within 1 h 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 Bieri 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 $\times$ 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 echinenone (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-

TABLE 2
Characteristics and biochemical indexes for the study participants*

	Men† (n = 4)		Women‡ (n = 7)	
	Before	After	Before	After
	mmol/L			
Triglycerides	1.50 ± 0.58	1.70 ± 0.30	0.73 ± 0.24	1.14 ± 0.20
Cholesterol	6.03 ± 0.58	5.51 ± 1.14	4.77 ± 0.54	4.77 ± 0.70
HDL cholesterol	0.93 ± 0.16	0.80 ± 0.18	1.42 ± 0.41	0.52 ± 0.09
VLDL cholesterol	0.70 ± 0.58	0.75 ± 0.16	0.33 ± 0.11	0.52 ± 0.09
LDL cholesterol	4.40 ± 1.06	4.03 ± 1.10	3.02 ± 0.30	3.02 ± 0.52

* $\bar{x}$ ± SD. There were no significant differences.
† Age 45.8 ± 4.7 y; BMI [wt (in kg)/stature^2 (mm)] 24.0 ± 1.8.
‡ Age 37.9 ± 8.4 y; BMI [wt (in kg)/stature^2 (in mm)] 21.5 ± 1.0.

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

TABLE 3
Plasma carotenoid concentrations in subjects on a low-carotenoid diet at baseline and before and after supplementation with a carotenoid complex*

	Baseline	Before supplementation	After supplementation
		nmol/L	
β-Carotene	860 ± 623	501 ± 344	1450 ± 873†‡
α-Carotene	307 ± 218	186 ± 121	665 ± 339†‡
Lycopene	896 ± 427	525 ± 300†	569 ± 299†

* $\bar{x}$ ± SD; n = 11.
† Significantly different from baseline, $P < 0.05$.
‡ Significantly different from before supplementation, $P < 0.05$.

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. Micozzi 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. Micozzi 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

1. Karnaukhov VN. Carotenoids: recent progress, problems and prospects. Comp Biochem Physiol 1990;95B:1–20.
2. Bendich A. Carotenoids and the immune response. J Nutr 1989;119:112–5.
3. Ong T, Whong WZ, Steward JD, Brockman HE. Comparative antimutagenicity of 5 compounds against 5 mutagenic complex mixtures in *Salmonella typhimurium* strain TA98. Mutat Res 1988;222:19–25.
4. Steinel HH, Baker RSU. Effects of β-carotene on chemically-induced skin tumors in HRA/SKH hairless mice. Cancer Lett 1990;51:163–8.
5. Lambert LA, Koch WH, Wayne G, Wamer WG, Kornhauser A. Antitumor activity in skin of SKH and Sencar mice by two dietary β-carotene formulations. Nutr Cancer 1990;13:213–21.
6. Alam BS, Alam SQ. The effect of different levels of dietary β-carotene on DMBA-induced salivary gland tumors. Nutr Cancer 1987;9:93–101.
7. Mathew-Roth MM. Carotenoid pigments and protection against photosensitization: new studies. Perspect Biol Med 1984;28:127–39.
8. Fuller CJ, Faulkner H, Bendich A, Parker RS, Roe DA. Effect of β-carotene supplementation on photosuppression of delayed-type hypersensitivity in normal young men. Am J Clin Nutr 1992;56:684–90.
9. Bertram JS, Pung A, Churley M, Kappock TJ, Wilkins LR, Cooney RV. Diverse carotenoids protect against chemically induced neoplastic transformation. Carcinogenesis 1991;12:671–8.
10. Ziegler RG. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 1989;119:116–22.
11. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 1981;290:201–8.
12. Ziegler RG, Subar AF, Craft NE, Ursin G, Patterson BH, Graubard BI. Does β-carotene explain why reduced cancer is associated with vegetable and fruit intake? Cancer Res 1992;52:2060S–6S.
13. Byers T, Perry G. Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. Annu Rev Nutr 1992;12:139–59.
14. Stahelin HB, Gey K, Eichholzer M, Ludin E. β-carotene and cancer prevention: the Basel Study. Am J Clin Nutr 1991;53:265S–9S.
15. Comstock GW, Helzlsouer KJ, Bush TL. Prediagnostic serum levels of carotenoids and vitamin E as related to subsequent cancer in Washington County, Maryland. Am J Clin Nutr 1991;53:260S–4S.
16. Smith AH, Waller KD. Serum beta-carotene in persons and their immediate families. Am J Epidemiol 1991;133:661–71.
17. Micozzi MS, Brown ED, Edwards BK, et al. Plasma carotenoid response to chronic intake of selected foods and β-carotene supplements in men. Am J Clin Nutr 1992;55:1120–5.
18. Friedwald WT, Levy RI, Fredrickson DC. Estimation of plasma low density lipoprotein cholesterol concentration without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
19. Bieri JG, Brown ED, Smith JC. Determination of individual carotenoids in human plasma by high performance liquid chromatography. J Liqu Chromatogr 1985;34:44–8.
20. Zar JH. Paired sample hypotheses. In: Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall, Inc, 1984:150–3.
21. Rock CL, Swendseid ME, Jacob RA, McKee RW. Plasma carotenoid levels in human subjects fed a low carotenoid diet. J Nutr 1992;122:96–100.
22. Tangney CC, Shekelle RB, Raynor W, Gale M, Betz EP. Intra- and interindividual variation in measurements of β-carotene, retinol and tocopherols in diet and plasma. Am J Clin Nutr 1987;45:764–9.
23. Brown ED, Micozzi MS, Craft NE, et al. Plasma carotenoids in normal men after a single ingestion of vegetables or purified β-carotene. Am J Clin Nutr 1989;49:1258–65.
24. Dimitrov NV, Meyer C, Ullrey DE, et al. Bioavailability of β-carotene in humans. Am J Clin Nutr 1988;48:298–304.
25. Cantilena LR, Stukel TA, Greenberg ER, Namm S, Nierenberg DW. Diurnal and seasonal variation of five carotenoids measured in human serum. Am J Clin Nutr 1992;55:659–63.