Effects of 4 y of oral supplementation with β-carotene on serum concentrations of retinol, tocopherol, and five carotenoids

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ABSTRACT β-Carotene has been studied widely as a potential cancer-preventive agent. Recent studies found that subjects who took β-carotene supplements orally had increases in their serum concentrations of α-carotene and lycopene that were large (>150% increase) and significantly greater than such increases in subjects who received placebo and that similar supplementation was associated with a decrease of ~37% in plasma lutein concentrations. A biologic interaction between β-carotene and other carotenoids was suggested. We measured concentrations of retinol, α-tocopherol, and five carotenoids in serum specimens from a random sample of subjects enrolled in a clinical trial of the use of antioxidant vitamins in preventing colon cancer. We used serum specimens obtained at enrollment and after the subjects took placebo (n = 54) or 25 mg β-carotene/d (n = 54) orally for 4 y. In a multivariate analysis, baseline serum concentrations of the analytes, sex, body mass index, diet, smoking status, and age were associated with variable changes in some analytes over the 4- to 5-year period but supplementation with β-carotene was related only to a mean increase in serum β-carotene itself of 151%. We excluded with 95% confidence an increase in lycopene >4.9% and an increase in α-carotene >17.6%, and a decrease in lutein >14.7% in subjects given β-carotene. These results confirm previous findings that supplementation with β-carotene given orally does not alter serum concentrations of retinol or α-tocopherol. The findings also indicate that β-carotene supplementation, which results in a moderate increase in serum β-carotene concentration, does not significantly change serum concentrations of other carotenoids. Am J Clin Nutr 1997;66:315–9.

KEY WORDS β-Carotene, α-carotene, lycopene, cryptoxanthin, lutein, zeaxanthin, retinol, α-tocopherol, supplementation

INTRODUCTION

β-Carotene, a naturally occurring carotenoid with both provitamin A and antioxidant properties, has been studied as a possible preventive agent for cancer and cardiovascular diseases (1–8). Some reports from clinical trials of this agent, however, raised concerns that supplementation with β-carotene given orally might increase blood concentrations of retinol (because β-carotene can be metabolized to retinol) or decrease serum concentrations of α-tocopherol (by unknown mechanisms) (9–11). In contrast, we (12) and others (13) reported that serum concentrations of retinol do not increase after supplementation with β-carotene and that serum concentrations of α-tocopherol appear to remain virtually unchanged after supplementation with β-carotene (14–16).

One recent study found that subjects who received 20 mg β-carotene/d orally for 24 mo had large increases in serum concentrations of lycopene (172–176%) and α-carotene (166–211%) and that these increases were significantly larger than increases in subjects given placebo (17). Smaller increases in serum lutein/zeaxanthin and β-cryptoxanthin were comparable in the treatment and placebo groups. The apparent increases in serum concentrations of lycopene and α-carotene associated with β-carotene supplementation persisted after statistical adjustment for baseline serum concentrations, changes in dietary intake, and other possible confounding variables. The authors hypothesized that β-carotene might affect the metabolism of lycopene and α-carotene, but not that of the two oxygen-containing carotenoids (xanthophylls), possibly by increasing absorption (bioavailability) of the hydrocarbon carotenoids. An earlier study also found that supplementation with 12 or 30 mg β-carotene/d in subjects consuming a specially defined low-carotene diet for 6 wk was associated with an increase in plasma α-carotene concentrations and a decline in plasma lutein concentrations and that these changes were significantly larger than changes in a group receiving placebo and consuming the defined diet (13).

To help define the effects of oral supplementation with β-carotene on serum concentrations of carotenoids, retinol, and α-tocopherol, we analyzed blood samples from a subgroup of patients enrolled in a randomized trial of the use of antioxidant vitamins in preventing recurrence of colorectal adenomas (16, 18). Changes in serum concentrations of retinol, α-tocopherol, and five carotenoids were determined in a subset of subjects who received placebo and were compared with changes in a subset of subjects who received 25 mg β-carotene/d for 4 y.

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SUBJECTS AND METHODS

Patients

The Polyp Prevention Study included patients at six clinical centers who had at least one adenoma (confirmed by histologic evaluation) removed from the large bowel between December 1984 and June 1988. The study was designed to explore the separate effects of two treatments (β-carotene and the combination of 1000 mg vitamin C and 400 IU dl-α-tocopherol acetate) on the rate of development of new colonic adenomas. Supplementation with β-carotene was provided in the form of gelatin capsules containing 25 mg β-carotene in soybean oil and small amounts of several preservatives (BASF, Wyandotte, MI). Of 2029 potentially eligible patients, 864 enrolled and 751 completed the 4-y study (18). Of patients who reported taking ≥ 85% of their pills during the study, we randomly selected 54 from the placebo group and 54 from the group given β-carotene. Of the 54 in each group, 27 had low (below median) serum β-carotene concentrations and 27 had high (above median) serum β-carotene concentrations at enrollment. This trial was approved by our institution’s Committee for the Protection of Human Subjects.

Analytic methods

During the trial we measured β-carotene concentrations in each serum sample at enrollment and at the end of the study, after the subjects had been taking β-carotene supplements or placebo orally for 4 y. We used a procedure designed to measure only β-carotene in serum or plasma. This involved extraction into an organic matrix, injection into an isocratic reversed-phase HPLC system, and monitoring at 436 nm (19). Results from these assays were used for the analyses reported here. The accuracy of the assays was confirmed by participation in a quality improvement program organized by the National Institute of Standards and Technology (NIST, Gaithersburg, MD).

Between 1984 and 1992 (the period during which plasma samples for this study were analyzed for β-carotene), we received 69 samples from the NIST for blinded analysis. Our mean (± SD) bias from the gold standard value for these 69 samples was −0.12 ± 11.38%. We tracked the precision of this assay by measuring samples from a plasma standard every day that an analysis was performed. During 1989 we analyzed portions of the same plasma sample on 70 d; the resulting mean (± SD) was 0.253 ± 0.011 μmol β-carotene/L (135.8 ± 5.8 ng/mL) (CV = 4.2%).

For all other analytes we used an assay that involved extraction into hexane, evaporation, resuspension into an organic matrix, and injection into an isocratic reversed-phase HPLC system with monitoring at 325 nm for retinol, 292 nm for α-tocopherol, and 450 nm for carotenoids. An internal standard was used as reported previously (20). For these measurements each subject’s serum samples from baseline and year 4 were analyzed on the same day (during 1995) to minimize the influence of between-day variations in the assay itself. The accuracy of this assay was confirmed by participation in a quality improvement program organized by NIST.

During 1995 (the period during which plasma samples for this study were analyzed for all analytes other than β-carotene), we received eight samples from the NIST for blinded analysis. Our mean (± SD) biases from the gold standard values for these eight samples were 5.8 ± 10.4% for retinol, 3.8 ± 7.6% for α-tocopherol, 11.8 ± 10.9% for lutein/zeaxanthin, 14.8 ± 10.2% for cryptoxanthin, −2.4 ± 15.2% for lycopene, and −8.5 ± 24.2% for α-carotene. We tracked the precision of this assay by measuring samples of a plasma standard every day that an analysis was performed. During 1995 we analyzed portions of the same plasma sample on 25 d; the resulting CVs were 5.7% for retinol, 5.4% for α-tocopherol, 5.0% for lutein/zeaxanthin, 5.2% for cryptoxanthin, 10.6% for lycopene, and 9.0% for α-carotene.

Our research assistant did not know whether a subject had been assigned to the placebo or supplementation group at the time the samples were analyzed for either β-carotene or the six other analytes.

Serum samples from all subjects were stored at −70 °C in polypropylene freezer tubes before analysis. Each tube had been thawed previously and opened (for other analyses) as many as two times or not at all. Our first subjects were enrolled in 1984 and enrollment continued until 1988. β-Carotene assays were run from 1984 through 1992 (to allow for the 4-y follow-up). All assays for the other six analytes were performed in 1995. Thus, serum samples analyzed in 1995 had been stored 3–11 y.

Statistical analyses

Two-sample t tests were used to compare changes in serum concentrations of retinol, α-tocopherol, lutein/zeaxanthin, cryptoxanthin, lycopene, α-carotene, and β-carotene between the β-carotene–supplemented and placebo groups. We then used multiple regression to compare the groups, adjusting for sex, age, study center, body mass index (in kg/m²), baseline serum concentrations of the analyte, year 4 of dietary intake of the analyte (mg/d), smoking status, and year 4 of vitamin use (21). To permit comparison of our results with those of Wahlqvist et al (17), we conducted another set of analyses with the following changes in covariates: vitamin use was dropped, dietary intake of the analyte was replaced by change in dietary intake (from baseline to year 4), and change in alcohol intake, change in total energy intake, change in fat intake, change in fiber intake, and baseline total cholesterol intake were added. Because the change in β-carotene had a skewed distribution, the Wilcoxon test (22), which makes no distributional assumptions, was used to compare the placebo and β-carotene groups.

RESULTS

Of the 108 patients selected, 6 were excluded from the analyses because of missing data on covariates. Of the remaining 102 patients, 53 (52%) received placebo and 49 (48%) received β-carotene supplements; 82 (80%) were men and 20 (20%) were women aged 40–78 y (median: 53 y). Body mass index at enrollment was 18.4–37.3 (median: 27.1). At enrollment, 37 subjects (36%) had never smoked, 50 (49%) were former smokers, and 15 (15%) were current smokers. In year 4, 84 subjects (82%) did not use vitamins, 13 (13%) were regular users, and 5 (5%) were irregular users. Selected patient characteristics at study entry in the placebo and β-carotene groups are shown in Table 1. There were no significant differences between the groups with respect to age at random assignment, sex, body mass index, or smoking status.
TABLE 1
Selected patient characteristics at study entry in the two groups

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Age at randomization (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>33.96 [18]</td>
<td>32.65 [16]</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81.13 [43]</td>
<td>79.59 [39]</td>
</tr>
<tr>
<td>Female</td>
<td>18.87 [10]</td>
<td>20.41 [10]</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.1–29.5</td>
<td>22.64 [12]</td>
<td>28.57 [14]</td>
</tr>
<tr>
<td>&gt;29.5</td>
<td>32.08 [17]</td>
<td>20.41 [10]</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>39.62 [21]</td>
<td>59.18 [29]</td>
</tr>
</tbody>
</table>

*There were no significant differences by chi-square test.

Plasma concentrations of the seven analytes examined did not differ significantly between the two groups at baseline (Table 2). After 4 y, during which the patients received either placebo or β-carotene, the only change in mean serum concentration that was significantly different between the two groups was the change in β-carotene. Patients given the supplements had a mean increase in serum β-carotene concentration of 151% (compared with a 7.4% decrease in the group given placebo). We found only small changes in all other analytes, except for cryptoxanthin, after supplementation with β-carotene. We excluded with 95% confidence a decrease > 17% or an increase > 18% in all analytes except cryptoxanthin in the supplementation group. In addition, the small changes in the β-carotene group were not significantly different from similar small changes in the placebo group. Both groups had larger changes in cryptoxanthin (increases of 17–27%) but these changes did not differ significantly between the two groups.

P values for the changes in the analytes after multivariate adjustment are given in Table 2. In this model supplementation with β-carotene given orally was associated with a change significantly larger than the change in the placebo group only for β-carotene itself and not for any other analyte. When the adjusting variables were changed to match the analysis of Wahlqvist et al (17), the results were similar: only β-carotene serum concentrations were increased by oral supplementation with β-carotene. Whereas in our model the adjusted increase in the supplementation group (compared with that in the placebo group) was 0.638 μmol β-carotene/L (0.343 μg/mL), the difference resulting from the model of Wahlqvist et al was 0.658 μmol/L (0.354 μg/mL).

DISCUSSION

We found that supplementation with 25 mg β-carotene/d given orally to a group of patients for 4 y produced changes that were significantly different from changes in a placebo group only with respect to serum concentrations of β-carotene.

We did not find that β-carotene supplementation was associated with changes greater than those produced by placebo in serum concentrations of retinol, α-tocopherol, or any of the other carotenoids we analyzed. Adjustment for multiple covariates did not affect these findings. In addition, on the basis of our results (mean changes and relatively small SEs in the group given supplements) we excluded with 95% confidence a decrease > 17% or an increase > 18% in any analyte other than cryptoxanthin; for cryptoxanthin, we excluded an increase > 34.1%.

We were curious why, in contrast with earlier studies (13, 17), we found a lack of effect of β-carotene supplementation on changes in serum concentrations of α-carotene, lycopene, and lutein/zeaxanthin. One possibility we considered was that our subjects might have had unusual baseline concentrations of β-carotene. The mean baseline serum concentration of β-carotene in our subset of 102 subjects (0.220 μg/mL) was similar to the mean baseline concentrations we reported for the entire patient population in this study that was randomly assigned to receive either placebo (0.352 μmol/L, or 0.189 μg/mL) or β-carotene (0.391 μmol/L, or 0.210 μg/mL) (18). Mean baseline concentrations of β-carotene were also similar to those in other studies: 0.335 μmol/L (0.180 μg/mL) in a study of patients with nonmelanoma skin cancers (23), 0.298 μmol/L (0.160 μg/mL) in a study of subjects with colorectal adenomas (17), and 0.303 μmol/L (0.163 μg/mL) in a study of 30 healthy men (13). Thus, our group of subjects had fairly typical enrollment (baseline) serum concentrations of β-carotene.

A second possibility was that the β-carotene supplement used in this study appeared to produce smaller increases in serum β-carotene than did the supplements in several other studies. In our study the increase of 0.614 μmol/L (0.330 μg/mL), an average increase of 151% in the β-carotene concentration in the 49 patients who received 25 mg β-carotene/d orally was similar to that in the larger study of similar subjects who received 0.675 μmol β-carotene/L (0.363 μg/mL), an increase of 173% (18). In a previous study we conducted in which a different group of subjects, who had had nonmelanoma skin cancer, received 50 mg β-carotene/d in a different formulation and in whom measurements were done after 1 y, we found that median β-carotene concentrations rose from 0.335 μmol/L (0.180 μg/mL) to 3.160 μmol/L (1.699 μg/mL), an increase of 843% (12).

In the study by Wahlqvist et al (17), supplementation with 20 mg β-carotene/d was provided for 2 y and was associated with an increase in serum β-carotene concentration of 1073% in men and 839% in women. Costantino et al (24) found an increase in serum β-carotene of almost 1000% after 4 mo of supplementation with 15 mg β carotene/d. Micozzi et al (13) reported similar large increases in plasma β-carotene in response to 6 wk of supplementation with 12 mg β-carotene/d. These increases were significantly larger than those we found resulting from comparable oral doses of β-carotene and were similar to changes we observed in our patients who received supplementation with 50 mg β-carotene/d.

One likely explanation for the large differences in steady state serum or plasma concentrations of β-carotene after supplementation with comparable doses (20–50 mg/d) may be that only our study and its parent study (18) used gelatin capsules containing β-carotene in oil. Costantino et al (24), Micozzi et al (13), and Wahlqvist et al (17) all used a formulation of
**Table 2**
Mean changes in plasma concentrations of retinol, α-tocopherol, and carotenoids by supplementation group with P values from t tests comparing changes between the two groups.

<table>
<thead>
<tr>
<th>Analyte and group</th>
<th>Plasma concentration</th>
<th>P</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Year 4</td>
<td>Change</td>
<td>Percentage change</td>
</tr>
<tr>
<td></td>
<td>μmol/L (μg/mL)</td>
<td>μmol/L (μg/mL)</td>
<td>μg/mL</td>
<td>%</td>
</tr>
<tr>
<td>Retinol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>2.499 (0.716)</td>
<td>2.492 (0.714)</td>
<td>-0.002 ± 0.016</td>
<td>-0.2</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>2.614 (0.749)</td>
<td>2.436 (0.698)</td>
<td>-0.051 ± 0.024</td>
<td>-6.8</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>28.58 (12.32)</td>
<td>28.33 (12.21)</td>
<td>-0.109 ± 0.495</td>
<td>-2.0</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>28.33 (12.21)</td>
<td>27.77 (11.97)</td>
<td>-0.241 ± 0.410</td>
<td>-0.9</td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.371 (0.211)</td>
<td>0.371 (0.211)</td>
<td>0.0001 ± 0.010</td>
<td>+0.1</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.378 (0.215)</td>
<td>0.357 (0.203)</td>
<td>-0.012 ± 0.010</td>
<td>-5.6</td>
</tr>
<tr>
<td>Cryptoxanthin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.148 (0.082)</td>
<td>0.186 (0.103)</td>
<td>0.022 ± 0.005</td>
<td>+26.7</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.168 (0.093)</td>
<td>0.197 (0.109)</td>
<td>0.016 ± 0.008</td>
<td>+16.8</td>
</tr>
<tr>
<td>Lycopene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.623 (0.335)</td>
<td>0.608 (0.327)</td>
<td>-0.007 ± 0.018</td>
<td>-2.2</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.578 (0.311)</td>
<td>0.545 (0.293)</td>
<td>-0.018 ± 0.017</td>
<td>-5.8</td>
</tr>
<tr>
<td>α-Carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.073 (0.039)</td>
<td>0.071 (0.038)</td>
<td>-0.001 ± 0.002</td>
<td>-1.9</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.073 (0.039)</td>
<td>0.074 (0.040)</td>
<td>0.001 ± 0.003</td>
<td>+2.2</td>
</tr>
<tr>
<td>β-Carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.415 (0.223)</td>
<td>0.383 (0.206)</td>
<td>-0.017 ± 0.018</td>
<td>-7.4</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.405 (0.218)</td>
<td>0.101 (0.548)</td>
<td>0.330 ± 0.056</td>
<td>+151.2</td>
</tr>
</tbody>
</table>

1. Groups did not differ significantly in concentration of any analyte at baseline, P > 0.30 (t tests).
2. i ± SE
3. Mean change expressed as a percentage of the mean baseline concentration.
4. Adjusted for age, sex, study center, Quetelet index, baseline plasma concentration, year 4 dietary intake, smoking status, and year 4 vitamin use.
5. P value for mean change in β-carotene was computed with the Wilcoxon test.

β-carotene that was made from dry gelatin beadlets in a capsule (water soluble, or micellar, β-carotene) whereas our study in patients with skin cancer (12) used β-carotene in the form of a 10% dry powder in a capsule. It appears that there is considerably less oral bioavailability of β-carotene when it is supplied in an oil matrix in a gelatin capsule. Thus, it is possible that supplementation with β-carotene that produces small increases in serum β-carotene is not associated with changes in other analytes whereas supplementation that produces large increases in β-carotene concentration might be associated with larger increases (e.g., in α-carotene and lycopene) or decreases (e.g., in lutein/zeaxanthin) in carotenoids.

A third possible difference between our study and previous studies is the length of supplementation. We continued β-carotene supplementation for 4 y whereas the other groups used supplementation periods of 6 wk (13) to 2 y (17). All three studies were continued for sufficient periods to allow β-carotene to attain a new steady state concentration in serum or plasma. However, it is hard to imagine a plausible biologic explanation for shorter periods of supplementation being associated with larger changes in serum or plasma concentrations of other carotenoids.

Fourth, these three studies also differed to some extent in the effects of placebo administration (plus dietary changes) on serum or plasma concentrations of analytes. In the study by Micoczi et al (13), placebo plus a controlled diet (constant and low in total carotenoid content) was associated with variable changes in plasma β-carotene (77%), α-carotene (72%), lutein/zeaxanthin (57%), and lycopene (69%) whereas in the study by Wahlgvist et al (17), the group given placebo had similarly variable changes in lutein/zeaxanthin (16–30%), β-cryptoxanthin (16–56%), lycopene (72–73%), α-carotene (13–51%), and β-carotene (4–24%). Thus, even though we followed subjects for the longest period (4 y) and did not include any specific dietary intervention, concentrations of all the analytes we investigated remained the most constant in the placebo group; they changed ≤ 7.4% for every analyte except cryptoxanthin, which increased ≈27%, possibly because of an increase in consumption of orange products. Although statistical tests can distinguish differences between treatment and placebo groups, the large changes in analyte concentrations in subjects who received placebo in the two other studies (13, 17) may indicate that other changes in diet also occurred.

Fifth, we must consider the possibility that laboratory artifacts with a variety of potential causes occurred. In our study β-carotene concentrations were determined as blood samples were received and were measured after relatively brief periods of storage between 1984 and 1992. All other analytes were measured in 1995 after storage periods of 3–11 y and after the tubes in which they were thawed and opened as many as two times or not at all. Length of storage and number of times a tube has been thawed may affect the stability of various analytes but should not preferentially affect one group of blood samples more than another. Although we previously summarized our work and that of others (25) showing that retinol, α-tocopherol, and β-carotene appear to be stable at −70 °C for...
≥ 1 y (and, for β-carotene, probably ≥ 2 y), there is little information available about the stability of these analytes for longer periods.

Micoczi et al (13) stored the blood samples in their study at −70 °C for 17–19 mo before analysis; Wahlqvist et al (17) also stored their samples at −70 °C but did not state the length of the storage period. It is not clear whether either or both studies included measurement of all samples from the same subject on the same day to minimize day-to-day variation in the assay. However, such between-day variation in the assay itself is unlikely to explain the large changes observed in either study because CVs of the assay of 2.8–10% [reported in one paper (13)] are typical of those in excellent laboratories.

Diurnal and seasonal factors can increase variability of results with respect to the analytes in single, randomly selected blood samples we studied here (26, 27). However, in our experience the increase in variability from these sources is on the order of several percentage points rather than on the order of increases or decreases of ≥ 100%.

In summary, our data suggest that supplementation with oral β-carotene in modest daily doses (25 mg) produced moderate increases in serum β-carotene (> 151%) that were not associated with changes in serum concentrations of retinol, α-tocopherol, or other carotenoids that were significantly different from the small changes observed in subjects taking placebo. It remains possible that supplementation with β-carotene, when such supplementation produces large increases in serum β-carotene concentrations (≥ 10-fold), may be associated with smaller increases in serum lycopene or α-carotene or decreases in serum lutein/zeaxanthin by unknown mechanisms. Such observations need to be confirmed in future clinical studies because studies already published have conflicting results with respect to changes in lutein/zeaxanthin and lycopene associated with β-carotene supplementation.

We thank Bernie Beaulieu for expert assistance in performing the various HPLC assays.

REFERENCES