Changes in serum carotenoids in subjects with colorectal adenomas after 24 mo of β-carotene supplementation[1–4]

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ABSTRACT The effect of β-carotene supplementation on major serum carotenoid fractions (lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene) was investigated in 224 people with colorectal adenomas (139 men, 85 women) recruited for the Australian Polyp Prevention Project (APPP). Each subject was randomly assigned to take either 20 mg β-carotene/d or placebo over 24 mo. Besides the expected increase in serum concentration of β-carotene (1073% in men, 839% in women), lycopene (176% in men) and α-carotene (211% in men and 166% in women) concentrations were also increased after body mass index, baseline concentration, change in respective carotenoid intake, and other confounding factors were adjusted for. The increase in serum concentrations of these carotenoids after β-carotene supplementation suggests that β-carotene may interact biologically with other carotenoids and such interaction would need to be taken into consideration when the protective effect of β-carotene supplementation for cancer or other diseases is examined. *Am J Clin Nutr* 1994;60:936–43.

KEY WORDS β-carotene supplementation, serum carotenoids concentration, intervention study

Introduction

Major carotenoids, except for lycopene, in serum have been reported to be positively correlated with each other (1). This is not surprising because α-carotene and β-carotene tend to be found together in foods such as green leafy vegetables, whereas lycopene is found primarily in tomatoes, which contain smaller amounts of other carotenoids. Data on carotenoid content in various vegetables and fruits show that high β-carotene–containing foods tend to contain high amounts of α-carotene and lutein/zeaxanthin (2, 3).

Several short-term and long-term β-carotene supplementation studies have focused on the increase in serum or plasma β-carotene concentration, whereas little is known about the effect of β-carotene supplementation on other carotenoids. The impairment of lutein absorption occurred in subjects being supplemented with 12 or 30 mg purified β-carotene while fed a low-carotenoid diet (4).

The aim of this study was to evaluate the influence of β-carotene supplementation over 24 mo on serum β-carotene and other major carotenoids in subjects under surveillance after removal of colorectal adenomas.

Subjects and methods

Study population

We evaluated serum concentrations of individual carotenoids in people participating in the Australian Polyp Prevention Project (APPP). This project was a double-blind randomized, placebo-controlled and dietary-intervention study (5). Because of limitations on the availability of serum samples and required information, a subpopulation of 224 people (139 men, 85 women) was included in the present study. This subpopulation was similar to the trial population in terms of age and sex and comprised 112

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subjects from Brisbane, 95 subjects from Melbourne, and 17 subjects from Sydney recruited from October 1985 to April 1988.

Eligible subjects were aged 30–75 y, having had at least one adenomatous polyp removed. Patients with chronic inflammatory bowel disease, familial adenomatous polyposis, and colorectal and other cancers (except nonmelanoma cancer) unless free from symptoms for 5 y, and those with special diets for renal, liver, or gallbladder disease were excluded.

With a 2 × 2 × 2 factorial design, each subject could be assigned into one of the following eight groups: *1*) wheat bran supplement + fat reduction + β-carotene supplement, *2*) wheat bran supplement + fat reduction + no β-carotene supplement, *3*) wheat bran supplement + no fat reduction + β-carotene supplement, *4*) wheat bran supplement + no fat reduction + no β-carotene supplement, *5*) no wheat bran supplement + fat reduction + β-carotene supplement, *6*) no wheat bran supplement + fat reduction + no β-carotene supplement, *7*) no wheat bran supplement + no fat reduction + β-carotene supplement, *8*) no wheat bran supplement + no fat reduction + no β-carotene supplement.

Each β-carotene capsule contained 20 mg β-carotene with 18 mg ascorbyl palmitate and 0.3 mg *dl*-α-tocopherol as stabilizers. Placebo capsules were identical to the active capsules except for β-carotene. The capsules were supplied in calender packs by Hoffmann-La Roche (Basel, Switzerland). Analysis of the carotenoid profile of the capsules by an HPLC method revealed only a single carotenoid, namely β-carotene. Compliance with β-carotene supplementation was monitored by self-reporting and capsule count. β-Carotene concentrations in serum also provided confirmatory evidence of compliance with capsule count.

All subjects gave informed consent and the study protocol was approved by the institutional ethics committee at each center.

Demographic, lifestyle, and medical information

At recruitment, subjects completed a questionnaire on weight, height, and demographic details, such as marital and occupational status, education attainment, smoking status, alcohol consumption, vitamin supplementation, and general health status.

Dietary intake

At entry, each subject was asked to complete a comprehensive food-frequency questionnaire so that usual food intake in the past 12 mo could be estimated. Nutrient intakes were assessed from the questionnaire by using a commercial nutrient-analysis program (*Nutritionist-III; N*-Squared Computing, Salem, OR). The food-frequency questionnaire was repeated at the 24th mo. Data on carotenoid composition in fruits and vegetables provided by the carotenoid food-composition database was used to estimate daily intake of individual carotenoids from food intake assessed by the food-frequency questionnaire (2).

Biochemical analyses

Fasting blood samples collected at entry to the study and at 24 mo were analyzed for serum carotenoids and lipids. After centrifugation at 2100 × *g* at 4 °C for 15 min, aliquot serum samples were stored at or below −70 °C until assayed. Serum total cholesterol and triglyceride were measured with a Progress selective chemistry analyzer (KONE Instruments Corporation, Espoo, Finland) by using commercial kits (Trace Scientific, Victoria, Australia). High-density-lipoprotein (HDL) cholesterol was measured as for serum total cholesterol, after the precipitation of apolipoprotein B–containing lipoproteins by using equal volumes of 20% polyethylene glycol 6000 (Merck-Schuchardt, Munich, Germany). Low-density-lipoprotein (LDL) cholesterol was derived by using the formula of Friedewald et al adapted to Système International (SI) units (6).

Serum concentrations of individual carotenoids were analyzed by a modification of the HPLC method of Thurnham et al (7). Purified standards of lutein (xanthophyll) extracted from alfalfa, lycopene from tomatoes, and all-*trans*-α-carotene and all-*trans*-β-carotene from carrots were supplied by Sigma Chemicals Co (St Louis). β-Cryptoxanthin standard was kindly donated by Hoffmann-La Roche. HPLC grade solvents (acetonitrile, methanol, chloroform and *n*-hexane) were purchased from Waters Associates (New South Wales, Australia).

Sample preparation was performed under subdued light. *n*-Octyl-α-naphthyl urethane (ONU), kindly provided by the Department of Human Nutrition, Deakin University (Geelong, Australia), was used as an internal standard. *n*-Hexane containing 500 mg butylated hydroxy toluene/L was used for extraction. Hexane extract was dried under nitrogen and the residue was dissolved in mobile phase just before injection into an HPLC system.

A Spherisorb ODS-2, 5-μm analytical column (Alltech Associates, New South Wales, Australia) was used in conjunction with a guard column. Mobile phase was acetonitrile: methanol: chloroform (48:48:4, by volume) run at a flow rate of 1.5 mL/min. A Lambda-Max model 481 LC spectrophotometer (Waters Associates, New South Wales, Australia) set at wavelength 450 nm was used to detect the absorbance of carotenoids. Quantitation was done by using the internal standard and comparing results with the standard curve by linear-regression analysis. Lutein and zeaxanthin could not be separated by this method; therefore, serum concentrations of lutein and zeaxanthin, mainly lutein, are presented as lutein concentrations.

Statistical analyses

The *Statistical Analysis System* (SAS Institute Inc, Cary, NC) software package was used for data analysis. Descriptive statistics including means and SDs were calculated for each continuous variable. A paired *t* test was used to evaluate differences in indexes of interest between the study entry and exit. Spearman correlation analyses were performed to determine the degree and direction of association between two variables. The significance level was set at 5%.

Results

Mean age and body fatness, expressed as body mass index (BMI; in kg/m^2), of subjects at the study entry are shown in **Table 1.** Of the 224 subjects, 139 (62%) were men and 85 (38%) were women. The age at entry ranged from 30 to 72 y ($\bar{x}$: 56 y) for men and from 33 to 73 y ($\bar{x}$: 56 y) for women. BMI ranged from 16.3 to 34.6 ($\bar{x}$: 25.4) in men and from 16.5 to 35.7 ($\bar{x}$: 24.1) in women. Subjects were similar in age and BMI with regard to randomization. There was a significant increase in BMI in women for both the supplement and placebo groups after 24 mo (data not shown). Serum lipid concentrations are also listed in Table 1. No significant differences in serum lipids between placebo and supplement groups were observed.

TABLE 1
Subject characteristics at study entry[1]

	Total (n = 139 M, 85 F)	Placebo group (n = 61 M, 42 F)	Supplement group (n = 78 M, 43 F)
Men			
Age (y)	56 ± 10	56 ± 9	55 ± 11
BMI[2]	25.4 ± 3.5	25.6 ± 3.4	25.3 ± 3.6
Serum total cholesterol (mmol/L)	6.3 ± 1.9	6.4 ± 1.3	6.3 ± 2.2
Serum LDL cholesterol (mmol/L)	4.4 ± 1.1	4.5 ± 1.1	4.3 ± 1.1
Serum HDL cholesterol (mmol/L)	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3
Serum triglyceride (mmol/L)	2.1 ± 5.0	1.7 ± 1.1	2.3 ± 6.7
Women			
Age (y)	56 ± 10	56 ± 10	56 ± 11
BMI[2]	24.1 ± 3.9	24.8 ± 4.0	23.4 ± 3.6
Serum total cholesterol (mmol/L)	6.6 ± 1.2	6.5 ± 1.3	6.8 ± 1.2
Serum LDL cholesterol (mmol/L)	4.6 ± 1.2	4.4 ± 1.3	4.8 ± 1.0
Serum HDL cholesterol (mmol/L)	1.4 ± 0.5	1.5 ± 0.6	1.3 ± 0.5
Serum triglyceride (mmol/L)	1.5 ± 0.9	1.4 ± 0.8	1.7 ± 1.0

[1] $\bar{x}$ ± SD. There were no significant differences between placebo and supplement groups.
[2] In kg/m^2.

Twenty-three percent of men did not smoke and 77% had smoked (49% were exsmokers and 28% were current smokers), whereas 55% of women did not smoke and 45% had smoked (22% were exsmokers and 23% were current smokers).

Table 2 shows mean daily intakes of nutrients and dietary carotenoids assessed by a comprehensive food-frequency questionnaire at study entry and exit. Changes in nutrient intake between the study entry and exit were more pronounced in men than in

TABLE 2
Nutrient intake at study entry and at exit[1]

	Men		Women	
Daily nutrient intake	At entry	At exit	At entry	At exit
Placebo group				
Total energy (MJ)	13 ± 5	11 ± 4[2]	9 ± 3	8 ± 3[3]
Protein (g)	125 ± 48	109 ± 45[4]	93 ± 43	82 ± 37
Fat (g)	123 ± 48	89 ± 52[2]	88 ± 48	62 ± 31[5]
Carbohydrate (g)	340 ± 139	293 ± 101[3]	253 ± 73	234 ± 85
Dietary fiber (g)	32 ± 13	33 ± 14	26 ± 9	29 ± 11
Retinol (mg)	1.1 ± 1.2	0.6 ± 0.4[5]	0.7 ± 0.8	0.4 ± 0.4
Alcohol (g)	39 ± 67	29 ± 39	6 ± 9	6 ± 10
Dietary carotenoids (mg)				
Lutein/zeaxanthin	2.7 ± 2.6	2.7 ± 2.0	2.1 ± 2.2	3.0 ± 4.7
β-Cryptoxanthin	0.3 ± 0.3	0.2 ± 0.3	0.3 ± 0.4	0.3 ± 0.5
Lycopene	2.5 ± 4.6	1.9 ± 1.9	1.7 ± 1.2	1.9 ± 1.2
α-Carotene	2.4 ± 3.9	2.0 ± 1.5	1.5 ± 1.4	1.9 ± 1.3
β-Carotene	4.7 ± 5.0	4.1 ± 2.8	3.6 ± 3.4	4.8 ± 3.1[5]
Supplement group				
Total energy (MJ)	13 ± 5	11 ± 5[2]	9 ± 3	8 ± 3[5]
Protein (g)	125 ± 68	110 ± 52	91 ± 36	83 ± 28
Fat (g)	119 ± 62	86 ± 50[2]	90 ± 36	68 ± 35[4]
Carbohydrate (g)	333 ± 138	302 ± 138	255 ± 104	226 ± 87
Dietary fiber (g)	28 ± 12	30 ± 11	28 ± 11	28 ± 11
Retinol (mg)	0.8 ± 0.6	0.6 ± 0.6[3]	0.9 ± 1.1	0.5 ± 0.3[3]
Alcohol (g)	43 ± 60	29 ± 38[5]	15 ± 30	10 ± 18
Dietary carotenoids (mg)				
Lutein/zeaxanthin	1.8 ± 1.2	1.9 ± 1.2	2.0 ± 1.5	2.3 ± 1.8
β-Cryptoxanthin	0.2 ± 0.3	0.2 ± 0.6	0.3 ± 0.6	0.2 ± 0.3
Lycopene	2.0 ± 1.8	1.8 ± 1.7	2.6 ± 3.1	2.7 ± 2.4
α-Carotene	1.2 ± 1.2	1.5 ± 1.5[3]	1.5 ± 1.2	1.5 ± 1.1
β-Carotene	2.9 ± 2.1	3.2 ± 2.4	3.5 ± 2.1	3.7 ± 2.2

[1] $\bar{x}$ ± SD. Placebo group (n = 61 men, 42 women), supplement group (n = 78 men, 43 women).
[2–5] Significantly different from value at study entry: [2] $P < 0.0001$, [3] $P < 0.05$, [4] $P < 0.001$, [5] $P < 0.01$.

TABLE 3
Serum carotenoid concentrations at study entry and at exit[1]

Serum carotenoid concentration (nmol/L)	Men		Women	
	At entry	At exit	At entry	At exit
Placebo group				
Lutein/zeaxanthin	547 ± 454	604 ± 494	512 ± 456	589 ± 523[2]
β-Cryptoxanthin	213 ± 273	249 ± 299[2]	366 ± 654	291 ± 292
Lycopene	271 ± 286	327 ± 341	179 ± 187	224 ± 201
α-Carotene	45 ± 41	55 ± 46[2]	37 ± 32	37 ± 32
β-Carotene	258 ± 275	270 ± 357	236 ± 247	235 ± 219
Supplement group				
Lutein/zeaxanthin	521 ± 619	509 ± 476	569 ± 681	660 ± 712
β-Cryptoxanthin	193 ± 183	247 ± 239[3]	281 ± 328	297 ± 242
Lycopene	255 ± 296	530 ± 521[4]	285 ± 666	440 ± 619[5]
α-Carotene	36 ± 39	85 ± 72[4]	47 ± 40	102 ± 85[4]
β-Carotene	280 ± 447	2139 ± 2666[4]	446 ± 1027	2367 ± 2448[4]

[1] $\bar{x}$ ± SD. Placebo group (n = 61 men, 42 women), supplement group (n = 78 men, 43 women).
[2-5] Significantly different from values at study entry: [2] $P < 0.05$, [3] $P < 0.001$, [4] $P < 0.0001$, [5] $P < 0.01$.

women. The major change was a reduction in fat intake, which also resulted in energy intake reduction. In men, reduction in protein intake was observed among the placebo group whereas a reduction in retinol intake was observed in both groups. After 24 mo, women in the placebo group and men in the supplement group reported increases in the intake of β-carotene and α-carotene, respectively.

Table 3 shows mean serum concentrations of five major carotenoids: lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene. After 24 mo of placebo supplementation there were significant increases in serum β-cryptoxanthin and α-carotene concentrations in men and in serum lutein/zeaxanthin concentrations in women. Serum concentrations of lycopene, α-carotene, and β-carotene at exit were significantly higher in those who were given the active β-carotene supplement. An increase in serum β-cryptoxanthin was also observed in men.

The serum concentration of β-carotene at study entry was found to be positively associated with other carotenoids, particularly α-carotene, as shown in **Table 4.** Spearman correlation coefficients ranged from 0.25 (lutein/zeaxanthin in women) to 0.81 (α-carotene in men). These relationships in men were also significant after 24 mo in both the placebo and supplement groups. In women, the relationship of β-carotene to α-carotene remained.

Relationships between serum concentrations and carotenoid intakes at study entry were investigated and Spearman correlation coefficients are listed in **Table 5.** Daily intakes of the major carotenoids, except lycopene, were positively associated with the serum concentrations of the respective carotenoids in men. The correlation coefficients ranged from 0.25 to 0.31. In women, a positive relationship was found only in β-cryptoxanthin (r = 0.41, $P < 0.0001$).

The serum carotenoid concentration at entry was a confounding factor to the percentage change in serum concentration of the respective carotenoid, particularly in men. In women, the baseline serum concentrations of lutein/zeaxanthin and β-carotene, however, were not related to the changes in the concentrations. **Table 6** shows the correlations between the percentage change

TABLE 4
Spearman correlation coefficients (r_s) between serum concentrations of β-carotene and other carotenoids at study entry and at exit[1]

Serum carotenoid concentration	Men	Women
At entry		
Lutein/zeaxanthin	0.38[2]	0.25[3]
β-Cryptoxanthin	0.50[2]	0.34[4]
Lycopene	0.47[2]	0.60[2]
α-Carotene	0.81[2]	0.80[2]
At exit		
Placebo group		
Lutein/zeaxanthin	0.30[3]	0.20
β-Cryptoxanthin	0.49[2]	0.29
Lycopene	0.52[2]	0.63[2]
α-Carotene	0.84[2]	0.75[2]
Supplement group		
Lutein/zeaxanthin	0.39[5]	0.28
β-Cryptoxanthin	0.58[2]	0.25
Lycopene	0.49[2]	0.26
α-Carotene	0.72[2]	0.78[2]

[1] At entry (n = 139 men, 85 women), placebo group (n = 61 men, 42 women), supplement group (n = 78 men, 43 women).
[2-5] Significantly different from zero: [2] $P < 0.0001$, [3] $P < 0.05$, [4] $P < 0.01$, [5] $P < 0.001$.

TABLE 5
Spearman correlation coefficients (r_s) between serum concentration and daily intake of carotenoid at study entry

Carotenoid	Men (n = 139)	Women (n = 85)
Lutein/zeaxanthin	0.26[1]	0.21
β-Cryptoxanthin	0.31[2]	0.41[3]
Lycopene	0.15	0.03
β-Carotene	0.27[1]	0.07
β-Carotene	0.25[1]	−0.01

[1-3] Significantly different from zero: [1] $P < 0.01$, [2] $P < 0.001$, [3] $P < 0.0001$.

TABLE 6
Spearman correlation coefficients (r_s) between percentage change in serum carotenoid concentration and selected confounders

Confounder at study entry	Lutein/zeaxanthin	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
Men (n = 139)					
Age at entry	0.10	−0.03	0.04	0.07	−0.02
BMI at entry	0.16	0.07	−0.10	0.02	0.04
Baseline serum concentration	−0.50[3]	−0.32[3]	−0.27[2]	−0.39[3]	−0.20[1]
Change in energy intake	−0.07	−0.02	0.10	0.01	0.02
Change in fat intake	0.11	−0.03	0.05	0.08	0.14
Change in dietary fiber intake	0.08	0.15	0.07	0.07	0.06
Change in corresponding carotenoid intake	0.26[2]	0.26[2]	0.12	0.18[1]	0.14
Change in alcohol consumption	0.01	0.01	0.21[1]	0.01	−0.04
Smoking status[4]	0.08	0.13	−0.01	−0.05	−0.08
Change in LDL cholesterol	0.16	0.18[1]	0.03	0.03	0.09
Change in HDL cholesterol	0.12	−0.08	0.15	0.01	0.02
Women (n = 85)					
Age at entry	0.07	−0.04	0.00	0.05	0.05
BMI at entry	0.04	−0.16	−0.06	−0.22[1]	−0.28[2]
Baseline serum concentration	−0.01	−0.50[3]	−0.32[2]	−0.22[1]	−0.10
Change in energy intake	0.01	−0.01	0.12	0.01	0.16
Change in fat intake	0.03	−0.05	0.19	0.04	0.14
Change in dietary fiber intake	0.09	0.14	0.03	−0.00	0.07
Change in corresponding carotenoid intake	−0.12	0.14	0.03	−0.00	−0.12
Change in alcohol consumption	−0.03	−0.15	−0.05	−0.21	−0.02
Smoking status[4]	0.11	0.11	0.15	0.23[1]	0.05
Change in LDL cholesterol	0.09	0.05	0.08	0.03	0.08
Change in HDL cholesterol	0.14	0.01	0.23[1]	−0.05	0.06

[1-3] Significantly different from zero: [1] $P < 0.05$, [2] $P < 0.01$, [3] $P < 0.0001$.
[4] Categorized variable: 0 = non-smokers, 1 = exsmokers, 2 = current smokers.

in serum concentrations and potential confounders, namely age and BMI at entry; changes in energy, fat, dietary fiber and the respective carotenoid intakes; change in alcohol consumption; smoking status; and changes in serum low-density-lipoprotein- (LDL) and high-density-lipoprotein- (HDL) cholesterol concentrations. In women, an inverse association was found between BMI and the changes in serum α-carotene and β-carotene concentrations ($r = -0.22$, $P < 0.05$; $r = -0.28$, $P < 0.01$, respectively), and a positive association was found between smoking status and the change in α-carotene concentration ($r = 0.23$, $P < 0.05$). In men, changes in serum concentration and daily intake were related in the case of lutein/zeaxanthin, β-cryptoxanthin, and α-carotene, whereas none of these relationships were found in women. The alteration of LDL-cholesterol concentration was positively associated with the change in serum β-cryptoxanthin concentration in men ($r = 0.18$, $P < 0.05$). No relationships between the changes in serum concentrations of HDL cholesterol and carotenoids were observed, except for lycopene in women ($r = 0.23$, $P < 0.05$).

Figure 1 shows mean percentage changes in serum carotenoid concentration after selected confounding factors listed in Table 6 were adjusted for. The serum concentrations of α-carotene and β-carotene in the supplement group were significantly elevated from baseline with the adjusted values 211% and 1073% in men, and 166% and 839% in women, compared with the placebo group of which increased values were 51% and 4% in men and 13% and 24% in women. In addition, a marked increase was also found in serum lycopene concentration in men (176% in supplement group and 72% in placebo group), after adjustment for the serum concentration at entry and change in alcohol consumption.

Discussion

Relationships among serum carotenoid concentrations

Positive relationships among serum concentrations of the major carotenoids, except for lycopene, were previously reported by Cantilena et al (1). It was postulated that the relationships were attributable to the occurrence of these carotenoids in the same food source. However, this may not adequately explain the finding in the present study that serum concentrations of lycopene and β-carotene were associated. Lycopene is available only in certain food items such as tomatoes, tomato products, and watermelon. Moreover, these relationships existed after 24 mo of β-carotene supplementation, particularly in men. This finding implies that there might be a regulatory mechanism controlling the balance or equilibrium of biological concentrations of β-carotene and other carotenoids.

Relationships between serum concentration and daily intake of carotenoids

A carotenoid food-composition database was used to estimate intakes of individual dietary carotenoids (2). The use of food-composition data based on a foreign food source may result in classification error insofar as the relationships between serum concentrations and intakes of nutrients are concerned. However, carotenoid contents in some vegetables from this database were comparable with those in Australian vegetables reported by Kajadphai (8). In addition, in the present study serum concentrations of carotenoids, except lycopene, were positively related to the corresponding carotenoid intakes. These findings are consistent

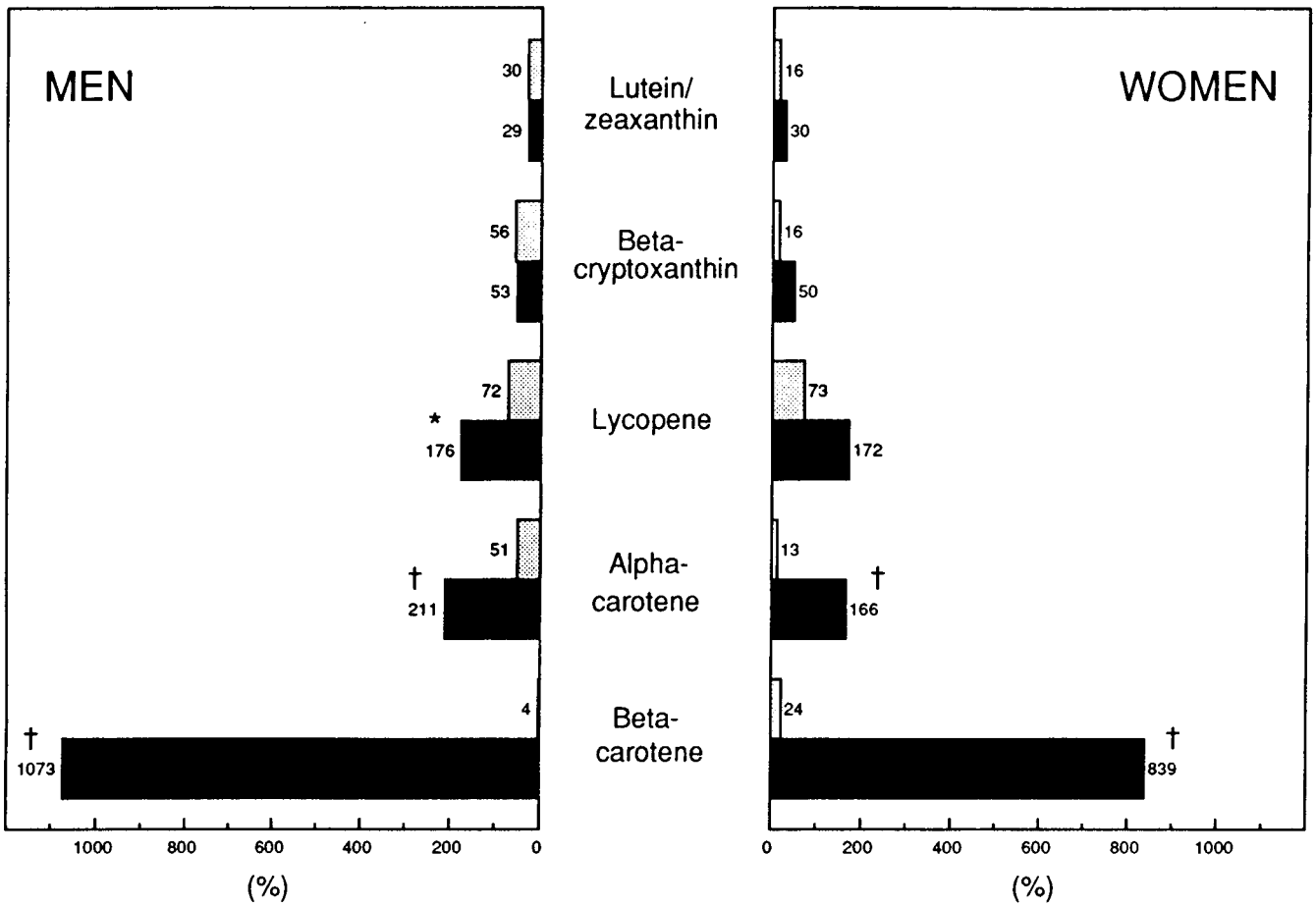

FIG 1. Comparison of percentage changes in serum carotenoid concentrations between supplement (■) and placebo (▨) groups after 24 mo of β-carotene supplementation in 139 men and 85 women, after adjustment for BMI, baseline concentration, change in carotenoid intake, and other confounding factors. *$P < 0.05$, †$P < 0.0001$.

with those reported earlier (9–12). Using the specified database, Forman et al (13) reported from a study of 57 male nonsmokers that carotenoid intakes were associated with serum concentrations of the respective carotenoids, except for lycopene. These results were similar to those in the present study. This indicates the usefulness of the database in the estimation of carotenoid intake.

Effect of sex, age, BMI, and baseline concentration on the response to β-carotene supplementation

It has been reported that the response to a β-carotene supplement is sex-dependent (14). Compared with men, women tend to have lower amounts of the intestinal mucosal dioxygenase enzyme that converts β-carotene to retinal and subsequently retinol (15). In the present study it was also observed that men and women responded to β-carotene supplementation differently.

In a previous study in which a supplement of 50 mg β-carotene/d was administered for 1 y, it was observed that age had no effect on changes in carotenoid concentration, and that leaner subjects had larger increases in plasma β-carotene concentration (14). The result is consistent with our finding that body fatness expressed as BMI was a confounding factor in the response to the β-carotene supplementation in women. However, it contrasts with a study showing that elderly women with a higher body fat percentage absorbed more β-carotene than did young women receiving the same single dose of 15 mg β-carotene (16).

It was suggested from previous supplementation studies that the serum concentration at entry was positively related to the concentration after supplementation (14, 17). This is also confirmed in the present study (data not shown). However, when the magnitude of the change was taken into account, a negative association was observed (Table 6). Subjects with higher baseline carotenoid concentrations tended to have smaller increases in serum concentrations compared with those with lower baseline concentrations.

Effect of β-carotene supplementation on serum β-carotene concentration

We observed that β-carotene supplementation for 24 mo resulted in an increase in serum β-carotene concentrations, 1073% in men and 839% in women (Fig 1). The 10-fold increase is consistent with other long-term supplementation studies. In a study of 220 apparently healthy Finnish men, aged 30–69 y, a supplement of 20 mg/d for 2 mo increased serum β-carotene 10-fold (17). About the same magnitude increase was reported after supplementation with 50 β-carotene/d for 1 y in a Skin Cancer Prevention Study (14). These results together suggest that the steady β-carotene serum concentration is usually ≈10-fold more

than the normal concentration, regardless of whether subjects are healthy, have a history of nonmelanoma skin cancer, or have had a colorectal polypectomy. Carotenodermia after intensive supplementation indicates the accumulation of β-carotene in skin after a steady concentration in serum has been reached (18–20). Adipose tissue, liver, and other tissues may also serve as a body pool for the storage and mobilization of carotenoids (21).

Effect of β-carotene supplementation on other carotenoids

An elevation in α-carotene concentration was observed in men after placebo supplementation. However, the increase was considerably higher in male and female subjects who took active β-carotene capsules (Fig 1). Although we found no α-carotene in the capsules by the HPLC method, contamination of α-carotene at a subdetectable level may be an explanation for the increase in serum α-carotene concentration. A similar result was reported by Micozzi et al (4), who found an increase in α-carotene concentration after supplementation with 12 or 30 mg β-carotene/d for 6 wk. An analytical artifact was suggested to be a possible explanation for this observation. We tested the HPLC method used in the present study by measuring concentrations of α-carotene and β-carotene in plain serum and the plain sera with various amounts of β-carotene standard solution to simulate the elevation of β-carotene concentration. No significant change in α-carotene values was observed. The analysis artifact, therefore, would not be an explanation for the increase of α-carotene. No lycopene was detected by HPLC in the capsules administered. And again, the analytical artifact could not be an explanation for the rise in lycopene concentration in men (176% in the supplement group vs 72% in the placebo group) because chromatograms showed the complete resolution of lycopene and β-carotene.

In the study of Micozzi et al (4), a decline in plasma lycopene concentration was reported after supplementation with 12- and 30-mg β-carotene capsules, carrots, and broccoli for 6 wk. The decline was due to the lycopene-restricted diet used in the study. But this was not the case in the present study, in which the subjects were not instructed to alter their diet with respect to carotenoid-containing foods.

The increase in serum concentrations of lycopene, and probably α-carotene, after β-carotene supplementation has led us to hypothesize that β-carotene affects the metabolism of other carotenoids. Lycopene, α-carotene, and β-carotene have been categorized as hydrocarbon carotenoids or carotenes (22). These carotenoids may share an absorption pathway, which may be different from that of oxygen-containing carotenoids or xanthophylls, such as lutein, zeaxanthin, and cryptoxanthin. When the system is boosted by β-carotene, the absorption of other carotenoids may also be elevated. Some carotenoids are directly absorbed and pass into the blood (23) and then deposited in the liver or organs such as kidney, adrenal gland, and testes (24).

Another possibility is that β-carotene has a biological function or functions in common with other carotenoids or their metabolites so that it is able to substitute for the others. In the case of β-carotene supplementation, the reserve or supply of carotenoids may be replenished and dominated by β-carotene, resulting in a suppression of the utilization or catabolism of other carotenoids for that function. It is unlikely that conversion to vitamin A is required for such a function or functions because lycopene, which was found to be increased, does not have provitamin A activity.

Until recently the production of a specific carotenoid de novo or by oxidative metabolism of ingested carotenoids had not been reported (25). In a study of in vivo metabolism of β-carotene, it has been suggested that the ionone rings of β-carotene were cleaved and resulted in the production of lycopene (26). The presence of lycopene in feces with the near absence of β-apo-8′-carotenal (one of the oxidative cleavage products) after β-carotene administration provides evidence that the cleavage of ionone rings is more likely to occur than that of the side chain of β-carotene. Increase in serum lycopene concentration, nevertheless, was not reported.

The activity of β-carotene to inhibit the oxidation of LDL has been widely studied (27–30). Several lines of evidence indicate that the increased susceptibility of LDL to oxidation is associated with the increased severity of coronary atherosclerosis (31, 32). In addition, it has been hypothesized that the singlet oxygen–quenching property of β-carotene contributes to its potential chemopreventive action (33). It has been reported that lycopene is the most effective singlet oxygen quencher when compared with β-carotene or α-tocopherol (34), and that β-cryptoxanthin and zeaxanthin play a role in preventing cigarette smoking–related cancer (35). Nevertheless, the biological or clinical significance of these carotenoids remains unverified. It would appear that much work needs to be done to clarify the mechanism of absorption, metabolism and biological functions of carotenoids other than β-carotene.

In summary, α-carotene and lycopene, in addition to β-carotene, were found to be increased after β-carotene supplementation over a 24-mo period after adjustment for baseline concentration, changes in dietary intake, and other confounders. A biological interaction between β-carotene and other carotenoids is therefore suggested and would need to be taken into account in the evaluation of any protective effect of β-carotene supplementation for cancer, coronary heart disease, or other diseases.

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