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Effects of Three-month Oral Supplementation of β-Carotene and Vitamin C on Serum Concentrations of Carotenoids and Vitamins in Middle-aged Subjects: A Pilot Study for a Randomized Controlled Trial to Prevent Gastric Cancer in High-risk Japanese Population

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Prior to a randomized controlled trial to prevent gastric cancer by oral supplementation of β-carotene and vitamin C in a high-risk Japanese population, we examined the serum response to three-month oral supplementation of β-carotene (0, 3, 30 mg/day) and vitamin C (0, 50, 1000 mg/day) by a three-by-three factorial design using 54 subjects (age range=40–69 years). Serum concentrations of carotenoids, α-tocopherol, and ascorbic acid were examined at baseline, and one, two, and three-month points. Both serum β-carotene and ascorbic acid were significantly higher in high-dose groups than in each placebo group during the supplementation. The serum β-carotene increased gradually (597–830% increase) during the study, whereas the serum ascorbic acid reached nearly a steady-state at the one-month point and remained stable thereafter (88–95% increase). No statistically significant interaction between β-carotene and vitamin C supplementations was observed either for serum β-carotene or for serum ascorbic acid. Among carotenoids and α-tocopherol examined, serum lycopene in the high-dose β-carotene group was significantly higher than in the placebo group at all points. No unfavorable change in carotenoids and α-tocopherol was observed in any group.

Key words: β-Carotene — Ascorbic acid — Supplements — Nutrient — Randomized controlled trial

Gastric cancer has long been the most common cancer in Japan.[1)] Several epidemiologic studies have reported protective effects of high intake of β-carotene and vitamin C (ascorbic acid) and their high serum or plasma concentration levels against gastric cancer.[2,3)] Vitamin C in fruits and vegetables is considered to be a preventative substance inhibiting nitrosamine formation and also acting as a free radical scavenger.[2,4)] Atrophic gastritis is considered to be a precursor of intestinal-type gastric cancer.[5)] A protective effect of β-carotene against the progression of atrophic gastritis has been reported.[6)]

Based on these findings, we planned a randomized controlled trial to test the preventative effect of oral β-carotene and vitamin C supplementation for a high-risk population, i.e., subjects with atrophic gastritis.[7)] However, the effects of oral supplementation of β-carotene and vitamin C on the serum concentrations have not yet been established in Japanese populations. Prior to the study, we conducted a pilot study using subjects of a health check-up program to evaluate the effect of supplementation of β-carotene and vitamin C on the serum levels.[8)]

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MATERIALS AND METHODS

Study design The study design has been reported elsewhere.[8)] Briefly, the subjects were recipients of a health check-up program in a local general hospital with chronic atrophic gastritis diagnosed on the basis of pepsinogen I less than 70 mg/ml and pepsinogen I/pepsinogen II ratio less then 3.0. The age ranged from 40 to 69 years. Among 90 subjects screened, 55 subjects, 35 men and 20 women, participated in the trial. The participants were randomly assigned to one of nine possible groups according to a three-by-three factorial design with three levels of β-carotene (0, 3, or 30 mg/day) and vitamin C (0, 50, or 1000 mg/day), and were asked to consume a total of four capsules (three for β-carotene and one for vitamin C) each day for three months. The appearance of the capsules was the same regardless of the content of β-carotene or vitamin C, and neither the participants nor the hospital staff were informed of the content. During the trial, clinically rele-

vant symptoms were observed in two cases, i.e., erythema of the body trunk and yellowing of the palms. The former subject dropped out, and the remaining 54 subjects, 34 men and 20 women, consumed 97% of the supplements and completed the trial. This study was started in January, 1995 and ended in September, 1995. The protocol of this study was submitted to the ethical committees of both the National Cancer Center and Hiraka General Hospital and approved by them.[8)]

Serum carotenoids, ascorbic acid, and α-tocopherol levels Serum was sampled at baseline, and one, two and three months after the supplementation started. The subjects were asked not to eat or drink anything except water after 21 o'clock on the day before the blood sampling. The serum was sampled between 9 and 11 o'clock in the morning. Sera for ascorbic acid measurement were stabilized by addition of *meta*-phosphoric acid. Serum concentrations of β-carotene, retinol, lutein, β-cryptoxanthin, lycopene, α-carotene, and α-tocopherol were determined by high-performance liquid chromatography. The level of serum ascorbic acid was analyzed fluorimetrically (iodine oxidation and condensation with 1,2-phenylenediamine). All samples were stored at −70 to −80°C and were analyzed simultaneously after the completion of the trial. All assays were conducted by persons who were blinded as to the intervention assignment and the questionnaire data.

Baseline data Body height and body weight were measured with light garments. Alcohol intake and cigarette consumption were assessed by a simple semiquantitative frequency questionnaire. The subjects who reported a frequency of alcoholic intake of once per month or more were considered as current drinkers. For smoking status, only current cigarette smokers were considered as smokers. Serum total and high-density-lipoprotein (HDL) cholesterol levels at baseline were analyzed enzymatically with an auto-analyzer.

Statistical analysis Means±standard deviations of age, body height, body weight, body mass index (BMI), serum cholesterol, HDL cholesterol, alcohol intake, and cigarette consumption at baseline were calculated by β-carotene or vitamin C supplementation group. Their differences between groups was examined by one-way analysis of variance (ANOVA). Percentages of men, habitual alcohol consumers, and current smokers at baseline were also calculated by supplementation group.

For serum carotenoids and α-tocopherol, the difference of the means between β-carotene supplementation groups at each point was examined using Dunnett's method of one-way ANOVA with the value of the placebo group as a reference. The difference of the means between points within a group was examined using Dunnett's method of repeated one-way ANOVA with the baseline value as a reference. The same analyses were performed for serum ascorbic acid between vitamin C supplementation groups. Interaction effect by β-carotene and vitamin C supplementations was examined by two-way ANOVA.

The effect of possible confounding factors was examined by multiple regression analysis with a backward elimination procedure. Sex, alcohol intake, cigarette consumption, serum β-carotene or ascorbic acid concentration at baseline, HDL cholesterol (for β-carotene only), and two dummy variables for supplementation group were included simultaneously in the model as independent variables. The change in serum β-carotene or ascorbic acid concentration from baseline at the one-, two-, or three-month point was used as the dependent variable for each model. For serum β-carotene and ascorbic acid concentrations, log-transformed values were used because of their positively skewed distributions. The significance level was set at <0.05. All analyses were done with SAS statistical software (SAS Institute Inc., Cary, NC).

RESULTS

Table I shows the baseline characteristics of subjects by vitamin supplementation group. For the variables examined, no statistically significant difference was observed between groups.

Table II shows the serum β-carotene and ascorbic acid concentrations at each point by supplementation group. At each point during supplementation, the high-dose groups showed a significantly higher concentration both for serum β-carotene and ascorbic acid ($P<0.001$). The serum β-carotene increased gradually (597−830% increase) during the supplementation, whereas the serum ascorbic acid reached nearly a steady state at the one-month point and remained stable thereafter (88−94% increase). No statistically significant difference was observed between the placebo and the low-dose group at any point of supplementation either for β-carotene or ascorbic acid. No statistically significant interaction effect by β-carotene and vitamin C supplementations was observed either for serum β-carotene or for serum ascorbic acid. Table II also shows the ranges and their percent changes. For β-carotene concentration, quite a wide range was observed for the percent change in both supplementation groups. In the high-dose group, the concentration only doubled for the subjects with the lowest response, whereas it increased by 20 times for the subjects with the highest response. For ascorbic acid concentration, some subjects did not respond at all to the supplementation. Although the response to the supplementation was different among the subjects, the ranges of percent change in the two supplementation groups were not as wide as those observed for β-carotene.

Table III shows the results of multiple regression analysis. For β-carotene, the dummy variables for supplementation groups and serum β-carotene concentration at baseline significantly and positively correlated with the change in

Table I. Baseline Characteristics of Subjects (Means±Standard Deviations unless Otherwise Indicated)

Supplementation	β-Carotene			Vitamin C			All subjects
Dose of supplementation	Placebo 0 mg/day	Low 3 mg/day	High 30 mg/day	Placebo 0 mg/day	Low 50 mg/day	High 1000 mg/day	
Number of subjects	17	19	18	18	16	20	54
Age (years)	53.9±7.8	53.1±7.3	52.1±6.7	52.6±6.2	53.4±8.2	53.1±7.5	53.0±7.2
Sex (% male)	65	58	67	72	56	60	63
Body weight (kg)	59.4±9.9	62.0±10.1	62.2±8.1	63.0±10.4	62.1±9.7	59.0±8.0	61.3±9.3
Body height (cm)	161.3±9.0	162.1±10.7	163.0±7.8	164.7±10.8	161.3±7.8	160.6±8.5	162.2±9.1
Body mass index (kg/m^2)	22.8±2.6	23.5±2.5	23.4±2.5	23.1±2.3	23.8±2.8	22.9±2.4	23.2±2.5
Serum cholesterol (mmol/liter)	5.17±0.78	4.92±0.70	5.36±0.87	5.11±0.86	5.02±0.51	5.28±0.92	5.14±0.79
HDL cholesterol (mmol/liter)	1.73±0.38	1.62±0.57	1.63±0.44	1.65±0.50	1.84±0.53	1.53±0.35	1.66±0.47
Alcohol intake (g/day)	18±23	24±27	16±22	28±27	17±24	14±20	19±24
Current drinkers (%)	71	74	61	83	56	65	69
Cigarette consumption (cigarettes/day)	3.5±8.6	5.5±9.8	4.6±11.4	4.1±9.1	5.3±12.3	4.5±8.9	4.6±9.9
Current smokers (%)	22	26	22	22	19	25	22

Table II. Serum Concentrations of β-Carotene and Ascorbic Acid by Supplementation Group: Means±Standard Deviations (SDs), Ranges, and Percent Changes from the Baseline

		Baseline		Duration of supplementation										
				One month				Two months				Three months		
	n	Mean±SD (Range)		Mean±SD (Range)			% change (Range)	Mean±SD (Range)			% change (Range)	Mean±SD (Range)			% change (Range)
Serum β-carotene (μmol/liter)														
β-Carotene supplementation														
Placebo (0 mg/day)	17	0.48±0.07	—	0.54±0.15	—	n.s.	12	0.61±0.28	—	n.s.	25	0.61±0.24	—	n.s.	25
		(0.40, 0.68)		(0.41, 0.94)			(−26, 54)	(0.40, 1.60)			(−20, 135)	(0.41, 1.38)			(−12, 104)
Low dose (3 mg/day)	19	0.47±0.13	n.s.	1.14±1.21	n.s.	n.s.	124	1.43±1.66	n.s.	n.s.	175	1.43±1.38	n.s.	n.s.	171
		(0.40, 0.96)		(0.41, 4.60)			(1, 909)	(0.44, 6.07)			(7, 1232)	(0.42, 5.29)			(3, 606)
High dose (30 mg/day)	18	0.56±0.24	n.s.	4.10±2.94	***	###	597	4.94±3.32	***	###	756	5.48±3.35	***	###	830
		(0.40, 1.21)		(0.59, 12.0)			(46, 1645)	(0.93, 11.7)			(98, 2176)	(0.96, 12.4)			(138, 1687)
Serum ascorbic acid (mmol/liter)														
Vitamin C supplementation														
Placebo (0 mg/day)	18	80.1±28.4	—	96.0±27.8	—	n.s.	31	98.2±25.0	—	n.s.	37	96.0±23.3	—	n.s.	31
		(24.8, 153.1)		(59.3, 185.1)			(−19, 248)	(65.9, 152.2)			(−29, 269)	(64.5, 146.3)			(−21, 240)
Low dose (50 mg/day)	16	83.5±34.6	n.s.	94.8±29.0	n.s.	n.s.	23	94.8±25.6	n.s.	n.s.	23	100.5±31.2	n.s.	n.s.	29
		(33.6, 175.2)		(64.9, 174.7)			(−20, 151)	(62.2, 150.8)			(−27, 95)	(71.7, 167.1)			(−5, 114)
High dose (1000 mg/day)	20	79.5±25.0	n.s.	139.7±33.5	***	###	88	141.4±35.8	***	###	95	143.7±38.0	***	###	94
		(38.3, 155.8)		(80.8, 198.0)			(7, 315)	(75.2, 213.5)			(−13, 276)	(68.1, 215.7)			(−4, 311)

Comparison between groups at each point: Dunnett's method of ANOVA with the placebo group as reference: n.s.: not significant (P≥0.05), *** P<0.001.
Comparison between points within a group: Dunnett's method of repeated ANOVA with the baseline concentration as reference: n.s.: not significant (P≥0.05), ### P<0.001.

the serum concentration at all three points. For vitamin C, the dummy variable for high-dose supplementation group positively and the serum ascorbic acid concentration at baseline negatively and significantly correlated with the change in the serum ascorbic acid concentration at all three points. Cigarette consumption marginally, but significantly, and positively correlated with serum ascorbic acid at the three-month point. The levels of determination coefficients and partial regression coefficients of the dummy variables both for high- and low-dose groups gradually increased until the three-month point for β-carotene, whereas they were nearly constant for vitamin C.

Table IV shows the serum concentrations of carotenoids and α-tocopherol by β-carotene supplementation group. In the case of lycopene alone, a significantly higher concentration was observed during the supplementation compared to the placebo group (P<0.05). A decrease in serum cryptoxanthin and a slight increase in serum lutein were observed when the mean at each point was compared to the baseline value by supplementation group. But no sta-

Table III. Multiple Regression Analysis with Backward Elimination Procedure for the Changes in Serum β-Carotene or Ascorbic Acid Concentration with Possible Determinant Factors at the Baseline (n=54)

Dependent variable	Duration of supplementation		
Independent variables[a)]	One month	Two months	Three months
Change from baseline in log-transformed serum β-carotene (μmol/liter)			
Determination coefficient	0.71	0.70	0.81
Dummy variable for supplementation (low dose=1, other=0)[b)]	0.25 *	0.30 *	0.33 ***
Dummy variable for supplementation (high dose=1, other=0)[b)]	0.91 ***	1.00 ***	1.10 ***
Log-transformed serum β-carotene at baseline (μmol/liter)[b)]	1.97 ***	2.16 ***	2.40 ***
Intercept	−0.73	−0.48	−0.79
Change from baseline in log-transformed serum ascorbic acid (mmol/liter)			
Determination coefficient	0.64	0.65	0.65
Dummy variable for supplementation (low dose=1, other=0)[b)]	—	—	—
Dummy variable for supplementation (high dose=1, other=0)[b)]	0.38 ***	0.38 ***	0.37 ***
Log-transformed serum ascorbic acid at baseline (mmol/liter)[b)]	−0.64 ***	−0.77 ***	−0.61 ***
Cigarette consumption (cigarettes/day)[b)]	—	—	0.007 *
Intercept	2.95	3.56	2.74

a) Dummy variable for sex (man=1, woman=2), alcohol intake (g/day), cigarette consumption (cigarettes/day), and HDL cholesterol (mmol/liter) (for β-carotene only) were also included in the models as independent variables.
b) Partial regression coefficient (β) and the significance level. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

tistically significant difference was observed between groups at each point for these carotenoids. Table IV also shows the ranges of the concentrations and their percent changes. Among the concentrations examined, the decrease in the highest values for cryptoxanthin was marked in all three groups.

DISCUSSION

In this study, both for β-carotene and vitamin C, only the high-dose groups showed a significant increase in the serum concentrations. The low-dose groups did not show a significant increase in the serum concentrations either for β-carotene or vitamin C. We estimated carotene and vitamin C intake levels using a self-administered semiquantitative food frequency questionnaire slightly modified from one developed for a large-scale prospective study in Japan.[9)] The supplemented doses in the low-dose groups might not be high enough, because they were similar to or lower than the estimated intake levels from foods, 3.2 mg/day for carotene and 136 mg/day for vitamin C in the subjects, and the number of subjects was not large enough to examine the effect of these relatively small increases in ingestion.

Serum β-carotene concentration increased by 597–830% in the high-dose group. The increase was within the range observed in three previous studies which examined the effect of β-carotene supplementation at similar doses, i.e., 842%,[10)] 664% in men and 431% in women,[11)] and 1470%.[12)] The serum concentration gradually increased during the supplementation, although a marked increase was observed at the one-month point (Table II). One study with 15 mg daily oral supplementation of β-carotene observed an increase plateauing after the four-month point.[13)] Another study with 30 mg daily oral supplementation of β-carotene for 42 days observed a gradual increase during the study, though most of the increase was observed within the first 21 days.[14)] The serum response, both for the level of increase and the time needed to reach a steady state, observed in this study was comparable to those in the previous studies.

Compared to β-carotene, the increase in serum ascorbic acid afforded by high-dose vitamin C supplementation was low, 88–94%, although it was highly significant. The previous study reported that an increase in vitamin C intake from 200 to 2500 mg/day caused a plasma increase by 1.2 to 1.5 μg/dl.[15)] This is comparable to the increase observed in this study, i.e., 64.2 mmol/liter (1.1 μg/dl) in the high-dose group (Table II). It appears that the serum level obtained in this study was almost saturated in the high-dose group. In contrast to β-carotene, the serum level of ascorbic acid reached nearly this level at the one-month point.

Several lifestyle factors have been postulated as predictors of serum β-carotene and/or serum ascorbic acid concentrations, i.e., sex, smoking, alcohol intake, HDL cholesterol, and dietary intakes of β-carotene or vitamin C.[11, 13, 16–20)] However, in this study, neither sex, alcohol intake, cigarette consumption, nor HDL cholesterol significantly correlated with the change in serum β-carotene or

Table IV. Serum Concentrations (μmol/liter) of Carotenoids, α-Carotene, and α-Tocopherol by β-Carotene Supplementation Group: Means±Standard Deviations (SDs), Ranges, and Percent Changes from the Baseline

		Baseline		Duration of supplementation								
				One month			Two months			Three months		
	n	Mean±SD (Range)		Mean±SD (Range)		% change (Range)	Mean±SD (Range)		% change (Range)	Mean±SD (Range)		% change (Range)
Retinol												
Placebo	17	2.09±0.66	—	2.16±0.80	— n.s.	3	2.16±0.71	— n.s.	5	2.20±0.70	— n.s.	7
(0 mg/day)		(0.99-3.43)		(1.14-3.97)		(−17, 44)	(1.06-3.81)		(−15, 54)	(1.04-3.71)		(−15, 36)
Low dose	19	2.23±0.85	n.s.	2.37±0.84	n.s. n.s.	10	2.17±0.62	n.s. n.s.	4	2.29±0.84	n.s. n.s.	7
(3 mg/day)		(0.62-3.89)		(1.14-3.85)		(−22, 85)	(1.10-3.50)		(−38, 88)	(1.13-4.15)		(−28, 83)
High dose	18	2.22±0.46	n.s.	2.18±0.59	n.s. n.s.	−2	2.16±0.56	n.s. n.s.	−3	2.11±0.48	n.s. n.s.	−4
(30 mg/day)		(1.31-2.87)		(1.17-3.17)		(−40, 30)	(1.47-3.10)		(−36, 25)	(1.25-2.83)		(−28, 29)
Lutein												
Placebo	17	0.42±0.13	—	0.57±0.13	— #	63	0.68±0.19	— ###	96	0.64±0.23	— ##	77
(0 mg/day)		(0.08-0.64)		(0.26-0.83)		(−36, 527)	(0.36-0.96)		(−20, 693)	(0.31-1.19)		(−17, 550)
Low dose	19	0.37±0.16	n.s.	0.49±0.14	n.s. n.s.	46	0.51±0.16	* #	55	0.54±0.23	n.s. #	61
(3 mg/day)		(0.18-0.76)		(0.26-0.78)		(−24, 139)	(0.33-0.90)		(−16, 147)	(0.23-1.29)		(−42, 212)
High dose	18	0.56±0.34	n.s.	0.60±0.34	n.s. n.s.	19	0.55±0.24	n.s. n.s.	9	0.62±0.38	n.s. n.s.	20
(30 mg/day)		(0.28-1.64)		(0.24-1.53)		(−47, 118)	(0.21-1.12)		(−59, 81)	(0.25-1.64)		(−45, 201)
Cryptoxanthin												
Placebo	17	0.14±0.09	—	0.09±0.04	— #	−28	0.07±0.03	— ##	−39	0.06±0.02	— ###	−46
(0 mg/day)		(0.05-0.37)		(0.04-0.20)		(−64, 12)	(0.03-0.16)		(−72, 0)	(0.03-0.09)		(−77, −7)
Low dose	19	0.15±0.17	n.s.	0.09±0.08	n.s. n.s.	−19	0.07±0.04	n.s. #	−29	0.07±0.04	n.s. #	−33
(3 mg/day)		(0.03-0.74)		(0.03-0.35)		(−76, 52)	(0.03-0.17)		(−78, 51)	(0.02-0.17)		(−86, 46)
High dose	18	0.21±0.16	n.s.	0.10±0.05	n.s. ###	−38	0.08±0.03	n.s. ###	−43	0.08±0.03	n.s. ###	−45
(30 mg/day)		(0.05-0.52)		(0.04-0.22)		(−79, 54)	(0.04-0.14)		(−84, 32)	(0.04-0.15)		(−87, 22)
Lycopene												
Placebo	17	0.12±0.03	—	0.13±0.04	— n.s.	3	0.13±0.03	— n.s.	4	0.13±0.03	— n.s.	8
(0 mg/day)		(0.11-0.22)		(0.11-0.28)		(−17, 26)	(0.10-0.20)		(−20, 60)	(0.11-0.23)		(−19, 58)
Low dose	19	0.13±0.04	n.s.	0.14±0.04	n.s. n.s.	16	0.15±0.04	n.s. n.s.	25	0.14±0.04	n.s. n.s.	18
(3 mg/day)		(0.11-0.23)		(0.11-0.27)		(−28, 62)	(0.11-0.25)		(−29, 74)	(0.11-0.24)		(−26, 75)
High dose	18	0.13±0.02	n.s.	0.17±0.06	* n.s.	33	0.18±0.07	** #	35	0.18±0.06	* #	38
(30 mg/day)		(0.11-0.18)		(0.10-0.31)		(−17, 138)	(0.11-0.32)		(−15, 145)	(0.11-0.33)		(−17, 153)
α-Carotene												
Placebo	17	0.18±0.02	—	0.19±0.02	— n.s.	6	0.21±0.04	— n.s.	13	0.21±0.04	— n.s.	16
(0 mg/day)		(0.15-0.23)		(0.16-0.25)		(−16, 21)	(0.16-0.31)		(−15, 43)	(0.15-0.30)		(−9, 67)
Low dose	19	0.18±0.04	n.s.	0.23±0.12	n.s. n.s.	23	0.25±0.17	n.s. n.s.	30	0.26±0.18	n.s. n.s.	34
(3 mg/day)		(0.16-0.31)		(0.16-0.66)		(−5, 149)	(0.16-0.85)		(−4, 216)	(0.16-0.94)		(−10, 201)
High dose	18	0.21±0.05	n.s.	0.24±0.07	n.s. n.s.	22	0.26±0.09	n.s. n.s.	28	0.26±0.08	n.s. n.s.	30
(30 mg/day)		(0.15-0.32)		(0.16-0.43)		(−20, 120)	(0.16-0.43)		(−33, 147)	(0.16-0.41)		(−25, 131)
α-Tocopherol												
Placebo	17	20.7±7.1	—	22.0±7.4	— n.s.	7	22.1±8.0	— n.s.	8	20.4±7.6	— n.s.	−1
(0 mg/day)		(12.4-40.4)		(14.2-42.0)		(−16, 29)	(14.0-42.4)		(−20, 49)	(13.0-38.6)		(−39, 32)
Low dose	19	18.0±4.4	n.s.	19.8±6.3	n.s. n.s.	13	18.7±5.7	n.s. n.s.	7	18.5±6.3	n.s. n.s.	6
(3 mg/day)		(8.70-25.9)		(11.2-37.2)		(−27, 107)	(11.0-35.9)		(−22, 146)	(10.3-36.7)		(−49, 151)
High dose	18	19.8±4.5	n.s.	19.4±5.4	n.s. n.s.	−1	18.8±3.7	n.s. n.s.	−3	20.0±5.2	n.s. n.s.	2
(30 mg/day)		(11.7-30.0)		(13.3-31.7)		(−22, 38)	(13.5-28.9)		(−32, 25)	(15.1-37.1)		(−31, 29)

Comparison between groups at each point: Dunnett's method of ANOVA with the placebo group as reference: n.s.: not significant ($P \geq 0.05$), * $P<0.05$, ** $P<0.01$.
Comparison between points within a group: Dunnett's method of repeated ANOVA with the baseline concentration as reference: n.s.: not significant ($P \geq 0.05$), # $P<0.05$, ## $P<0.01$, ### $P<0.001$.

ascorbic acid after the level of supplementation and the concentration at baseline were controlled for, with the sole exception of cigarette consumption (Table III). This may be due to the small number of study subjects.

The concentration at baseline correlated with the subsequent change in the opposite direction between β-carotene and ascorbic acid, i.e., positively and negatively respectively, after possible confounding factors and the level of supplementation were controlled for (Table III). Because the correlation was negative for vitamin C, the supplemented dose seems to be high enough to obtain the maximal level in serum. On the other hand, the results supported the wide inter-individual variation of bioavailability for β-carotene observed in several previous

studies.[13, 17, 21] In this case, side effects caused by extreme response to supplementation should be monitored carefully. During the study, no apparent side effect was observed except in two cases, as mentioned previously.[7] On the other hand, we observed some subjects who showed no response to the supplementations with β-carotene or vitamin C (Table II). Quite a wide range of response was observed, especially for β-carotene. These differential responses among subjects may explain the differential diet (β-carotene and vitamin C intakes)-disease (gastric cancer) association at an individual level. Determinant factors of the difference should be examined in future studies.

We also examined the changes in serum concentrations of other carotenoids and α-tocopherol. Among them, only lycopene showed a significant increase in the high-dose β-carotene group compared to the placebo group (Table IV). An increase in serum lycopene by β-carotene supplementation was reported in previous studies.[12, 22, 23] A significant increase was also reported for serum or plasma concentration of α-carotene[12, 14, 22] and lutein/zeaxanthin[11] after the supplementation of β-carotene. On the other hand, some other studies reported contradictory results, i.e., decrease in lutein/zeaxanthin[14, 22–24] and lycopene.[14] No significant change was observed for α-tocopherol in this study. Most of the previous studies also reported no significant change,[10, 12, 25, 26] except one study.[27] Because the changes in concentration of these carotenoids and α-tocopherol were much smaller than those of β-carotene, a larger sample size may be necessary to examine the effect of β-carotene supplementation on the changes in their serum concentrations.

This study confirmed that a significant increase in serum concentrations of β-carotene and ascorbic acid could be obtained by oral 30 mg/day β-carotene and 1000 mg/day vitamin C supplementation, respectively, without any interaction effect or apparent side effect on serum concentrations of other carotenoids and α-tocopherol in middle-aged Japanese subjects.

But, in response to a National Cancer Institute press report indicating potential harm from β-carotene supplementation in January, 1996,[28] the β-carotene supplementation, which had been re-started after this pilot trial had been completed, was halted and the same protocol modification was done in the main trial which had already been started.[7]

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