ORIGINAL PAPER

Nagoya J. Med. Sci. **84**. 607–620, 2022
doi:10.18999/nagjms.84.3.607

Association between serum carotenoids and premature mortality in a population-based case-control study

Ryosuke Fujii[1], Koji Suzuki[1], Hiroya Yamada[2], Miyuki Kawado[2], Shuji Hashimoto[2], Yoshiki Tsuboi[1], Kenji Wakai[3], Hiroyasu Iso[4], Yoshiyuki Watanabe[5], Yoshihisa Fujino[6], Akiko Tamakoshi[7] and JACC Study Group

[1]*Department of Preventive Medical Sciences, Fujita Health University School of Medical Sciences, Toyoake, Japan*
[2]*Department of Hygiene, Fujita Health University School of Medicine, Toyoake, Japan*
[3]*Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[4]*Public Health, Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Suita, Japan*
[5]*Department of Nursing, Faculty of Health and Medical Sciences, Kyoto University of Advanced Science, Kyoto, Japan*
[6]*Department of Environmental Epidemiology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan*
[7]*Department of Public Health, Hokkaido University Faculty of Medicine, Sapporo, Japan*

ABSTRACT

Carotenoids are abundant pigments mainly contained in vegetables and fruits, and show antioxidant properties by quenching free radicals in human body. Few studies have investigated associations between serum carotenoid levels and premature mortality. The objective of this study was to investigate the association between serum carotenoid level and premature mortality in a Japanese population. This study included 446 Japanese adults (174 men, aged of 40–64) recruited as participants in the Japan Collaborative Cohort (JACC) Study. Serum carotenoid level was measured by high-performance liquid chromatography. Premature mortality was defined as death before 65 years old during the follow-up period. Premature mortality was ascertained in 60 men (34.5%) and 65 women (23.9%). In men, compared to the 1st tertile of serum β-cryptoxanthin and provitamin A, those who were in the 3rd tertile had lower risks of premature all-cause mortality (OR, 95% CI: 0.19, 0.07–0.47 for β-cryptoxanthin, and 0.24, 0.09–0.61 for provitamin A). In women, compared to the 1st tertile of serum β-cryptoxanthin, those who were in the 3rd tertile had higher risks of premature all-cause mortality (OR, 95% CI: 1.94, 1.00–4.03). These significant associations were observed in analyses for premature cancer mortality. We found significant associations between higher levels of serum β-cryptoxanthin and provitamin A and lower risks of premature mortality among Japanese men, while a different directional association was found in women. Although these findings suggest roles of serum carotenoids on premature mortality, further studies are needed to validate this association in other populations.

Keywords: carotenoid, premature mortality, case-control study, cancer mortality, epidemiology

Received: July 21, 2021; accepted: November 10, 2021

Corresponding Author: Koji Suzuki, PhD
Department of Preventive Medical Sciences, Fujita Health University School of Medical Sciences, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake 470-1192, Japan
Tel: +81-562-93-2537, Fax: +81-562-93-4595, E-mail: ksuzuki@fujita-hu.ac.jp

Abbreviations:
CI: confidence interval
CVD: cardiovascular disease
OR: odds ratio

INTRODUCTION

Fruit and vegetable intake is associated with reduced risks of total and cause-specific mortality.[1-3] Such preventive effects of dietary vegetable and fruit intake may be attributable to antioxidant properties derived from carotenoids and other vitamins. Carotenoids are abundant as pigments in vegetables and fruits, and show antioxidant properties by quenching free radicals.[4] In addition to playing a role as an antioxidant, serum level of carotenoid is thought to offer an objective and reliable marker of vegetable intake than food frequency questionnaire.[5] The half-life of serum carotenoids is usually a few weeks, and serum carotenoid level can thus reflect the relatively long-term intake of vegetables and fruits.[6,7] In line with previous studies on dietary intake of vegetables and fruit, serum carotenoids levels are associated with decreased risk of mortality and incidence from cancer and cardiovascular disease (CVD) in US, European, and Japanese populations.[8-16]

Life expectancy in developed countries has increased substantially over a few decades, especially in Japan (81.09 years for men and 87.26 years for women in 2017).[17] However, there are a certain number of people who die before the age of 60 to 70, and they are defined as premature mortality. In fact, more than 25% of deaths are premature mortality even in high income countries.[18] In other words, reducing premature mortality is a matter of concern not only in low-middle income countries, but also in high income countries. From an epidemiological viewpoint, understanding the clinical and general background of premature mortality may be the first step in reducing premature mortality. Previous studies have reported several risk factors for premature mortality, including smoking, drinking alcohol, low income, poor educational level, decreased mental health, dietary intake, and preexisting condition.[19-25] However, no previous study is available on association between serum carotenoids, as biomarkers of dietary vegetable and fruit intake, and premature mortality. Investigating this association with a long-term follow-up period will provide insight into whether prediagnostic serum carotenoid levels may be a long-term marker for predicting reduced healthy life expectancy. Therefore, we aimed to assess the association between serum carotenoid levels and premature mortality in a Japanese population.

MATERIALS AND METHODS

Study design and participants

The baseline survey of the Japan Collaborative Cohort (JACC) Study was conducted in 45 study sites across Japan between 1988 and 1990. A total of 110,585 adults between 40 and 79 years old living in study sites were enrolled to the study. Details of this cohort study have been published elsewhere.[26] We collected information from a self-administered questionnaire regarding demographic features (sex, age, educational level, and study site), lifestyle (smoking status, drinking status, and leisure-time activity) and clinical history of non-communicable diseases (cancer, CVD, or diabetes). We conducted follow-up of eligible participants to identify each new death from the baseline survey through to the end of 2009. We reviewed death certificates

and classified the underlying causes of death as coded by the National Vital Statistics according to the *International Statistical Classification of Diseases and Related Health Problems 10th Revision* (*ICD-10*). All deaths during follow-up were ascertained by population registries in each municipality. We defined participants who died from all causes before 65 years old during follow-up as all-cause premature mortality (case) in this study. In Japan, the population aged 65 and above is summarized in vital statistics as the "elderly". Therefore, we defined those who died before this period as cases in this study. In addition, we also examined those who died from cancer before 65 years old as premature cancer mortality (*ICD-10*, C00-97). Deaths caused by external injury (*ICD-10*, S00-T98, V01-Y98, and Z00-Z98) were not included as cases, because we focused on natural premature death. We defined the following two types of participants as control in this study: 1) those still alive at the end of the follow-up survey; or 2) those who died 65 years old or older during the follow-up survey. We targeted 80,629 individuals aged 40–64 years at the baseline survey from 1988 to 1990 and followed up them until 2009. The selection for participants were retrospectively performed after the end of the follow-up period. Of the 68,925 individuals in the blood sample collection areas, we collected blood samples from 30,193 individuals. Of these, we excluded 4,761 participants from whom blood samples were unavailable because the samples had been used in previous case-control studies for cancer incidence, then 25,432 participants were available for this study.[27-29] Among those who are available in serum

Table 1 Premature mortality causes coded for *ICD-10*

Premature mortality (*ICD-10* code)	**Number (n)**	**Percentage (%)**
A	1	0.8
B	1	0.8
C	80	64.0
D	3	2.4
E	2	1.6
F	1	0.8
G	6	4.8
H	0	0.0
I	15	12.0
J	6	4.8
K	5	4.0
L	0	0.0
M	2	1.6
N	2	1.6
Q	1	0.8
R	0	0.0
S	0	0.0
T	0	0.0
Unknown	0	0.0
Total	125	100.0

samples, controls (n = 926) were randomly selected from the strata of sex, age group for each 10 years, study area, and year of sample collection. 346 cases from the whole populations are identified during the follow-up period. From 1,272 participants (346 cases and 926 controls), a total of 619 participants was available for measurement of serum carotenoid after measurement of other biomarkers, but randomly selected from cases and controls. A total of 173 participants were excluded due to the following reasons: clinical history of cancer, CVD, or diabetes (n = 35) for selecting healthy adults at the baseline survey, lack of information for anthropometric indices (n = 17) or blood pressure (n = 99); or relocation away from a study site (n = 22). Finally, a total of 446 participants (125 cases and 321 controls) were included in our statistical analysis. The breakdown of premature mortality (n = 125) in this study is shown in Table 1. Written informed consent was obtained from all participants from 36 of 45 study sites. From the remaining 9 sites, we obtained group-based informed consent from the area leader. The design and protocol of this study was approved by the ethics committee at Hokkaido University School of Medicine (No. 14-044).

Measurement of serum carotenoids

All samples were collected by trained staff during the baseline survey in each study site. Serum concentration of six carotenoids (zeaxanthin/lutein, canthaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene) were measured by high-performance liquid chromatography (Waters 2487 Dual Absorbance Detector, Waters Co., Milford, MA, USA) as described elsewhere.[30] We calculated total carotenes by totaling concentrations of α- and β-carotenes and lycopene, total xanthophylls by totaling concentrations of β-cryptoxanthin, canthaxanthin, and zeaxanthin/lutein, and provitamin A by totaling concentrations of α- and β-carotenes and β-cryptoxanthin.

Statistical analysis

We performed statistical analyses after stratification by sex, because serum carotenoid levels differed significantly between men and women. Student's t-test and the Wilcoxon rank-sum test were used to compare continuous variables between controls and the premature mortality group. For categorical variables, we performed Fisher's exact test to check whether proportions of variables differed between controls and the premature mortality group. We used logistic regression analysis to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for premature mortality. All participants were categorized into tertiles (low, middle, or high) according to serum carotenoid level of controls in each sex. ORs were calculated for the middle and highest tertile against the lowest tertile as a reference. To assess linear trends in OR across tertiles, we also performed logistic regression analysis with an explanatory variable coding each tertile as a continuous variable (1, 2, or 3). These regression analyses were performed after adjusting for potential confounders, including, age, study site, years of education, diastolic blood pressure, body mass index, smoking status (current smoker vs never/ex-smoker), alcohol drinking (current drinker vs never/ex-drinker), and physical activity in leisure time (none vs >1 h/week). All statistical analyses were two-sided and values of $p<0.05$ were considered statistically significant. Statistical analyses were performed using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of participants

The demographic data of participants at the baseline survey are summarized by sex (Table 2). During follow-up, premature mortality was identified in 60 men and 65 women. Mean age

(standard deviation) in men and women at baseline survey was 50.4 (7.1) years and 50.8 (7.1) years, respectively. No significant differences in lifestyle risk factors such as smoking, drinking alcohol, or exercise were apparent between case and control in either sex.

Table 2 Basic characteristics of the participants at the baseline survey

	Men (n = 174)		**Women (n = 272)**	
	Control (n = 114)	**Case (n = 60)**	**Control (n = 207)**	**Case (n = 65)**
Age, year	51.5 (7.5)	48.6 (5.8)	51.8 (7.2)	47.7 (5.7)
Years of education, year	16.7 (2.2)	16.9 (2.0)	16.6 (2.0)	17.0 (2.2)
BMI, kg/m^2	23.0 (3.0)	23.2 (3.2)	22.9 (2.9)	23.6 (3.9)
SBP, mmHg	127.7 (14.5)	130.5 (17.0)	125.7 (18.3)	126.8 (18.2)
DBP, mmHg	79.3 (10.5)	80.3 (13.2)	76.6 (10.7)	76.7 (13.5)
Zeaxanthin/Lutein, μmol/L[a]	0.82 [0.62, 1.12]	0.82 [0.67, 1.06]	0.95 [0.73, 1.22]	0.85 [0.69, 1.12]
Canthaxanthin, μmol/L[a]	0.04 [0.03, 0.06]	0.04 [0.03, 0.06]	0.05 [0.03, 0.06]	0.05 [0.04, 0.07]
β-cryptoxanthin, μmol/L[a]	0.17 [0.10, 0.31]	0.10 [0.05, 0.18]	0.26 [0.16, 0.44]	0.38 [0.18, 0.60]
Lycopene, μmol/L[a]	0.08 [0.07, 0.14]	0.08 [0.04, 0.13]	0.14 [0.10, 0.20]	0.12 [0.09, 0.19]
α-carotene, μmol/L[a]	0.06 [0.04, 0.10]	0.05 [0.03, 0.07]	0.10 [0.06, 0.14]	0.09 [0.06, 0.13]
β-carotene, μmol/L[a]	0.26 [0.16, 0.49]	0.18 [0.10, 0.40]	0.59 [0.37, 0.87]	0.54 [0.36, 0.79]
Total carotene, μmol/L[a]	0.39 [0.28, 0.71]	0.30 [0.20, 0.62]	0.81 [0.56, 1.21]	0.78 [0.53, 1.06]
Xanthophylls, μmol/L[a]	1.04 [0.82, 1.52]	1.03 [0.81, 1.27]	1.33 [1.03, 1.73]	1.32 [1.06, 1.68]
Provitamin A, μmol/L[a]	0.56 [0.37, 0.89]	0.38 [0.22, 0.63]	1.03 [0.73, 1.51]	1.07 [0.79, 1.46]
Total carotenoid, μmol/L[a]	1.49 [1.18, 2.19]	1.38 [1.00, 1.97]	2.14 [1.67, 2.95]	2.12 [1.66, 2.85]
	n (%)	**n (%)**	**n (%)**	**n (%)**
Smoking status				
Never/Ever	55 (48.2%)	23 (38.3%)	198 (95.7%)	59 (90.8%)
Current	59 (51.8%)	37 (61.7%)	9 (4.3%)	6 (9.2%)
Alcohol consumption				
Never/Ever	25 (21.9%)	10 (16.7%)	158 (76.3%)	54 (83.1%)
Current	89 (78.1%)	50 (83.3%)	49 (23.7%)	11 (16.9%)
Leisure-time exercise				
> 1hr/week	25 (21.9%)	9 (15.0%)	45 (21.7%)	12 (18.5%)

[a]Values are summarized as median and interquartile range; otherwise continuous variables are summarized as mean and standard deviation.
BMI: body mass index
DBP: diastolic blood pressure
SBP: systolic blood pressure

Carotenoids and all-cause premature mortality

Crude and adjusted ORs for all-cause premature mortality are shown according to the tertiles of serum carotenoids (Table 3 and 4). In men, higher levels of serum β-cryptoxanthin and provitamin

A were associated with lower risks of all-cause premature mortality compared to the lowest tertile in crude analyses (OR, 95% CI: 0.33, 0.15–0.72 and 0.26, 0.11–0.61, respectively) (Table 3). In women, serum β-cryptoxanthin was marginally associated with all-cause premature mortality compared to the lowest tertile in crude analyses (OR, 95% CI: 1.89, 0.98–3.72) (Table 4). In men, adjusted ORs for all-cause premature mortality in the highest tertiles of β-cryptoxanthin and provitamin A were significantly lower compared to the lowest tertile (OR, 95% CI: 0.19,

Table 3 Associations between premature all-cause mortality according to tertile of serum carotenoid level (Men)

			Crude		Adjusted[a]	
		Case/Control	OR (95% CI)	trend *p*	OR (95% CI)	trend *p*
Zeaxanthin/Lutein	Low	18/38	Reference		Reference	
	Middle	27/38	1.50 (0.71–3.20)	0.69	2.47 (1.05–6.08)	0.68
	High	15/38	0.83 (0.36–1.89)		1.25 (0.50–3.13)	
Canthaxanthin	Low	22/37	Reference		Reference	
	Middle	16/38	0.71 (0.32–1.55)	0.95	0.69 (0.29–1.63)	0.95
	High	22/38	0.97 (0.46–2.05)		1.02 (0.43–2.46)	
β-cryptoxanthin	Low	36/38	Reference		Reference	
	Middle	12/38	0.33 (0.15–0.72)	0.003	0.21 (0.08–0.53)	<0.001
	High	12/38	0.33 (0.15–0.72)		0.19 (0.07–0.47)	
Lycopene	Low	28/40	Reference		Reference	
	Middle	14/36	0.56 (0.25–1.20)	0.21	0.71 (0.29–1.70)	0.47
	High	17/38	0.64 (0.30–1.34)		0.75 (0.32–1.74)	
α-carotene	Low	28/39	Reference		Reference	
	Middle	20/39	0.71 (0.34–1.47)	0.06	0.69 (0.30–1.54)	0.07
	High	12/36	0.46 (0.20–1.03)		0.43 (0.17–1.07)	
β-carotene	Low	30/38	Reference		Reference	
	Middle	13/38	0.43 (0.19–0.94)	0.06	0.52 (0.21–1.22)	0.18
	High	15/38	0.50 (0.23–1.06)		0.58 (0.24–1.36)	
Total carotene	Low	31/38	Reference		Reference	
	Middle	13/38	0.42 (0.19–0.91)	0.04	0.46 (0.19–1.09)	0.09
	High	15/38	0.48 (0.22–1.03)		0.48 (0.20–1.14)	
Xanthophylls	Low	21/38	Reference		Reference	
	Middle	26/38	1.24 (0.60–2.58)	0.29	1.38 (0.62–3.10)	0.61
	High	13/38	0.62 (0.27–1.40)		0.76 (0.30–1.86)	
Provitamin A	Low	34/38	Reference		Reference	
	Middle	15/38	0.44 (0.20–0.93)	0.001	0.41 (0.18–0.94)	0.002
	High	9/38	0.26 (0.11–0.61)		0.24 (0.09–0.61)	
Total carotenoid	Low	25/38	Reference		Reference	
	Middle	19/38	0.76 (0.36–1.60)	0.20	0.78 (0.34–1.76)	0.41
	High	15/38	0.60 (0.27–1.30)		0.70 (0.29–1.67)	

[a]Adjustment for age, study site, years of education, diastolic blood pressure, body mass index, smoking status, drinking status, and leisure-time exercise.
CI: confidence intervals
OR: odds ratio

0.07–0.47 and 0.24, 0.09–0.61, respectively) (Table 3). Conversely, in women, significantly higher risk was observed for the highest tertile of serum β-cryptoxanthin level compared to the lowest tertile (OR, 95% CI: 1.94, 1.00–4.03) (Table 4). Although higher levels of β-cryptoxanthin and provitamin A were associated with lower risk of premature mortality in men (trend $p < 0.001$ and 0.002) (Table 3), a positive association between β-cryptoxanthin and premature mortality was observed in women (trend $p = 0.05$) (Table 4).

Table 4 Associations between premature all-cause mortality according to tertile of serum carotenoid level (Women)

			Crude		Adjusted[a]	
		Case/Control	OR (95% CI)	trend *p*	OR (95% CI)	trend *p*
Zeaxanthin/Lutein	Low	31/68	Reference	0.09	Reference	0.62
	Middle	16/71	0.49 (0.24–0.97)		0.55 (0.26–1.14)	
	High	18/68	0.58 (0.29–1.13)		0.88 (0.41–1.85)	
Canthaxanthin	Low	24/69	Reference	0.67	Reference	0.34
	Middle	15/73	0.59 (0.28–1.21)		0.64 (0.29–1.36)	
	High	26/65	1.15 (0.60–2.21)		1.46 (0.72–3.01)	
β-cryptoxanthin	Low	18/68	Reference	0.04	Reference	0.05
	Middle	13/71	0.69 (0.31–1.51)		0.65 (0.28–1.50)	
	High	34/68	1.89 (0.98–3.72)		1.94 (1.00–4.03)	
Lycopene	Low	22/68	Reference	0.59	Reference	0.87
	Middle	24/71	1.04 (0.54–2.05)		1.09 (0.53–2.25)	
	High	18/68	0.82 (0.40–1.66)		1.06 (0.48–2.35)	
α-carotene	Low	24/69	Reference	0.37	Reference	0.47
	Middle	24/70	0.99 (0.51–1.90)		0.84 (0.41–1.73)	
	High	17/68	0.72 (0.35–1.45)		0.76 (0.35–1.62)	
β-carotene	Low	26/68	Reference	0.23	Reference	0.84
	Middle	22/71	0.81 (0.42–1.56)		0.78 (0.38–1.60)	
	High	17/68	0.65 (0.32–1.30)		0.95 (0.44–2.05)	
Total carotene	Low	25/68	Reference	0.22	Reference	0.67
	Middle	24/71	0.92 (0.48–1.77)		0.93 (0.46–1.89)	
	High	16/68	0.64 (0.31–1.29)		0.84 (0.38–1.85)	
Xanthophylls	Low	19/68	Reference	0.89	Reference	0.34
	Middle	26/71	1.31 (0.67–2.61)		1.38 (0.66–2.90)	
	High	20/68	1.05 (0.52–2.16)		1.45 (0.67–3.17)	
Provitamin A	Low	18/68	Reference	0.42	Reference	0.18
	Middle	23/71	1.22 (0.61–2.49)		1.28 (0.60–2.79)	
	High	24/68	1.33 (0.67–2.71)		1.70 (0.79–3.76)	
Total carotenoid	Low	21/69	Reference	0.62	Reference	0.62
	Middle	27/60	1.27 (0.66–2.47)		1.30 (0.63–2.68)	
	High	17/68	0.82 (0.40–1.69)		1.20 (0.54–2.64)	

[a]Adjustment for age, study site, years of education, diastolic blood pressure, body mass index, smoking status, drinking status, and leisure-time exercise.
CI: confidence intervals
OR: odds ratio

Carotenoids and cancer premature mortality

Crude and adjusted ORs for premature cancer mortality with respect to the tertiles of serum carotenoids (Table 5 and 6). In men, the highest tertiles of β-cryptoxanthin and provitamin A showed lower ORs for premature cancer mortality in crude analyses (OR, 95% CI: 0.28, 0.08–0.78 and 0.28, 0.08–0.78, respectively) (Table 5). On the other hand, in women, the highest tertile of β-cryptoxanthin showed a significantly higher OR of premature cancer mortality

Table 5 Associations between premature cancer mortality according to tertile of serum carotenoid level (Men)

			Crude		Adjusted[a]	
		Case/Control	OR (95% CI)	trend *p*	OR (95% CI)	trend *p*
Zeaxanthin/Lutein	Low	8/38	Reference		Reference	
	Middle	17/38	2.13 (0.84–5.76)	0.84	4.51 (1.46–15.79)	0.51
	High	7/38	0.88 (0.28–2.67)		1.59 (0.45–5.82)	
Canthaxanthin	Low	11/37	Reference		Reference	
	Middle	9/38	0.80 (0.29–2.14)	0.89	0.80 (0.26–2.43)	0.68
	High	12/38	1.06 (0.42–2.74)		1.28 (0.42–4.02)	
β-cryptoxanthin	Low	18/38	Reference		Reference	
	Middle	9/38	0.50 (0.19–1.23)	0.02	0.34 (0.10–1.01)	0.001
	High	5/38	0.28 (0.08–0.78)		0.10 (0.02–0.37)	
Lycopene	Low	18/40	Reference		Reference	
	Middle	6/36	0.37 (0.12–0.99)	0.08	0.46 (0.14–1.39)	0.19
	High	8/36	0.47 (0.17–1.17)		0.51 (0.17–1.49)	
α-carotene	Low	15/39	Reference		Reference	
	Middle	9/39	0.60 (0.23–1.51)	0.24	0.47 (0.16–1.36)	0.31
	High	8/36	0.58 (0.21–1.50)		0.60 (0.18–1.91)	
β-carotene	Low	16/38	Reference		Reference	
	Middle	7/38	0.44 (0.15–1.15)	0.13	0.52 (0.16–1.55)	0.44
	High	8/38	0.50 (0.18–1.28)		0.68 (0.21–2.10)	
Total carotene	Low	17/38	Reference		Reference	
	Middle	7/38	0.41 (0.14–1.07)	0.09	0.43 (0.13–1.29)	0.22
	High	8/38	0.47 (0.17–1.19)		0.51 (0.15–1.59)	
Xanthophylls	Low	12/38	Reference		Reference	
	Middle	15/38	1.25 (0.52–3.06)	0.17	1.31 (0.49–3.60)	0.38
	High	5/38	0.42 (0.12–1.24)		0.51 (0.14–1.72)	
Provitamin A	Low	18/38	Reference		Reference	
	Middle	8/38	0.44 (0.16–1.12)	0.01	0.37 (0.12–1.09)	0.02
	High	5/38	0.28 (0.08–0.78)		0.26 (0.07–0.85)	
Total carotenoid	Low	13/38	Reference		Reference	
	Middle	10/38	0.77 (0.29–1.96)	0.45	0.76 (0.25–2.24)	0.89
	High	9/38	0.69 (0.26–1.80)		0.93 (0.30–2.92)	

[a]Adjustment for age, study site, years of education, diastolic blood pressure, body mass index, smoking status, drinking status, and leisure-time exercise.

CI: confidence intervals

OR: odds ratio

compared to the lowest tertile in crude analyses (OR, 95% CI: 2.80, 1.30–6.47) (Table 6). After adjusting for covariates, in men, logistic regression analyses showed significantly lower ORs for premature cancer mortality in the highest tertiles of serum β-cryptoxanthin and provitamin A (OR, 95% CI: 0.10, 0.02–0.37 and 0.26, 0.07–0.85, respectively) (Table 5). Linear trends were also observed in these carotenoids (β-cryptoxanthin and provitamin A) (p = 0.001 and 0.02, respectively) (Table 5). In women, the highest tertile of β-cryptoxanthin showed a significantly

Table 6 Associations between premature cancer mortality according to tertile of serum carotenoid level (Women)

			Crude		Adjusted[a]	
		Case/Control	OR (95% CI)	trend p	OR (95% CI)	trend p
Zeaxanthin/Lutein	Low	19/68	Reference		Reference	
	Middle	13/71	0.66 (0.29–1.42)	0.52	0.73 (0.31–1.67)	0.66
	High	15/68	0.79 (0.37–1.68)		1.25 (0.53–2.94)	
Canthaxanthin	Low	18/69	Reference		Reference	
	Middle	10/73	0.53 (0.22–1.20)	0.76	0.55 (0.22–1.33)	0.31
	High	19/65	1.12 (0.54–2.33)		1.58 (0.70–3.61)	
β-cryptoxanthin	Low	10/68	Reference		Reference	
	Middle	9/71	0.86 (0.32–2.27)	0.005	0.82 (0.29–2.27)	0.004
	High	28/68	2.80 (1.30–6.47)		3.23 (1.40–8.03)	
Lycopene	Low	16/68	Reference		Reference	
	Middle	17/71	1.02 (0.47–2.19)	0.74	1.09 (0.48–2.49)	0.51
	High	14/68	0.87 (0.39–1.93)		1.36 (0.56–3.36)	
α-carotene	Low	17/69	Reference		Reference	
	Middle	16/70	0.93 (0.43–1.99)	0.65	0.85 (0.37–1.92)	0.84
	High	14/68	0.84 (0.38–1.83)		0.92 (0.39–2.17)	
β-carotene	Low	20/68	Reference		Reference	
	Middle	14/71	0.67 (0.31–1.42)	0.26	0.67 (0.29–1.52)	0.91
	High	13/68	0.65 (0.29–1.40)		1.00 (0.42–2.36)	
Total carotene	Low	19/68	Reference		Reference	
	Middle	16/71	0.81 (0.38–1.70)	0.26	0.80 (0.35–1.79)	0.84
	High	12/68	0.63 (0.28–1.39)		0.94 (0.39–2.25)	
Xanthophylls	Low	11/68	Reference		Reference	
	Middle	19/71	1.65 (0.74–3.84)	0.33	1.81 (0.77–4.49)	0.07
	High	17/68	1.55 (0.68–3.63)		2.35 (0.96–6.01)	
Provitamin A	Low	13/68	Reference		Reference	
	Middle	15/71	1.11 (0.49–2.52)	0.33	1.14 (0.47–2.82)	0.09
	High	19/68	1.46 (0.67–3.25)		2.11 (0.89–5.23)	
Total carotenoid	Low	15/69	Reference		Reference	
	Middle	17/70	1.12 (0.52–2.44)	0.97	1.08 (0.47–2.53)	0.29
	High	15/68	1.01 (0.46–2.25)		1.64 (0.68–4.01)	

[a]Adjustment for age, study site, years of education, diastolic blood pressure, body mass index, smoking status, drinking status, and leisure-time exercise.
CI: confidence intervals
OR: odds ratio

higher OR compared to the lowest tertile (OR, 95% CI: 3.23, 1.40–8.03), and statistical analysis for linear trends also yielded a significant result (p = 0.004) (Table 6).

DISCUSSION

In this study, we prospectively examined associations between serum carotenoids and premature mortality (all-cause and cancer mortality). Higher levels of serum β-cryptoxanthin and provitamin A were associated with lower risks of premature mortality in men, whereas higher levels of serum β-cryptoxanthin with higher risks in women. For cancer premature mortality, significant beneficial association of β-cryptoxanthin and provitamin A were observed in men, but higher levels of β-cryptoxanthin was associated with higher risk in women.

Beta-cryptoxanthin is a common carotenoid with high levels seen in citrus fruit such as tangerines, persimmons and oranges. Citrus *unshiu* MARC, a specific species of mandarin cultivated in Japan, is known as a major source of β-cryptoxanthin. In fact, Sugiura and his colleagues found that higher intake of Japanese mandarins was associated with higher serum β-cryptoxanthin levels.[31] In men, we found that higher serum levels are associated with lower premature mortality. One of the plausible biological functions explaining this association is an antioxidant by accepting energy from singlet oxygen, one type of reactive oxygen species.[32] In Japan, cross-sectional and prospective studies have collectively reported protective roles of β-cryptoxanthin against a wide range of diseases and clinical conditions.[33-35] In addition to previous studies in Japan, a reasonable body of evidence has been accumulated around the world,[36-38] which shows that beta-cryptoxanthin levels among Japanese controls (0.31 μmol/L) were much higher than those of US controls (0.19 μmol/L).[16,39] A recent systematic review including 69 prospective studies reported that an increment of 15 μg/dL in serum β-cryptoxanthin may reduce the risk of all-cause mortality and cancer, with relative risks of 0.84 and 0.74, respectively, but showed no significant effect on CVD mortality.[36] Our results were line with this meta-analysis for all-cause mortality and premature cancer mortality, although we could neither analyze nor compare premature CVD mortality due to the limited sample size. Taking these information together, serum β-cryptoxanthin can be a marker not only for mortality but also premature mortality in men.

On the other hand, in women, it was surprised that higher levels of serum β-cryptoxanthin at baseline were associated with a higher risk of premature mortality in women. To the best of our knowledge, there is no study showing harmful effects of β-cryptoxanthin in women. Regarding surprising results, one possible explanation is that higher levels of beta-cryptoxanthin is associated with better bone repairing indices among postmenopausal women. This beneficial effect for bone metabolism seems to be stronger than males and is found only in groups with beta-cryptoxanthin levels higher than a certain threshold.[40] Then, if postmenopausal women with higher levels of beta-cryptoxanthin tend to use it for bone repair, serum levels may be similar to or lower than the low group. This possibility can lead to opposite results compared to men. Another explanation is that high levels of serum β-cryptoxanthin in women cases may have adverse effects on human health as well as conflicting evidence of higher levels of serum β-carotene on lung cancer in randomized controlled trials (RCT).[41,42] In these previous clinical trials on β-carotene supplementations, blood levels of β-carotene were two to six times higher than the 95th percentile of β-carotene in a representative sample of the US population. In the present study, we found that the mean levels of serum β-cryptoxanthin in woman cases were 1.5-folds higher than those of controls. Although the biological backgrounds underlying adverse effects of β-carotene are still unclear, excessive β-cryptoxanthin may have a similar adverse effect because both β-carotene and β-cryptoxanthin are categorized in provitamin A. Even though

the research design and the target population are different between previous clinical trials and this study, caution is thus warranted when interpreting the result of β-cryptoxanthin and further studies are needed to evaluate this association in other populations.

Provitamin A (α-carotene, β-carotene, and β-cryptoxanthin) are the natural precursor for retinoids. In this study, significant association between serum provitamin A and premature mortality from all-causes and cancer appears to be derived from β-cryptoxanthin in men. In other words, this suggests that the effects of α-carotene and β-carotene are not significantly associated with premature mortality. Several epidemiological studies reported similar results in different health outcomes. In European and North American populations, the pooled analysis of large cohort studies showed that only β-cryptoxanthin among carotenoids with provitamin A activity (α-carotene and β-carotene) was associated with lung cancer.[43] Additionally, a meta-analysis also indicated stronger effects of dietary intake and blood levels of β-cryptoxanthin on lung cancer than that of α-carotene and β-carotene.[44] The plausible mechanism underlying this stronger effect of β-cryptoxanthin among provitamin A carotenoids can be its high bioavailability.[45] This difference of biological function can explain the results that only β-cryptoxanthin and provitamin A were associated with premature mortality in this study.

Various limitations of this study should be discussed. First, the process for selecting cases and controls was incomplete as shown in the Method. Although we needed to select cases and controls before utilizing serum samples from 30,193 participants, we conducted this study after excluding 4,761 participants due to the sample availability. This might not be much biased in our results because these excluded participants included both cancer cases and age- and sex-matched participants. However, this incomplete process seems a limitation in this study. Second, study subjects were limited compared to the overall population of study participants in the JACC Study, which may have resulted in sampling bias. It is not easy to describe how this biased our results because effect and directions by sampling bias can be defined by how different the sampling people was from whole participants. Third, we investigated associations in an entirely Japanese population, even though participants were from every part of Japan. The associations identified thus cannot be generalized to other populations and should be evaluated in other studies. Forth, our sample size was relatively small. We therefore could not estimate the risk of serum carotenoids for premature CVD mortality, other causes, or particular types of cancer. Fifth, seasonal variations in some carotenoids may have affected the results. In particular, serum levels of β-cryptoxanthin were affected by mandarin intake and were higher in January.[46] In this study, the timing of blood collection varied between each research facility. To remove seasonal variation in carotenoids as much as possible, we adjusted for each study site in multivariable regression analyses. Regarding a stored duration, after collecting samples in 1988–1990, we stored serum samples at –80 degrees and measured in 2015. Although this long-term storage may deteriorate the quality of samples, interpretation with qualitative groups (ie three different groups for each carotenoid; low, middle, and high) may be valid. Sixth, we performed multiple testing for the association between each serum carotenoids levels and premature mortality. When we applied the Bonferroni correction for *P*-values (adjusted P-value: 0.05 / 10 = 0.005), significant associations with total premature mortality still remains in serum β-cryptoxanthin and provitamin A in men (trend $p < 0.001$ and 0.002). On the other hand, the association between serum β-cryptoxanthin and total premature mortality was not significant in women (trend $p = 0.05$). Summing up these limitations, further studies are expected to validate this association using a larger population.

In conclusion, we found that serum β-cryptoxanthin and provitamin A played protective roles against premature all-cause and cancer mortality in men. Although serum β-cryptoxanthin seems to be a risk factor for premature mortality in women, the biological mechanisms underlying this association remain unclear. Care is therefore needed in interpreting this result, but the present

study may provide an insight that serum β-cryptoxanthin and provitamin may be a biomarker for premature mortality in Japanese populations.

ACKNOWLEDGMENTS

The authors sincerely express their appreciation to Drs Kunio Aoki and Yoshiyuki Ohno, professors emeritus of Nagoya University Graduate School of Medicine and former chairpersons of the JACC Study, for their encouragement and support during this study. We are also greatly indebted to Dr Haruo Sugano, former director of the Cancer Institute, Tokyo, who made substantial contributions to the initiation of the JACC Study; Dr Tomoyuki Kitagawa, director emeritus of the Cancer Institute of the Japanese Foundation for Cancer Research and former project leader of the Grant-in-Aid for Scientific Research on Priority Areas of Cancer; and Dr Kazuo Tajima of Aichi Cancer Center, previous project leader of the Grant-in-Aid for Scientific Research on Priority Areas of Cancer Epidemiology. We wish to thank Dr Chigusa Date for contributing to the validation of the food frequency questionnaire and estimation of nutrient intake in the JACC Study.

ADDITIONAL INFORMATION

Funding source

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) (Monbu-Kagaku Sho); Grants-in-Aid for Scientific Research on Priority Areas of Cancer; and Grants-in-Aid for Scientific Research on Priority Areas of Cancer Epidemiology from MEXT (Monbu-Kagaku Sho) (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022, 18014011, 20014026, 20390156, and 26293138). This study was also supported by JSPS KAKENHI Grant Number JP 16H06277.

Data availability

The datasets used in the current study are not publicly available due to ethical restrictions, but are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1 Boffetta P, Couto E, Wichmann J, et al. Fruit and vegetable intake and overall cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst.* 2010;102(8):529–537. doi:10.1093/jnci/djq072.

2 Leenders M, Sluijs I, Ros MM, et al. Fruit and vegetable consumption and mortality: European prospective investigation into cancer and nutrition. *Am J Epidemiol.* 2013;178(4):590–602. doi:10.1093/aje/kwt006.

3 Aune D, Giovannucci E, Boffetta P, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. *Int J Epidemiol.* 2017;46(3):1029–1056. doi:10.1093/ije/dyw319.

4 Paiva, SA, Russell, RM. Beta-carotene and other carotenoids as antioxidants. *J Am Coll Nutr.* 1999;18(5):426–433. doi:10.1080/07315724.1999.10718880.
5 Carlsen MH, Karlsen A, Lillegaard IT, et al. Relative validity of fruit and vegetable intake estimated from an FFQ, using carotenoid and flavonoid biomarkers and the method of triads. *Br J Nutr.* 2011;105(10):1530–1538. doi:10.1017/S0007114510005246.
6 Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K. Which plasma antioxidants are most related to fruit and vegetable consumption? *Am J Epidemiol.* 2001;154(12):1113–1118. doi:10.1093/aje/154.12.1113.
7 Rock CL, Swendseid ME, Jacob RA, McKee RW. Plasma carotenoid levels in human subjects fed a low carotenoid diet. *J Nutr.* 1992;122(1):96–100. doi:10.1093/jn/122.1.96.
8 Wakai K, Suzuki K, Ito Y, et al. Serum carotenoids, retinol, and tocopherols, and colorectal cancer risk in a Japanese cohort: effect modification by sex for carotenoids. *Nutr Cancer.* 2005;51(1):13–24. doi:10.1207/s15327914nc5101_3.
9 Ito Y, Kurata M, Suzuki K, Hamajima N, Hishida H, Aoki K. Cardiovascular disease mortality and serum carotenoid levels: a Japanese population-based follow-up study. *J Epidemiol.* 2006;16(4):154–160. doi:10.2188/jea.16.154.
10 Suzuki K, Inoue T, Hioki R, et al. Association of abdominal obesity with decreased serum levels of carotenoids in a healthy Japanese population. *Clin Nutr.* 2006;25(5):780–789. doi:10.1016/j.clnu.2006.01.025.
11 Suzuki K, Honjo H, Ichino N, et al. Association of serum carotenoid levels with urinary albumin excretion in a general Japanese population: The Yakumo Study. *J Epidemiol.* 2013;23(6):451–456. doi:10.2188/jea.je20130058.
12 Suzuki K, Ishii J, Kitagawa F, et al. Association of serum carotenoid levels with N-terminal pro-brain-type natriuretic peptide: a cross-sectional study in Japan. *J Epidemiol.* 2013;23(3):163–168. doi:10.2188/jea.je20120087.
13 Huang J, Weinstein SJ, Yu K, Männistö S, Albanes D. Serum Beta Carotene and Overall and Cause-Specific Mortality. *Circ Res.* 2018;123(12):1339–1349. doi:10.1161/CIRCRESAHA.118.313409.
14 Mignone LI, Giovannucci E, Newcomb PA, et al. Dietary carotenoids and the risk of invasive breast cancer. *Int J Cancer.* 2009;124(12):2929–2937. doi:10.1002/ijc.24334.
15 Karppi J, Laukkanen JA, Mäkikallio TH, Ronkainen K, Kurl S. Low β-carotene concentrations increase the risk of cardiovascular disease mortality among Finnish men with risk factors. *Nutr Metab Cardiovasc Dis.* 2012;22(10):921–928. doi:10.1016/j.numecd.2012.01.008.
16 Fujii R, Tsuboi Y, Maeda K, Ishihara Y, Suzuki K. Analysis of Repeated Measurements of Serum Carotenoid Levels and All-Cause and Cause-Specific Mortality in Japan. *JAMA Netw Open.* 2021;4(6):e2113369. doi:10.1001/jamanetworkopen.2021.13369.
17 Ministry of Health, Labour and Welfare, Government of Japan. Abridged Life Tables for Japan 2017, https://www.mhlw.go.jp/english/database/db-hw/lifetb17/index.html. Accessed September 6, 2020.
18 GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* 2016;388(10053):1659–1724. doi:10.1016/S0140-6736(16)31679-8.
19 Jha P, Ramasundarahettige C, Landsman V, et al. 21st-century hazards of smoking and benefits of cessation in the United States. *N Engl J Med.* 2013;368(4):341–350. doi:10.1056/NEJMsa1211128.
20 GBD 2016 Alcohol Collaborators. Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet.* 2018;392(10152):1015–1035. doi: 10.1016/S0140-6736(18)31310-2.
21 Cooper RS, Kennelly JF, Durazo-Arvizu R, Oh HJ, Kaplan G, Lynch J. Relationship between premature mortality and socioeconomic factors in black and white populations of US metropolitan areas. *Public Health Rep.* 2001;116(5):464–473. doi:10.1093/phr/116.5.464.
22 Alicandro G, Frova L, Sebastiani G, Boffetta P, La Vecchia C. Differences in education and premature mortality: a record linkage study of over 35 million Italians. *Eur J Public Health.* 2018;28(2):231–237. doi:10.1093/eurpub/ckx125.
23 John A, McGregor J, Jones I, et al. Premature mortality among people with severe mental illness - New evidence from linked primary care data. *Schizophr Res.* 2018;199:154–162. doi:10.1016/j.schres.2018.04.009.
24 van den Brandt PA. The impact of a Mediterranean diet and healthy lifestyle on premature mortality in men and women. *Am J Clin Nutr.* 2011;94(3):913–920. doi:10.3945/ajcn.110.008250.
25 Muller DC, Murphy N, Johansson M, et al. Modifiable causes of premature death in middle-age in Western Europe: results from the EPIC cohort study. *BMC Med.* 2016;14:87. doi:10.1186/s12916-016-0630-6.
26 Tamakoshi A, Ozasa K, Fujino Y, et al. Cohort profile of the Japan Collaborative Cohort Study at final

follow-up. *J Epidemiol*. 2013;23(3):227–232. doi:10.2188/jea.je20120161.
27 Watanabe Y, Iwamura A, Shimada YJ, et al. Transforming Growth Factor-beta1 as a Predictor for the Development of Hepatocellular Carcinoma: A Nested Case-Controlled Study. *EBioMedicine.* 2016;12:68–71. doi:10.1016/j.ebiom.2016.09.001.
28 Pham TM, Fujino Y, Nakachi K, et al. Relationship between serum levels of insulin-like growth factors and subsequent risk of cancer mortality: findings from a nested case-control study within the Japan Collaborative Cohort Study. *Cancer Epidemiol.* 2010;34(3):279–284. doi:10.1016/j.canep.2010.03.017.
29 Pham TM, Fujino Y, Kikuchi S, et al. A nested case-control study of stomach cancer and serum insulin-like growth factor (IGF)-1, IGF-2 and IGF-binding protein (IGFBP)-3. *Eur J Cancer.* 2007;43(10):1611–1616. doi:10.1016/j.ejca.2007.04.014.
30 Ito Y, Ochiai J, Sasaki R, et al. Serum concentrations of carotenoids, retinol, and alpha-tocopherol in healthy persons determined by high-performance liquid chromatography. *Clin Chim Acta.* 1990;194(2-3):131–144. doi:10.1016/0009-8981(90)90128-f.
31 Sugiura M, Kato M, Matsumoto H, Nagao A, Yano M. Serum Concentration of β-Cryptoxanthin in Japan Reflects the Frequency of Satsuma Mandarin (*Citrus unshiu Marc.*) Consumption. *J Health Sci.* 2002;48(4):350–353.doi: 10.1248/jhs.48.350.
32 Burri BJ, La Frano MR, Zhu C. Absorption, metabolism, and functions of β-cryptoxanthin. *Nutr Rev.* 2016;74(2):69–82. doi:10.1093/nutrit/nuv064.
33 Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Yano M. High serum carotenoids associated with lower risk for the metabolic syndrome and its components among Japanese subjects: Mikkabi cohort study. *Br J Nutr.* 2015;114(10):1674–1682. doi:10.1017/S0007114515003268.
34 Nakamura M, Sugiura M, Aoki N. High beta-carotene and beta-cryptoxanthin are associated with low pulse wave velocity. *Atherosclerosis.* 2006;184(2):363–369. doi:10.1016/j.atherosclerosis.2005.04.006.
35 Sugiura M, Nakamura M, Ikoma Y, et al. The homeostasis model assessment-insulin resistance index is inversely associated with serum carotenoids in non-diabetic subjects. *J Epidemiol.* 2006;16(2):71–78. doi:10.2188/jea.16.71.
36 Aune D, Keum N, Giovannucci E, et al. Dietary intake and blood concentrations of antioxidants and the risk of cardiovascular disease, total cancer, and all-cause mortality: a systematic review and dose-response meta-analysis of prospective studies. *Am J Clin Nutr.* 2018;108(5):1069–1091. doi:10.1093/ajcn/nqy097.
37 Montonen J, Knekt P, Järvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. *Diabetes Care.* 2004;27(2):362–366. doi:10.2337/diacare.27.2.362.
38 De Waart FG, Schouten EG, Stalenhoef AF, Kok FJ. Serum carotenoids, alpha-tocopherol and mortality risk in a prospective study among Dutch elderly. *Int J Epidemiol.* 2001;30(1):136–143. doi:10.1093/ije/30.1.136.
39 Min KB, Min JY. Serum carotenoid levels and risk of lung cancer death in US adults. *Cancer Sci.* 2014;105(6):736–743. doi:10.1111/cas.12405.
40 Yamaguchi M, Igarashi A, Uchiyama S, et al. Effect of β-Crytoxanthin on Circulating Bone Metabolic Markers: Intake of Juice (*Citrus Unshiu*) Supplemented with β-Cryptoxanthin Has an Effect in Menopausal Women. *J Health Sci.* 2006;52(6):758–768.doi: 10.1248/jhs.52.758
41 Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med.* 1996;334(18):1150–1155. doi:10.1056/NEJM199605023341802.
42 Albanes D, Heinonen OP, Taylor PR, et al. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst.* 1996;88(21):1560–1570. doi:10.1093/jnci/88.21.1560.
43 Männistö S, Smith-Warner SA, Spiegelman D, et al. Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies. *Cancer Epidemiol Biomarkers Prev.* 2004;13(1):40–48. doi:10.1158/1055-9965.epi-038-3.
44 Gallicchio L, Boyd K, Matanoski G, et al. Carotenoids and the risk of developing lung cancer: a systematic review. *Am J Clin Nutr.* 2008;88(2):372–383. doi:10.1093/ajcn/88.2.372.
45 Burri BJ, Chang JS, Neidlinger TR. β-Cryptoxanthin- and α-carotene-rich foods have greater apparent bioavailability than β-carotene-rich foods in Western diets. *Br J Nutr.* 2011;105(2):212–219. doi:10.1017/S0007114510003260.
46 Sugiura M, Matsumoto H, Kato M, Ikoma Y, Yano M, Nagao A. Multiple linear regression analysis of the seasonal changes in the serum concentration of beta-cryptoxanthin. *J Nutr Sci Vitaminol(Tokyo).* 2004;50(3):196–202. doi: 10.3177/jnsv.50.196.