Serum carotenoids and mortality from lung cancer: a case-control study nested in the Japan Collaborative Cohort (JACC) Study

Yoshinori Ito,[1] Kenji Wakai,[2] Koji Suzuki,[1] Akiko Tamakoshi,[2] Nao Seki,[3] Masahiko Ando,[2] Yoshikazu Nishino,[4] Takaaki Kondo,[5] Yoshiyuki Watanabe,[6] Kotaro Ozasa[6] and Yoshiyuki Ohno[2] for the JACC Study Group

[1]Department of Public Health, Fujita Health University School of Health Sciences, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, [2]Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, [3]Division of Public Health, Department of Infectious Disease Control and International Medicine, Niigata University Graduate School of Medical and Dental Science, 1-757 Asahimachidori, Niigata 951-8510, [4]Division of Epidemiology, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, [5]Department of Public Health/Heath Information Dynamics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550 and [6]Department of Social Medicine and Cultural Sciences, Research Institute for Neurological Diseases and Geriatrics, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566

(Received August 1, 2002/Revised October 7, 2002/Accepted October 23, 2002)

To investigate whether high serum levels of carotenoids, tocopherols, and folic acid decrease risk of lung cancer in Japanese, we conducted a case-control study nested in the Japan Collaborative Cohort (JACC) Study. A total of 39 140 subjects provided serum samples at baseline between 1988 and 1990. We identified 147 cases (113 males and 34 females) of death from lung cancer during an 8-year follow-up. Of the subjects who survived to the end of this follow-up, 311 controls (237 males and 74 females) were selected, matched to each case of lung cancer death for gender, age and participating institution. We measured serum levels of antioxidants in cases of lung cancer death and controls. Odds ratios (ORs) for lung cancer death were estimated using conditional logistic models. The risk of lung cancer death for the highest quartile of serum α-carotene, β-carotene, lycopene, β-cryptoxanthin, and canthaxanthin was significantly or marginally significantly lower than for the lowest quartile: the ORs, adjusted for smoking and other covariates, were 0.35 (95% confidence interval (CI), 0.14–0.88), 0.21 (0.08–0.58), 0.46 (0.21–1.04), 0.44 (0.17–1.16) and 0.37 (0.15–0.91), respectively. The ORs for the highest serum levels of zeaxanthin/lutein and folic acid tended to be low, but the differences were not statistically significant. Serum total cholesterol was also inversely related to risk of lung cancer death: the OR for the highest vs. the lowest quartile was 0.39 (95% CI, 0.19–0.79). Higher serum levels of carotenoids such as α- and β-carotenes may play a role in preventing death from lung cancer among Japanese. (Cancer Sci 2003; 94: 57–63)

Many epidemiological studies suggest that dietary vegetables rich in β-carotene decrease human lung cancer risk.[1, 2] Several studies have shown that high dietary intake of carotenoids other than β-carotene is associated with low risk of lung cancer.[2–5] In Japan, high intake of green and yellow vegetables was found to be significantly associated with low mortality rates of lung cancer among a large-scale cohort followed for over 15 years.[6] However, clinical trials have found no inverse association between administration of synthetic β-carotene and lung cancer incidence.[4] Recent studies have found that serum carotenoids such as cryptoxanthin and lutein have clearer inverse associations with lung cancer risk than does β-carotene.[7] This indicates the need for further investigation of the role of carotenoids other than β-carotene. Presently, there is little available data concerning the role of these carotenoids, particularly in Japan.

Thus, in order to examine the association between death from lung cancer and serum levels of carotenoids and other antioxidants, we conducted a case-control study nested in a large-scale Japanese cohort. In this cohort, we found that, among male smokers, risk of death from lung cancer was lower for those who frequently consume vegetables and fruits rich in carotenoids.[8]

Materials and Methods

Subjects and blood samples. The study subjects were recruited in the Japan Collaborative Cohort (JACC) Study for Evaluation of Cancer Risk Sponsored by Monbukagakusho (the Ministry of Education, Culture, Sports, Science and Technology of Japan). The details of the JACC Study are described elsewhere.[9] A questionnaire about health and lifestyles was distributed to the participants. The questions addressed personal and family medical histories, smoking, alcohol consumption, dietary intake of major foods, and anthropometric factors.[8, 9]

In addition to completing the questionnaire survey, participants in the present study gave peripheral-blood samples at health-screening check-ups sponsored by municipalities between 1988 and 1990. In total, 39 140 subjects aged 40 to 79 years (35.3% of the 110 792 respondents to the questionnaire survey) provided blood samples. There were no apparent differences in responses to the questionnaire survey of smoking, drinking, or dietary habits between the respondents who gave blood samples (group G) and respondents in the same age range (40 to 79 years) who did not gave blood samples (group N). Percentages of current smokers were as follows: group G, 52.3% for males and 3.8% for females; group N, 53.0% for males and 6.7% for females. Percentages of regular alcohol drinkers were as follows: group G, 76.0% for males and 21.3% for females; group N, 74.9% for males and 27.1% for females. Percentages of daily intake frequency of green leaf vegetables were as follows: group G, 31.0% for males and 34.5% for females; group N, 29.0% for males and 33.2% for females. Sera were separated from the samples at laboratories in or near the surveyed municipalities as soon as possible after the blood was drawn. Serum of each participant was divided into 3 to 5 tubes (100 to 500 μl per tube), and serum samples were stored in deep freezers at −80°C until used in 2001. Informed consent was obtained from all participants. This study was approved by the Ethical Board of the Nagoya University School of Medicine.

Selection of cases and controls. Losses of cohort members, and their underlying cause of death, were identified by reviewing all death certificates in each area once per year, with permission from the Director-General of the Prime Minister's Office. This

E-mail: yoshiito@fujita-hu.ac.jp

Table 1. Baseline characteristics of 147 cases by lung cancer death and 311 controls by sex

Parameter		Males		Females		Total	
		Cases (%)	Controls (%)	Cases (%)	Controls (%)	Cases (%)	Controls (%)
Total number		113 (100.0)	237 (100.0)	34 (100.0)	74 (100.0)	147 (100.0)	311 (100.0)
Age (year)	40–49	2 (1.8)	5 (2.1)	3 (8.8)	6 (8.1)	5 (3.4)	11 (3.5)
	50–59	12 (10.6)	25 (10.5)	8 (23.5)	18 (24.3)	20 (13.6)	43 (13.8)
	60–69	58 (51.3)	127 (53.6)	17 (50.0)	36 (48.6)	75 (51.0)	163 (52.4)
	70–79	41 (36.3)	80 (33.8)	6 (17.6)	14 (18.9)	47 (32.0)	94 (30.2)
Smoking status	Current	78 (69.0)	117 (49.4)	4 (11.8)	3 (4.1)	82 (55.8)	120 (38.6)
	Former	30 (26.5)	65 (27.4)	0 (0.0)	1 (1.4)	30 (20.4)	66 (21.2)
	Never	3 (2.7)	50 (21.1)	26 (76.5)	62 (83.8)	29 (19.7)	112 (36.0)
	Unknown	2 (1.8)	5 (2.1)	4 (11.8)	8 (10.8)	6 (4.1)	13 (4.2)
Alcohol drinking habit	Current	69 (61.1)	159 (67.1)	6 (17.6)	17 (23.0)	75 (51.0)	176 (56.6)
	Occasional	15 (13.3)	15 (6.3)	0 (0.0)	1 (1.4)	15 (10.2)	16 (5.1)
	Never	29 (25.7)	56 (23.6)	26 (76.5)	51 (68.9)	55 (37.4)	107 (34.4)
	Unknown	0 (0.0)	7 (3.0)	2 (5.9)	5 (6.8)	2 (1.4)	12 (3.9)
Body mass index (kg/m^2)	<20.0	23 (20.4)	38 (16.0)	3 (8.8)	6 (8.1)	26 (17.7)	44 (14.1)
	20.0–24.9	69 (61.1)	143 (60.3)	24 (70.6)	45 (60.8)	93 (63.3)	188 (60.5)
	25.0–	12 (10.6)	33 (13.9)	4 (11.8)	18 (24.3)	16 (10.9)	51 (16.4)
	Unknown	9 (8.0)	23 (9.7)	3 (8.8)	5 (6.8)	12 (8.2)	28 (9.0)
Medical history (yes)	Myocardial infarction	3 (2.7)	6 (2.5)	1 (2.9)	5 (6.8)	4 (2.7)	11 (3.5)
	Apoplexy	0 (0.0)	4 (1.7)	1 (2.9)	2 (2.7)	1 (0.7)	6 (1.9)
	Liver diseases	8 (7.1)	21 (8.9)	3 (8.8)	7 (9.5)	11 (7.5)	28 (9.0)
	Kidney diseases	2 (1.8)	9 (3.8)	0 (0.0)	3 (4.1)	2 (1.4)	12 (3.9)
	Diabetes	7 (6.2)	13 (5.5)	0 (0.0)	2 (2.7)	7 (4.8)	15 (4.8)

verification was performed by the present investigators personally, with administrative assistance from public health nurses in each area who were well acquainted with the vital status of the local inhabitants, including out-migration and health conditions (including screening findings).[9] Underlying causes of death coded according to the International Classification of Diseases and Injuries (ICD) 9th version (from the baseline to 1994) and stored in the JACC computer database were re-coded in 1999 according to the ICD 10th version (from 1995 to the present), using a computer program specifically developed for converting the ICD 9th code to the ICD 10th code. Out-migration was also verified annually by the present investigators personally, by reviewing population registers, with administrative assistance from public health nurses in each area.[9] Of the initial 110 792 participants, we identified 3242 (2.9%) move-outs during the study period to December 31, 1997. The follow-up for these move-outs was conducted on the day of migration. Although the loss from follow-up could not be methodologically identified, the verification of vital status and out-migration was believed to be substantially accurate because of the firmly established population registration system in Japan.[9] We estimated the follow-up rate to be about 97% (out-migration: 2.7% for males and 3.1% for females) during the study period,[9] the final day of which was December 31, 1997. Eligible case subjects were defined as those who died from lung cancer (International Statistical Classification of Diseases and Related Health Problems 10th Revision: C34). Because cell types of lung cancer were seldom specified on death certificates, we analyzed lung cancers as a whole.

During the 8-year follow-up, 196 deaths from lung cancer were identified among the subjects who had provided baseline serum samples. Of these, we excluded 9 subjects with a previous history of any cancer, 17 who died within 2 years after the baseline survey, and 23 without suitable samples. For each case of lung cancer death (hereafter referred to as "cases"), 2 or 3 controls were selected from the survivors, matching for gender, age (as near as possible), and participating institution. Of the initial 418 matched controls, we excluded 4 with a previous history of cancer, 31 without sufficient samples, and 72 whose matched cases were excluded. Thus, 147 cases and 311 controls were included in the analysis. Sufficient serum samples for determination of folic acid were available for 102 cases and 244 controls.

Determination of serum carotenoids, retinol, tocopherols, and folic acid. All the samples were analyzed by trained staff blinded to case-control status. Serum total cholesterol was determined using an autoanalyzer. Serum concentrations of carotenoids, retinol, and tocopherols were measured by high-performance liquid chromatography (HPLC), as described elsewhere.[10] All of the samples were stored in deep freezers at −80°C for about 10 years, and serum levels of carotenoids, retinol, and tocopherols were measured using the same equipment for all specimens; the ranges of repeatability and day-to-day variation were 4.6% to 6.9% and 6.3% to 20.0% (coefficients of variation), respectively, for the assays of carotenoids, retinol and tocopherols.[10] We could not separately measure serum levels of zeaxanthin and lutein or β- and γ-tocopherols, and therefore report the combined levels as zeaxanthin/lutein and β-/γ-tocopherols, respectively. We calculated total carotenes as the sum of α- and β-carotenes and lycopene, calculated total xanthophylls as the sum of β-cryptoxanthin, canthaxanthin and zeaxanthin/lutein, and calculated total provitamin A as the sum of α- and β-carotenes and β-cryptoxanthin. Total carotenoids were calculated as total carotenes plus total xanthophylls. Serum folic acid levels were determined using a cloned enzyme donor immunoassay.[11]

Statistical analysis. Body mass index (BMI) at baseline was calculated from reported height and weight: BMI=(weight in kg)/(height in m)^2. Mean differences between lung cancer cases and controls were examined by *t* test after converting serum levels of carotenoids, retinol, tocopherols, folic acid, and total cholesterol to logarithmic values. Analysis of covariance (ANCOVA)

Table 2. Comparison of serum levels of carotenoids, folic acid, retinol, tocopherols, and total cholesterol between 147 cases of lung cancer (113 males and 34 females) and 311 controls (237 males and 74 females)[1)]

Serum component		Cases		Controls		*P* for difference[2)]	
		G. mean	5%–95%	G.mean	5%–95%	Univariate	Multivariate
α-Carotene	(μmol/liter)	0.05	0.01–0.19	0.06	0.01–0.22	0.020	0.014
β-Carotene	(μmol/liter)	0.29	0.04–1.51	0.35	0.04–1.73	0.089	0.015
Lycopene	(μmol/liter)	0.18	0.04–0.74	0.21	0.05–0.92	0.18	0.11
Total carotenes	(μmol/liter)	0.56	0.11–2.28	0.66	0.11–2.76	0.096	0.028
β-Cryptoxanthin	(μmol/liter)	0.15	0.02–0.93	0.16	0.02–0.78	0.42	0.50
Zeaxanthin/lutein	(μmol/liter)	0.84	0.35–2.34	0.88	0.29–2.36	0.50	0.47
Canthaxanthin	(μmol/liter)	0.03	0.02–0.08	0.04	0.01–0.08	0.16	0.13
Total xanthophylls	(μmol/liter)	1.11	0.44–2.84	1.14	0.39–2.84	0.56	0.75
Provitamin A	(μmol/liter)	0.53	0.10–2.15	0.60	0.07–2.73	0.21	0.086
Total carotenoids	(μmol/liter)	1.74	0.55–4.71	1.87	0.48–5.20	0.27	0.23
Folic acid[3)]	(nmol/liter)	12.39	6.34–28.77	12.89	5.89–29.68	0.49	0.42
Retinol	(μmol/liter)	2.30	1.33–3.56	2.42	1.39–3.68	0.11	0.80
α-Tocopherol	(μmol/liter)	23.17	13.69–41.49	23.12	12.75–37.60	0.95	0.045
β-/γ-Tocopherols	(μmol/liter)	2.70	0.90–6.14	2.98	1.08–6.33	0.078	0.20
Total cholesterol	(mmol/liter)	4.71	3.26–6.49	4.92	3.52–6.73	0.021	0.004

1) Data were represented as the geometric mean (G. mean) values and 5–95 percentile ranges (5%–95%).
2) "*P* for difference" indicates the *P* value for difference of the geometric mean value between cases and controls by *t* test (univariate) and that adjusted for gender, age, participating institution, smoking and alcohol drinking habits, body mass index, and serum total cholesterol (except for analysis of total cholesterol itself) by analysis of covariance (multivariate).
3) The numbers of cases and controls were 76 and 165 for males and 26 and 59 for females.

was also performed after controlling for gender, age, participating institutions, smoking habit, alcohol consumption, BMI, and serum cholesterol level.[12)] Two conditional logistic regression models were used to calculate odds ratios (ORs) for lung cancer death.[12)] Variables adjusted in the models were as follows: model 1, gender, age, participating institution, and smoking habit; model 2, gender, age, participating institution, smoking habit, alcohol consumption, BMI, and serum cholesterol level or total carotenoids levels (for analyses involving total cholesterol).

Cases and controls were categorized into 4 groups, according to quartile levels of carotenoids, retinol, tocopherols, folic acid, and total cholesterol: Q1 (lowest) to Q4 (highest). However, the control subjects were not precisely divided into 4 equal groups, because some controls had identical serum values. ORs were calculated for Q2, Q3, and Q4 vs. Q1. To test for linear trends across the quartiles, we coded each quartile as 0, 1, 2 or 3, and then incorporated it into the logistic model as a single variable. Because smoking is a very important risk factor for lung cancer, it was adjusted in all analyses using the following detailed strata: subjects who had never smoked; former smokers who had not smoked for 0 to 4 years, 5 to 9 years, 10 to 14 years, 15 to 19 years, or 20 years or more; current smokers with pack-years of 0 to 19, 20 to 39, 40 to 59, 60 to 79, 80 to 99, or 100 or more; and unknown smoking habit. All *P* values were two-sided, and all analyses were performed using the Statistical Analysis System.[12)]

Results

Subject characteristics. Table 1 shows the distribution of subjects by gender, age, smoking habit, alcohol consumption, BMI, and medical history at baseline. About half of the subjects were in their 60s, and about 30% were in their 70s. For both genders, current smokers comprised a much greater proportion of cases than controls, whereas current drinkers comprised a slightly smaller proportion of cases than controls. A greater proportion of females than males had high BMI (≥25.0 kg/m^2), and a greater proportion of controls than cancer cases had high BMI. There was no apparent difference in disease history between cases and controls.

Comparison of serum levels of carotenoids, retinol, folic acid, and lipids between lung cancer cases and controls. Serum levels of carotenoids and other substances at baseline were compared between lung cancer cases and matched controls, as shown in Table 2. For all subjects, after adjusting for gender, age, participating institution, smoking habit, alcohol consumption, BMI, and serum cholesterol level or total carotenoids levels (for analyses involving total cholesterol), we found that serum levels of α- and β-carotenes, total carotenes, and total cholesterol were significantly lower in cancer cases than in controls. Lycopene, canthaxanthin, and provitamin A tended to be lower in cancer cases, but the difference was not significant. Serum level of α-tocopherol was rather higher in cases than in controls, when the covariates were considered. Among controls, all serum components other than retinol and canthaxanthin were lower in males than in females (data not shown).

ORs for lung cancer death. The ORs of serum carotenoids and other components for lung cancer mortality are shown in Tables 3 and 4. Risk calculated using model 1 was significantly or marginally significantly lower for the highest serum levels of α- and β-carotenes, total carotenes, β-cryptoxanthin, zeaxanthin/lutein, canthaxanthin, total carotenoids, β-/γ-tocopherols and total cholesterol, compared to the lowest levels. With the exception of β-carotene, total carotenes, and total cholesterol, ORs were lower for moderate levels (Q2 and/or Q3) of serum components than for the lowest levels. The ORs tended to be lower (though not significantly) for the highest serum levels of folic acid, retinol, and α-tocopherol.

Table 3. Odds ratios and 95% confidence intervals (CI) for mortality from lung cancer by serum carotenoid levels

Serum carotenoid	Group	Range	Cases	Controls	Model 1[1)] OR (95% CI)	Trend *P*	Model 2[2)] OR (95% CI)	Trend *P*
α-Carotene	Q1	<0.035	43	69	1.00	0.017	1.00	0.018
(μmol/liter)	Q2	0.035–0.066	46	86	0.68 (0.37–1.27)		0.58 (0.30–1.13)	
	Q3	0.067–0.103	27	73	0.37 (0.17–0.81)[4)]		0.37 (0.16–0.85)[4)]	
	Q4	0.104–	31	83	0.40 (0.17–0.92)[4)]		0.35 (0.14–0.88)[4)]	
β-Carotene	Q1	<0.19	41	77	1.00	0.017	1.00	0.006
(μmol/liter)	Q2	0.19–0.38	44	78	0.66 (0.34–1.28)		0.60 (0.30–1.22)	
	Q3	0.39–0.75	40	78	0.66 (0.32–1.37)		0.54 (0.24–1.20)	
	Q4	0.76–	22	78	0.28 (0.11–0.70)[5)]		0.21 (0.08–0.58)[5)]	
Lycopene	Q1	<0.10	39	75	1.00	0.18	1.00	0.088
(μmol/liter)	Q2	0.10–0.20	36	73	0.90 (0.47–1.73)		0.75 (0.38–1.52)	
	Q3	0.21–0.37	40	81	0.80 (0.42–1.53)		0.76 (0.38–1.53)	
	Q4	0.38–	32	82	0.61 (0.29–1.27)		0.46 (0.21–1.04)[3)]	
Total carotenes	Q1	<0.37	37	77	1.00	0.068	1.00	0.023
(μmol/liter)	Q2	0.37–0.69	53	78	1.13 (0.60–2.11)		1.00 (0.51–1.95)	
	Q3	0.70–1.29	32	78	0.78 (0.39–1.57)		0.64 (0.30–1.37)	
	Q4	1.30–	25	78	0.46 (0.19–1.12)[3)]		0.34 (0.13–0.90)[4)]	
β-Cryptoxanthin	Q1	<0.09	44	77	1.00	0.086	1.00	0.11
(μmol/liter)	Q2	0.09–0.18	38	78	0.58 (0.27–1.24)		0.52 (0.23–1.19)	
	Q3	0.19–0.34	34	78	0.50 (0.22–1.12)[3)]		0.38 (0.16–0.92)[4)]	
	Q4	0.35–	31	78	0.43 (0.18–1.07)[3)]		0.44 (0.17–1.16)[3)]	
Zeaxanthin/lutein	Q1	<0.66	48	77	1.00	0.19	1.00	0.34
(μmol/liter)	Q2	0.66–0.89	29	78	0.40 (0.20–0.81)[4)]		0.35 (0.17–0.75)[5)]	
	Q3	0.90–1.18	31	77	0.39 (0.19–0.81)[4)]		0.37 (0.17–0.79)[4)]	
	Q4	1.19–	39	79	0.54 (0.26–1.13)[3)]		0.58 (0.26–1.29)	
Canthaxanthin	Q1	<0.026	44	70	1.00	0.011	1.00	0.046
(μmol/liter)	Q2	0.026–0.036	35	84	0.51 (0.26–1.00)[3)]		0.59 (0.29–1.19)	
	Q3	0.037–0.052	40	79	0.52 (0.26–1.05)[3)]		0.57 (0.27–1.20)	
	Q4	0.053–	28	78	0.31 (0.14–0.70)[5)]		0.37 (0.15–0.91)[4)]	
Total xanthophylls	Q1	<0.84	44	77	1.00	0.43	1.00	0.74
(μmol/liter)	Q2	0.84–1.18	31	78	0.48 (0.23–0.99)[4)]		0.42 (0.19–0.93)[4)]	
	Q3	1.19–1.61	39	78	0.67 (0.31–1.42)		0.62 (0.28–1.37)	
	Q4	1.62–	33	78	0.57 (0.26–1.27)		0.65 (0.27–1.56)	

1) Odds ratios (95% confidence interval), adjusted for gender, age, participating institution, and smoking habit.
2) Odds ratios (95% confidence interval), adjusted for gender, age, participating institution, smoking and alcohol drinking habits, body mass index, and serum cholesterol level.
3) $P<0.10$, *4*) $P<0.05$, *5*) $P<0.01$.
Controls were not precisely divided into four even groups due to identical measurement values.

The risk calculated using model 2 was significantly or marginally significantly lower for subjects with the highest serum levels of α- and β-carotenes, lycopene, total carotenes, β-cryptoxanthin, canthaxanthin, total carotenoids, and total cholesterol, compared to the lowest levels, but was not lower for subjects with the highest serum levels of retinol and α-tocopherol. The ORs for the highest serum levels of provitamin A, folic acid, and β-/γ-tocopherols appeared to be lower but did not reach statistical significance. The decreased risk calculated for the highest serum cholesterol level using model 2 remained significant after further adjustment for total carotenoids levels (OR, 0.46; 95% CI, 0.21–0.98).

When the analysis was limited to men, we obtained findings similar to those for all subjects. Risk adjusted for smoking and other potential confounders was significantly or marginally significantly lower for the highest quartile of α- and β-carotenes, total carotenes, β-cryptoxanthin, canthaxanthin and total cholesterol: compared to the lowest quartile, the ORs were 0.28 (95% CI, 0.11–0.75), 0.28 (0.10–0.83), 0.33 (0.12–0.93), 0.36 (0.12–1.07), 0.34 (0.12–0.96), and 0.50 (0.22–1.11), respectively (data not shown in the tables; adjusted for age, participating institution, smoking and alcohol drinking habits, BMI, and serum cholesterol levels or total carotenoids levels).

Discussion

This study was conducted using a nested case-control design, controlling for gender, age and participating institution, because serum levels of antioxidants such as carotenoids seemed to decrease during 10-year storage. The available reports regarding quantitative stability of carotenoids during long-term storage at −70°C are inconclusive.[13, 14)] To assess the degradation of serum components in stored sera, we previously compared serum levels of carotenoids, retinol and tocopherols at the time of collection and after 9 years of storage at −80°C.[15)] That study (using 46 subjects) showed the following decreases in mean percentages of serum components: retinol and α-tocopherol, less than 5% decrease; α- and β-carotenes, less than 15%; lyco-

Table 4. Odds ratios and 95% confidence intervals (CI) for mortality from lung cancer by serum levels of carotenoids, folic acid, retinol, tocopherols, and total cholesterol

Serum component	Group	Range	Cases	Controls	Model 1[1)] OR (95% CI)	Trend *P*	Model 2[2)] OR (95% CI)	Trend *P*
Provitamin A	Q1	<0.34	39	77	1.00	0.11	1.00	0.087
(μmol/liter)	Q2	0.34–0.67	46	78	0.79 (0.39–1.58)		0.73 (0.35–1.54)	
	Q3	0.68–1.21	33	78	0.63 (0.29–1.36)		0.52 (0.22–1.20)	
	Q4	1.22–	29	78	0.49 (0.19–1.23)		0.44 (0.16–1.22)	
Total carotenoids	Q1	<1.34	46	77	1.00	0.023	1.00	0.030
(μmol/liter)	Q2	1.34–1.94	35	78	0.43 (0.22–0.86)[4)]		0.35 (0.16–0.76)[5)]	
	Q3	1.95–3.01	42	78	0.55 (0.27–1.14)[3)]		0.47 (0.22–1.01)[3)]	
	Q4	3.02–	24	78	0.28 (0.12–0.68)[5)]		0.27 (0.10–0.70)[5)]	
Folic acid	Q1	<9.29	30	53	1.00	0.27	1.00	0.24
(nmol/liter)	Q2	9.29–12.46	25	58	0.77 (0.34–1.79)		0.65 (0.26–1.67)	
	Q3	12.47–18.35	25	56	0.57 (0.23–1.41)		0.46 (0.16–1.30)	
	Q4	18.36–	22	57	0.58 (0.21–1.66)		0.52 (0.15–1.75)	
Retinol	Q1	<2.01	36	77	1.00	0.62	1.00	0.83
(μmol/liter)	Q2	2.01–2.41	39	75	1.28 (0.70–2.35)		1.34 (0.70–2.55)	
	Q3	2.42–2.94	42	81	1.12 (0.60–2.10)		1.41 (0.71–2.77)	
	Q4	2.95–	30	78	0.84 (0.43–1.66)		1.02 (0.49–2.14)	
α-Tocopherol	Q1	<18.82	36	73	1.00	0.46	1.00	0.35
(μmol/liter)	Q2	18.82–23.52	43	81	1.10 (0.59–2.06)		1.23 (0.63–2.40)	
	Q3	23.53–29.15	36	79	0.82 (0.40–1.68)		1.20 (0.51–2.80)	
	Q4	29.16–	32	78	0.83 (0.39–1.73)		1.59 (0.64–3.95)	
β-/γ-Tocopherols	Q1	<2.29	47	77	1.00	0.090	1.00	0.33
(μmol/liter)	Q2	2.29–3.15	32	72	0.50 (0.26–0.98)[4)]		0.56 (0.28–1.14)	
	Q3	3.16–4.21	40	80	0.74 (0.39–1.42)		0.85 (0.43–1.66)	
	Q4	4.22–	28	82	0.42 (0.19–0.94)[4)]		0.56 (0.24–1.29)	
Total cholesterol	Q1	<4.29	44	74	1.00	0.037	1.00	0.013
(mmol/liter)	Q2	4.29–4.89	37	79	0.70 (0.38–1.27)		0.71 (0.38–1.33)	
	Q3	4.90–5.66	41	80	0.79 (0.44–1.43)		0.68 (0.36–1.26)	
	Q4	5.67–	25	78	0.43 (0.22–0.86)[4)]		0.39 (0.19–0.79)[5)]	

1) Odds ratios (95% confidence interval), adjusted for gender, age, and participating institution, and smoking habit.
2) Odds ratios (95% confidence interval), adjusted for gender, age, participating institution, smoking and alcohol drinking habits, body mass index, and serum cholesterol level (except for analysis of total cholesterol itself).
3) $P<0.10$, *4*) $P<0.05$, *5*) $P<0.01$.
Controls were not precisely divided into four even groups due to identical measurement values.

pene, β-cryptoxanthin and zeaxanthin/lutein, less than 20%. Furthermore, mean serum values of components determined in the present study were comparable to those found in previous studies in Japan using fresher specimens.[10, 15, 16)]

Differences between genders among the present controls for serum levels of α- and β-carotenes, lycopene, xanthophylls, tocopherols, and retinol were similar to those seen in other populations.[10, 15, 16)] Serum levels of α- and β-carotenes, β-cryptoxanthin, and zeaxanthin/lutein were lower among current smokers and regular alcohol drinkers in the present study (data not shown), a finding also reported in other investigations.[17)] In addition, serum levels of carotenoids and tocopherols are associated with BMI, and serum total cholesterol levels closely correlate with serum levels of carotenoids because serum carotenoids are carried by lipoprotein in the blood.[18)] We, therefore, tested for differences in serum levels of carotenoids and other components between cases and controls, and estimated the risk of lung cancer death associated with these serum levels, adjusting for gender, smoking habit, alcohol consumption, BMI, and serum cholesterol levels, using ANCOVA and logistic regression analysis.

In the present study, higher serum levels of carotenoids such as α- and β-carotenes, lycopene, β-cryptoxanthin, canthaxanthin, and zeaxanthin/lutein were significantly or marginally significantly associated with lower lung cancer mortality. Previous studies have demonstrated that serum levels of β-carotene are lower in lung cancer cases.[19, 20)] Although the mechanisms of carcinogenesis are complex, β-carotene is considered to be a crucial factor.

Intervention trials have found that high-dose administration of synthetic β-carotene is associated with an increased incidence of lung cancer in male smokers[21)] and industrial workers.[22)] A trial conducted by American physicians found no inverse association between synthetic β-carotene administration and lung cancer incidence.[23)] A high dose of synthetic β-carotene elevates serum β-carotene levels more than 10-fold, and then produces prooxidant activities in biological systems, depending on the redox potential, which is affected by other antioxidant interactions and oxygen tension.[4, 24)] It has been reported that synthetic β-carotene administration also increases levels of cell proliferation indicators such as c-jun and c-fos proteins in the lungs of ferrets.[25)] Thus, the available data suggest that high-dose administration of synthetic β-carotene alone is associated with high risk of lung cancer incidence.[4, 26)]

Although we could not establish dietary habits before the time of blood collection in this study, serum levels of caro-

tenoids such as β-carotene were also indicated to be positively associated with intake of vegetables and fruits.[27] The finding that β-carotene has antioxidant activity and enhances immunity related to carcinogenesis indicates that β-carotene may protect against oxidative stress, such as damage to cell membranes, enzymes and nucleic acids caused by activated oxygen species and free radicals.[18, 28] According to some reports, most carotenoids possess antioxidant activities and anticarcinogenic activity,[18, 29, 30] a finding consistent with the inverse association between high serum levels of α- and β-carotenes and lung cancer death found in the present study. Furthermore, α- and β-carotenes can enhance cell-mediated immune responses.[31] There have been reports that higher serum levels of carotenoids other than β-carotene obtained through high intake of vegetables and fruits are associated with lower risk of lung cancer.[7, 32, 33] High intake of vegetables and fruits can increase serum levels of various carotenoids, and other biofactors such as vitamins, by about a few tenths of a percent, and these biofactors may play a role in decreasing cancer incidence.[18, 29]

In a study of bioactivity of α-carotene, the promotion stage of lung carcinogenesis in mice was found to be suppressed more effectively by α-carotene than β-carotene.[34] In addition, mandarin juice, which is rich in β-cryptoxanthin, has chemopreventive effects against mouse lung tumorigenesis.[35] In follow-up studies, high serum levels of β-cryptoxanthin have been associated with reduced risk of lung cancer,[7] and high intake of β-cryptoxanthin.[33] In the light of these reports, our finding that serum levels of α-carotene and β-cryptoxanthin have an inverse association with lung cancer mortality should be followed up, because of the potential for application of these protective substances in prevention of lung cancer.

In the present study, furthermore, serum canthaxanthin levels were inversely associated with lung cancer risk, and high serum levels of lycopene and zeaxanthin/lutein tended to be associated with lower risk. These carotenoids also possess antioxidant activities.[20, 29, 30, 36] Some studies have shown that high intake of tomatoes or high levels of lycopene can reduce the risk of mortality from lung cancer: significant reduction was found in multiple US populations, and in China and Spain; non-significant reduction was found in the UK, Norway, and Finland.[37] It has been reported that bioavailability of lutein, a major carotenoid in green-leaf vegetables, is 5 times higher than that of β-carotene.[38] No inverse association between serum canthaxanthin and lung cancer has been found, although canthaxanthin has been shown to induce apoptosis in human cell lines.[39]

In contrast, there have been no reports of clear association between lower ORs for lung cancer and higher serum levels of folic acid. It has been shown that folic acid scavenges free radicals,[40] and that high intake of folate helps reduce the risk of lung cancer.[33, 41] For example, an association between low mortality from lung cancer and high intake of folate has been found in a Netherlands cohort study.[41] However, the alpha-tocopherol, beta-carotene cancer prevention (ATBC) study found no significant association between lung cancer incidence and serum levels of folate among elderly men.[42] We found that serum folic acid levels tended to be inversely associated with lung cancer death. However, further investigations are needed to clarify this issue because the sample size for serum folic acid in the present study was limited.

No associations have been found between the risk of lung cancer and intake of vitamin E (α-tocopherol) or vitamin A (retinol).[33, 43] In all 9 population studies, cases of lung cancer were found to have lower serum β-carotene levels, compared to controls, whereas only a few studies reported that cases of lung cancer death had lower serum levels of retinol and α-tocopherol.[44] In a previous follow-up study of Japanese subjects, we found that higher serum levels of α-tocopherol and retinol were not significantly associated with mortality from cancer of all sites,[45] a finding similar to that of the present study. Finally, serum cholesterol levels were inversely associated with mortality from lung cancer, after adjusting for smoking and other covariates, a finding consistent with previous reports.[46, 47] This association could not be explained by serum carotenoid levels, because the inverse association remained after adjusting for serum total carotenoids levels.

In conclusion, the present results indicate that serum carotenoids such as α- and β-carotenes, lycopene, canthaxanthin and β-cryptoxanthin are associated with reduced risk of death from lung cancer. Serum levels of α- and β-carotenes appear to be particularly promising as biomarkers to predict mortality of lung cancer in Japanese inhabitants.

The present members of the JACC Study and their affiliations are as follows: Dr. Akiko Tamakoshi (present chairman of the study group), Nagoya University Graduate School of Medicine; Dr. Mitsuru Mori, Sapporo Medical University School of Medicine; Dr. Yutaka Motohashi, Akita University School of Medicine; Dr. Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr. Yosikazu Nakamura, Jichi Medical School; Dr. Hiroyasu Iso, Institute of Community Medicine, University of Tsukuba; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Yutaka Inaba, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, Showa University School of Medicine; Dr. Hiroshi Suzuki, Niigata University Graduate School of Medical and Dental Science; Dr. Hiroyuki Shimizu, Gifu University School of Medicine; Dr. Hideaki Toyoshima, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, Nagoya City University Graduate School of Medicine; Dr. Yoshinori Ito, Fujita Health University School of Health Sciences; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Akio Koizumi, Graduate School of Medicine and Faculty of Medicine, Kyoto University; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Dr. Yoshiyuki Watanabe, Research Institute for Neurological Diseases and Geriatrics, Kyoto Prefectural University of Medicine; Dr. Tsuneharu Miki, Kyoto Prefectural University of Medicine; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Mukogawa Women's University; Dr. Kiyomi Sakata, Wakayama Medical University; Dr. Takayuki Nose, Tottori University Faculty of Medicine; Dr. Norihiko Hayakawa, Research Institute for Radiation Biology and Medicine, Hiroshima University; Dr. Takesumi Yoshimura, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan; Dr. Katsuhiro Fukuda, Kurume University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; Dr. Hideo Shio, Shiga Medical Center; Dr. Yoshiyuki Ohno, Nagoya University Graduate School of Medicine; Dr. Tomoyuki Kitagawa, Cancer Institute of the Japanese Foundation for Cancer Research; Dr. Toshio Kuroki, Gifu University; Dr. Kazuo Tajima, Aichi Cancer Center Research Institute; and Dr. Kei Nakachi, Radiation Effects Research Foundation. The past investigators of the study group were listed previously in reference 9 except for the following seven members, Dr. Takashi Shimamoto, Institute of Community Medicine, University of Tsukuba; Dr. Heizo Tanaka, Medical Research Institute, Tokyo Medical and Dental University; Dr. Shigeru Hisamichi, Tohoku University Graduate School of Medicine; Dr. Masahiro Nakao, Kyoto Prefectural University of Medicine; Dr. Takaichiro Suzuki, Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases; Dr. Tsutomu Hashimoto, Wakayama Medical University and Dr. Teruo Ishibashi, Asama General Hospital. The authors wish to express their sincere appreciation to Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine, and former chairman of the JACC Study Group, and also to Dr. Haruo Sugano, former Director of the Cancer Institute of the Japanese Foundation for Cancer Research, both of whom greatly contributed to the initiation of this study. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (C) (2) (No. 12218216) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The JACC Study has been supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, and 11181101).

1. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer* 1992; **18**: 1–29.
2. Ziegler RG, Mayne ST, Swanson CA. Nutrition and lung cancer. *Cancer Causes Control* 1996; **7**: 157–77.
3. Le Marchand L, Hankin JH, Kolonel LN, Beecher GR, Wilkens LR, Zhao LP. Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 1994; **2**: 183–7.
4. Vainio H, Rautalahti M. An international evaluation of the cancer preventive potential of carotenoids. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 725–8.
5. Michaud DS, Freskanich D, Rimm EB, Colditz GA, Spiezer FE, Willett WC, Giovannucci E. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* 2000; **72**: 990–7.
6. Hirayama T. Diet and cancer. *Nutr Cancer* 1979; **1**: 67–81.
7. Yuan JM, Ross RK, Chu XD, Gao YT, Yu MC. Prediagnostic levels of serum beta-cryptoxanthin and retinol predict smoking-related lung cancer risk in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 767–73.
8. Ozasa K, Watanabe Y, Ito Y, Suzuki K, Tamakoshi A, Seki N, Nishino Y, Kondo T, Wakai K, Ando M, Ohno Y. for the JACC Study Group. Dietary habits and risk of lung cancer death in a large-scale cohort study (JACC Study) in Japan by sex and smoking habit. *Jpn J Cancer Res* 2001; **92**: 1259–69.
9. Ohno Y, Tamakoshi A, the JACC Study Group. Cohort study for evaluation of cancer risk sponsored by Monbusho (JACC Study). *J Epidemiol* 2001; **11**: 144–50.
10. Ito Y, Ochiai J, Sasaki R, Suzuki S, Kushuhara Y, Morimitsu Y, Otani M, Aoki K. Serum concentrations of carotenoids, retinol, and α-tocopherol in healthy persons determined by high-performance liquid chromatography. *Clin Chim Acta* 1990; **194**: 131–44.
11. Ishiwata Y, Endo N, Ikeda R, Yasuda K. Fundamental studies on the determination of serum vitamin B_{12} and folate using Chemilumianalyzer ACS-180. *Jpn J Clin Lab Assoc* 1995; **20**: 29–37 (in Japanese).
12. SAS Institute, Inc. SAS/STAT user's guide (ver. 6, 4th ed.). Cary, NC: SAS Institute, Inc.;1989.
13. Tangney CC, Shekelle RB, Raynor W, Gale M, Betz EP. Intra- and inter-individual variation in measurements of β-carotene, retinol, and tocopherols in diet and plasma. *Am J Clin Nutr* 1987; **45**: 764–9.
14. Hunter D. Biochemical indicators of dietary intake. In: Willett W, editor. Nutrition epidemiology. New York: Oxford University Press; 1990. p. 143–216.
15. Ito Y, Suzuki K, Ichino N, Imai H, Sakamoto H, Hokama M, Nishii M, Nakano H. The risk of *Helicobacter pylori* infection and atrophic gastritis from food and drink intake: a cross-sectional study in Hokkaido, Japan. *Asia Pac J Cancer Prev* 2000; **1**: 147–56.
16. Ito Y, Shimizu H, Yoshimura T, Hashimoto T, Hayakawa N, Shinohara R, Takatsuka N, Tokui N, Kasamatsu T, Suzuki K. Relationship between serum levels of lipid peroxides and carotenoids among residents in Japan. *Vitamins* 1997; **71**: 427–34 (in Japanese).
17. Ito Y, Sasaki R, Suzuki S, Yasui T, Hishida H, Otani M, Aoki K. Serum carotenoid levels and its sex differences in the residents living in a southern area of Hokkaido. *Vitamins* 1994; **68**: 351–63 (in Japanese).
18. Bendich A, Olson JA. Biological actions of carotenoids. *FASEB J* 1987; **3**: 1927–32.
19. van Poppel G, Goldbohm RA. Epidemiologic evidence for β-carotene and cancer prevention. *Am J Clin Nutr* 1995; **62** Suppl: 1393S–402S.
20. Comstock GW, Alberg AJ, Huang HY, Wu K, Burke AE, Hoffman SC, Norkus EP, Gross M, Cutler RG, Morris JS, Spate VL, Helzlsouer KJ. The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 907–16.
21. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994; **330**: 1029–35.
22. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. Effect of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996; **334**: 1150–5.
23. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996; **334**: 1145–9.
24. Palozza P. Prooxidant actions of carotenoids in biologic systems. *Nutr Rev* 1998; **56**: 257–65.
25. Wolf G. The effect of low and high doses of β-carotene and exposure to cigarette smoke on the lungs of ferrets. *Nutr Rev* 2002; **60**: 88–90.
26. Wang X-D, Russell RM. Procarcinogenic and anticarcinogenic effects of β-carotene. *Nutr Rev* 1999; **57**: 263–72.
27. Hann CS, Rock CL, King I, Drewnowski A. Validation of the healthy eating index with use of plasma biomarkers in a clinical sample of women. *Am J Clin Nutr* 2001; **74**: 479–86.
28. Bendich, A. Antioxidant vitamins and their functions in immune responses. In: Bendich A, Phillips M, Tengerdy RP, editors. Antioxidant nutrients and immune functions. New York and London: Plenum Press; 1990. p. 35–55.
29. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radic Biol Med* 1999; **26**: 1034–53.
30. Gerster H. Anticarcinogenic effects of common carotenoids. *Int J Vitam Nutr Res* 1992; **63**: 93–121.
31. Hughes DA. Effects of carotenoids on human immune function. *Proc Nutr Soc* 1999; **58**: 713–8.
32. Eichholzer M, Stahelin HB, Gey KF, Ludin E, Bernasconi F. Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year follow-up of the prospective Basel study. *Int J Cancer* 1996; **66**: 145–50.
33. Stefani ED, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Carzoglio JC, Ronco A, Olivera L. Dietary antioxidants and lung cancer risk: a case-control study in Uruguay. *Nutr Cancer* 1999; **34**: 100–10.
34. Murakoshi M, Nishino H, Satomi Y, Takayasu J, Hasegawa T, Tokuda H, Iwashima A, Okuzumi J, Okabe H, Kitano H, Iwasaki R. Potent preventive action of α-carotene against carcinogenesis: spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by α-carotene than by β-carotene. *Cancer Res* 1992; **52**: 6583–7.
35. Kohno H, Tajima M, Sumida T, Azuma Y, Ogawa H, Tanaka T. Inhibitory effect of mandarin juice rich in β-cryptoxanthin and hesperidin on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary tumorigenesis in mice. *Cancer Lett* 2001; **174**: 141–50.
36. Khachik F, Beecher GR, Smith JC. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J Cell Biochem* 1995; **22** Suppl: 236–46.
37. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J Natl Cancer Inst* 1999; **91**: 317–31.
38. van het Hof KH, Brouwer IA, West CE, Haddeman E, Steegers-Theunissen RPM, van Dusseldrop M, Weststrate JA, Eskes TKAB, Hautvast JGAJ. Bioavailability of lutein from vegetables is 5 times higher than that of β-carotene. *Am J Clin Nutr* 1999; **70**: 261–8.
39. Palozza P, Maggiano N, Calviello G, Lanza P, Piccioni E, Ranelletts GM. Canthaxanthin induces apotosis in human cancer cell lines. *Carcinogenesis* 1998; **19**: 373–6.
40. Joshi R, Adhikari S, Patro BS, Chattopadhyay S, Mukherjee T. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radic Biol Med* 2001; **30**: 1390–9.
41. Voorrips LE, Goldbohm RA, Brants HA, von Poppel GA, Sturmans F, Hermus RJ, van den Brandt PA. A prospective cohort study on antioxidant and folate intake and male lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2000; **6**: 357–65.
42. Hartman TJ, Woodson K, Stolzenberg-Solomon R, Virtamo J, Selhub J, Barrett MJ, Albanes D. Association of B-vitamins pyridoxal 5′-phosphate (B_6), B_{12}, and folate with lung cancer risk in older men. *Am J Epidemiol* 2001; **153**: 688–94.
43. Ocke MC, Bueno-de-Mesquita HB, Feskens EJM, van Staveren WA. Repeated measurements of vegetables, fruits, β-carotene, and vitamins C and E in relation to lung cancer. *Am J Epidemiol* 1997; **145**: 358–65.
44. Comstock G, Bush T, Helzlsouer K. Serum retinol, beta-carotene, vitamin E, and selenium as related to subsequent cancer of specific sites. *Am J Epidemiol* 1992; **135**: 115–21.
45. Ito Y, Suzuki K, Suzuki S, Sasaki R, Otani M, Aoki K. Serum antioxidants and subsequent mortality rates of all causes or cancer among a rural Japanese inhabitants. *Int J Vitam Nutr Res* 2002; **72**: 237–50.
46. Kritchevsky SD, Kritchevsky D. Serum cholesterol and cancer risk an epidemiologic perspective. *Annu Rev Nutr* 1992; **12**: 391–416.
47. Siemianowicz K, Gminiski J, Stajszczyk M, Wojakowski W, Goss M, Machalski M, Telega A, Brulinski K, Magiera-Molendowska H. Serum total cholesterol and triglyceride levels in patients with lung cancer. *Int J Mol Med* 2000; **5**: 201–5.