RESEARCH COMMUNICATION

A Population-based Follow-up Study on Mortality from Cancer or Cardiovascular Disease and Serum Carotenoids, Retinol and Tocopherols in Japanese Inhabitants

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Abstract

Objective: Observational epidemiologic studies have shown that a high intake of dietary and high serum levels of carotenoids are associated with a reduced risk of mortality from cancer and cardiovascular disease. To investigate whether high serum levels of carotenoids can reduce mortality rates, a population-based follow-up study was conducted among Japanese inhabitants. Materials and Methods: Three thousand two hundred and fifty-four subjects (1,260 males and 1,994 females) aged from 39 to 85 years who had attended health check-up programs from 1989 to 1995 were recruited from the Japanese population. Serum levels of carotenoids, retinol and tocopherols were separately determined by high-performance liquid chromatography. Hazard ratios for serum values of carotenoids, retinol and tocopherols were estimated by Cox's proportional hazard model after adjusting for sex, age, and other confounding factors. Results: During the 11.7-year follow-up period, 140 deaths (86 males and 54 females) from cancer of all sites were identified among the cohort subjects, including 41 from lung , 17 from stomach , 16 from colorectal and 12 from liver cancer, as well as 89 deaths from cardiovascular disease, including 45 from heart disease and 37 from stroke. High serum values of carotenoids including xanthophylls were apparently associated with low hazard ratios for mortality rates of cancer of all sites or of cardiovascular disease. High serum values of β-carotene, total carotene, provitamin A and total carotenoid for colorectal cancer or stroke also appeared to be related to low hazard ratios. Those of retinol and tocopherols were not associated with any reduction in risk of mortality from cancer or cardiovascular disease. Conclusions: Our follow-up study demonstrated that a typical Japanese diet related to elevating serum levels of carotenoids with provitamin A activity may significantly reduce risk of mortality from cancer of certain sites or cardiovascular disease, especially colorectal cancer or stroke, while high serum levels of some xanthophylls, retinol and tocopherols do not.

Key Words: Follow-up studies - mortality - cancer – colorectal cancer – stroke – carotenoids - tocopherols

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Introduction

Many epidemiologic studies have shown that a high dietary intake of vegetables and fruits rich in carotenoids can reduce the risk of human cancer mortality (Hirayama, 1979; Peto et al., 1981; Block et al.,1992; van Poppel et al., 1995; Riboli et al., 2003; Sauvaget et al., 2003; Donaldson, 2004; Vainio et al., 2006), including death from lung (Feskanich et al., 2000; Neuhouser et al., 2003; Smith-Warner et al., 2003; Mannisto et al., 2004), stomach (Lunet, et al., 2005), and colorectal cancer (Potter, 1996; Sanjoaquin et al., 2004; Satia-About et al., 2003; Lin et al., 2005). A high intake of vegetables and fruits is also associated with a reduced mortality from cardiovascular disease (Ness et al., 1997; Liu et al., 2000; Bazzano, 2000; Genkinger et al., 2004), coronary heart disease (Knekt et al., 1994; Joshipura et al., 2001), and stroke (Joshipura et al., 1999; Johnsen et al., 2003).

Some studies showed that high serum levels of carotenoids such as β-carotene (BC) also had an association with a low mortality rate from cancer (Comstock et al., 1992; Vianio et al., 1998), including lung cancer (Comstock et al., 1997; Holick et al., 2002; Ito et al., 2003), stomach cancer (Abnet et al., 2003; Yuan et al., 2004), cardiovascular disease (Voutilainen et al., 2006), coronary heart disease (Morris et al., 1994; Street et al., 1994; Kritchevsky, 1999; Tavani et al., 1999, Knekt et al., 2004), and stroke (Hak et al., 2004; Chang et al., 2005). We previously found that high serum

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levels of α-carotene (AC) and BC among Japanese male inhabitants can significantly reduce the risk of mortality from lung cancer in a case-control study nested in a large-scale Japanese cohort (Ito et al., 2005). In addition, high serum levels of AC and BC were also associated with low mortality rates from cardiovascular disease in Japanese inhabitants (Ito et al., 2006). Recent studies have also found that serum levels of carotenoids such as β-cryptoxanthin (CR) and lutein have a clearly inverse association with the risk of lung cancer (Yuan et al., 2001). Moreover, an association was shown among Europeans between low serum levels of lycopene (LY) and a high risk of coronary heart disease or stroke (Kohlmeier et al., 1997; Rissanen et al., 2001, 2002; Hak et al., 2004; Sesso et al., 2004).

It is well known that serum levels of AC and BC are affected by daily lifestyle factors such as diet, smoking and alcohol consumption (Aoki et al., 1987; Stryker et al., 1988; Ito et al., 1991, 1994),which were related with a risk factor for cancer or cardiovascular disease. Recently, several intervention studies of BC administration for the prevention of cancer or cardiovascular disease have been conducted among Europeans (The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994; Hennekens et al., 1996; Omenn et al, 1996), and the obtained results were then evaluated (Vianio et al., 1998; Morris et al., 2003; Taylor et al., 2005). The BC supplementation study among healthy women proved neither beneficial nor harmful to health or in terms of the incidence of cancer and cardiovascular disease (Lee et al., 1999). Moreover, follow-up studies among male subjects also failed to show any overall protective association between BC administration and the incidence of cancer or coronary heart disease among male subjects (Goodman et al., 2004; Tornwall et al., 2004; Hak et al., 2004).

It has been shown that carotenoids, including AC, BC and CR, can provide an antioxidant activity and an enhancement of immunity related to protecting against mortality from cancer or cardiovascular disease (Gerster, 1992; Stahl and Sies, 2003; Bendich, 1990, 2004; Hughes, 1999). Except for our recent studies, there were no reports on whether high serum levels of carotenoids, retinol or tocopherols were associated with a reduced risk of mortality from cancer or cardiovascular disease in a population-based follow-up study on Japanese inhabitants (Ito et al., 2005, 2006).

In the present study, we investigated effects of serum levels of carotenoids, including xanthophylls, retinol (RE) and tocopherols, with respect to deaths from specific types of cancer, such as lung, stomach, colorectal and liver, or from cardiovascular disease.

Materials and Methods

Subjects and Health Check-up Programs

The study subjects were recruited from the inhabitants of a rural area of southern Hokkaido, Japan, who attended for the first time health check-up programs (CHEP) from 1988 to 1995. They were 3,254 participants (1,260 males and 1,994 females) who were aged from 39 to 85 years of age. The subjects were not randomly selected, but the distribution by age was similar to that of the town population. The CHEP was applied to those individuals age 40 years and over, but 39 year-olds were accepted if they turned 40 years old within the year. Table 1 shows the age and sex distributions, smoking habits, and alcohol consumption of the subjects at baseline, and the causes of deaths during follow-up. About 30% of the subjects were aged 39-69 years, while those 70 to 85 years comprised only less than 10% of the cohort. The proportions of current smokers and regular drinkers (alcohol consumed daily = regular drinkers) were 49.6 and 67.1% for males and 10.0% and 26.2% for females, respectively. The percentages of subjects with some symptoms were 37.1% for high BMI (above 25 kg/m^2), 38.3% for high serum cholesterol (above 220 mg/dl), 25.4% for high serum TG (above 150 mg/dl), 6.3% or 3.5% for high alanine aminotransferase (ALT: GPT) or aspartate aminotransferase (AST : GOT) activity (above 41 IU/L), and 14.9% for high BUN (above 21 mg/dl). In addition, the percentages of subjects, who responded with a history of cancer, cardiovascular disease or other related diseases were 1.5% for cancer, 24.1% for hypertension, 5.7% for heart disease, 1.1% for stroke, 4.8% for diabetes, 9.1% for liver disease, 5.2% for kidney disease, 14.6% for stomach disease, and 8.0% for tuberculosis, respectively.

Collection of CHEP Information

The informed consent to our study for using the information obtained from questionnaires and serum collected at the CHEP was accepted by individual signature (Ito et al., 1997, 2002). A questionnaire regarding health status, personal medical-treatment history of diseases, and daily lifestyles was distributed to the participants a few days or a few months before CHEP, and answers were checked and revised by public health nurses at the CHEP (Ito et al., 1997, 2002, 2006). The responses to smoking status and alcohol consumption were obtained by individual interviews with the nurses using a three-level questionnaire [1. current smoker (cigarettes smoked daily); 2. former smoker (quit smoking); 3. never smoker, and 1. regular drinker (alcohol consumed daily); 2. irregular drinker (alcohol consumed occasionally); and 3. non drinker (alcohol never consumed)]. This study was approved by the Ethical Board of the Nagoya University School of Medicine.

Follow-up of the cohort

The cohort was followed until December 2003 using mortality records obtained with the permission of the Agency of General Affairs and the Ministry of Health and Warfare. Death certificates were examined to check the direct and indirect causes of death, which were classified using the International Classification of Diseases (ICD) 9 from 1989 to 1994 and ICD 10 from 1995 to 2003. The number of residents who had moved away, excluding the young age group, was small (male: 8% and female: 10.4%) (Ito et al., 2002). We calculated sample death rates using person-years.

During the eight- to fourteen-year follow-up period (mean: 11.7- person years), 321 deaths occurred among the cohort (197 males and 124 females). Of these, 142 deaths (87 males and 55 females) from cancer and 92 deaths (53 males and 39 females) from cardiovascular disease were attributed to cancer, including 41 lung cancer, 18 stomach cancer, 16 colorectal cancer, 13 liver cancer, and to cardiovascular disease including 46 heart disease, 40 stroke, 63 from other diseases, and 24 from accidents, as shown in Table 1.

Table 1. Characteristics of All Study Subjects

Item	Male (%)	Female (%)	Overall (%)
Total	1,260 (100)	1,994 (100)	3,254 (100)
Age39-49	417 (33.1)	694 (34.8)	1,111 (34.1)
50-59	341 (27.1)	597 (29.9)	938 (28.8)
60-69	365 (29.0)	565 (28.3)	930 (28.6)
70-85	137 (10.9)	138 (6.9)	275 (8.5)
Smoking Status			
Current smoker	625 (49.6)	200 (10.0)	825 (25.4)
Former smoker	347 (27.5)	97 (4.9)	444 (13.6)
Never smoker	288 (22.9)	1,697 (85.1)	1,985 (61.0)
Alcohol Consumption			
Regular drinker	845 (67.1)	522 (26.2)	1,371 (42.1)
Irregular drinker	156 (12.4)	85 (4.3)	241 (7.4)
Non drinker	259 (20.6)	1,384 (69.4)	1,642 (50.5)
Physiology Data			
Body Mass Index (BMI)			
25 kg/m2 ≤	444 (35.2)	762 (38.2)	1,206 (37.1)
Total Cholesterol (TC)			
220 mg/dl ≤	421 (33.4)	825 (41.4)	1,246 (38.3)
Triglyceride (TG)			
150 mg/dl ≤	381 (30.2)	446 (22.4)	827 (25.4)
Alanine Aminotransferase (ALT))			
41 U/l ≤	121 (9.6)	85 (4.3)	206 (6.3)
Aspartate Aminotransferase (AST)			
41 U/l ≤	60 (4.8)	54 (2.7)	114 (3.5)
Blood Urea Nitrogen (BUN)			
21 mg/dl ≤	191 (15.2)	180 (9.0)	485 (14.9)
History of Diseases			
Cancer	8 (0.6)	42 (2.1)	50 (1.5)
Hypertersion	302 (24.0)	481 (24.1)	783 (24.1)
Heart disease	76 (6.0)	109 (5.5)	185 (5.7)
Stroke	25 (2.0)	11 (0.6)	36 (1.1)
Diabetes	81 (6.4)	76 (3.8)	157 (4.8)
Liver disease	131 (10.4)	164 (8.2)	295 (9.1)
Kideny disease	50 (4.0)	118 (5.9)	168 (5.2)
Stomach disease	271 (21.5)	205 (10.3)	476 (14.6)
Tuberculosis	123 (9.8)	138 (6.9)	261 (8.0)
Deaths			
All Causes	197 (100)	124 (100)	321 (100)
Cancer of All Sites	87 (44.2)	55 (44.4)	142 (44.2)
Lung	33 (16.8)	8 (6.5)	41 (12.8)
Stomach	15 (7.6)	3 (2.4)	18 (5.6)
Colorectal	9 (4.6)	7 (5.6)	16 (5.0)
Liver	7 (3.6)	6 (4.8)	13 (4.1)
Other Sites	23 (11.7)	31 (25.0)	54 (16.8)
Cardiovascular	53 (26.9)	39 (31.5)	92 (28.7)
Heart Disease	22 (11.2)	24 (19.4)	46 (14.3)
Ischemic	15 (7.6	21 (16.9)	36 (11.2)
Stroke	28 (14.2)	12 (9.7)	40 (12.5)
Other	3 (1.5)	4 (3.2)	7 (2.2)
Accidents	13 (6.6)	11 (8.9)	24 (7.5)
Other	44 (22.3)	19 (15.3)	63 (19.6)

Blood Samples and Determination Methods for Serum Component Levels

Fasting serum samples were taken at the CHEP, and each serum level of carotenoids, RE, and tocopherols was separately determined by HPLC, as previously reported (Ito et al., 1990). Serum levels of zeaxanthin/lutein (ZL) or total carotene (TCA) were the sum of zeaxanthin and lutein levels or the sum of AC, BC, and LY levels, respectively. Serum levels of provitamin A (PVA), total xanthophyll (TXA) or total carotenoid (TCAR) were the sum of AC, BC and CR levels, CR, ZL and canthaxanthin (CX) levels, or TCA and TXA levels, respectively. Serum levels of total cholesterol (TC), triglyceride (TG), ALT activity, and other biomedical tests were determined using an auto-analyzer (SMAC, Technicon Co., Ltd., or TBA-80M, Toshiba Co., Ltd.). Each serum value of carotenoids, retinol, tocopherols, TC, TG and ALT activity was transformed logarithmically.

Statistical Analysis

Among the 3,254 recruited inhabitants, 3,204 (1,252 males and 1,952 females) were enrolled for analyses of cancer mortality after excluding the 14 subjects who answered with a history of cancer. Three hundred and eleven deaths (193 males and 118 females) from all causes, 140 (86 males and 54 females) from cancer of all sites, 41 from lung, 17 from stomach, 16 from colorectal, 12 from liver, and 54 from other cancers were identified. For analyses of mortality from cardiovascular disease, 3,039 participants (1,163 males and 1,876 females) from 3,254 follow-up subjects were also enrolled after excluding 215 subjects (97 males and 118 females) who responded with a history of cardiovascular disease. Seventy-five deaths from cardiovascular disease including 39 from heart disease, 30 from ischemic heart disease, and 31 from stroke were identified among the follow-up cohort subjects.

Statistical analyses of the mean value differences between the survivors and those who died of cancer were performed by analysis of variance (ANOVA) after adjusting for sex, age and smoking status using the statistical package, JMP ver. 4.0. Mortality rates from cancer were calculated as the number of deaths per person-year for each serum level of carotenoids, RE and tocopherols after each serum value was transformed logarithmically. Hazard ratios per each logarithmically transformed serum value of carotenoids, RE and tocopherols for mortality from all causes or from cancer of all sites were calculated using Cox's proportional hazard model (JMP ver. 4.0) after adjusting for sex, age and smoking status (model 1), or sex, age, smoking status, alcohol consumption and serum values of TC, TG, and ALT activity (model 2). On the stepwise analyses, mortality rates for cancer were calculated as the number of deaths over person-years after equally dividing the subjects into the three groups according to high, moderate and low serum levels of

carotenoids, RE and tocopherols by sex. The hazard ratios for these groups were estimated by the Cox proportional hazard model (JMP ver. 4.0) after adjusting for the factors mentioned above (model 1 and 2)(as follows; Model 1: adjusting for sex, age and smoking status, and Model 2: adjusting for sex, age, smoking status, alcohol consumption, and serum levels of TC, TG and ALT activity.) Statistical analyses for mean value differences between those who survived and those who died of cardiovascular disease were also performed using analysis of variance (ANOVA: JMP ver. 4.0) after adjusting for sex, age, smoking status, and alcohol consumption. Mortality rates from cardiovascular disease were calculated using the same method as analyses of cancer mortality. Hazard ratios per each logarithmically transformed serum value of carotenoids, RE and tocopherols for mortality from cardiovascular diseases were calculated using Cox's proportional hazard model (JMP ver. 4.0) as follows; Model 1: adjusting for sex, age, smoking status and alcohol consumption, and Model 2: adjusting for sex, age, smoking status, alcohol consumption, body mass index (BMI), systolic blood pressure, and serum levels of TC, TG and ALT activity. On the stepwise analyses, mortality rates from cardiovascular disease were calculated as the number of deaths over person-years after equally dividing the subjects into the four groups according to highest, high, moderate and low serum levels of carotenoids, retinol and tocopherols by sex. The hazard ratios for these groups were estimated by the Cox proportional hazard model (JMP ver. 4.0) in the same way as mentioned above in the analyses of cancer mortality. Statistical significance was set at $p < 0.05$.

Results

Comparison of serum values of carotenoids, retinol, and tocopherols

Geometric mean values of serum carotenoids, including

Table 2. Comparison on Serum Carotenoid, Retinol, and Tocopherol Levels between Survivors and Dead from All Causes or Cancer in Follow-up Subjects

Components (μmol/l)		Alive	Death due to All Causes	Death due to Cancer				
				All sites	Lung	Stomach	Colorectal	Liver
α-Carotene (AC)	mean	0.121	0.097	0.093	0.079	0.092	0.095	0.067
	p value		<0.0001	<0.0001	0.007	0.0231	0.155	0.0017
β-Carotene (BC)	mean	0.727	0.565	0.539	0.497	0.425	0.451	0.350
	p value		<0.0001	<0.0001	0.0056	0.0103	0.028	0.003
Lycopene (LY)	mean	0.289	0.212	0.199	0.191	0.165	0.188	0.170
	p value		<0.0001	<0.0001	0.0017	0.0053	0.0437	0.0268
Total Carotene (TCA)	mean	1.214	0.937	0.899	0.828	0.760	0.804	0.637
	p value		<0.0001	<0.0001	0.001	0.0088	0.027	0.0022
β-Cryptoxanthin (CR)	mean	0.327	0.261	0.263	0.247	0.168	0.281	0.286
	p value		0.0001	0.0015	0.0273	0.0004	0.4936	0.6006
Zeaxanthin&Lutein (ZL)	mean	1.064	0.994	0.944	0.990	0.846	0.959	1.008
	p value		0.0423	0.0139	0.4446	0.096	0.4832	0.765
Canthaxanthin (CX)	mean	0.043	0.040	0.041	0.043	0.027	0.042	0.031
	p value		0.0928	0.3151	0.959	0.0049	0.8761	0.1006
Total Xanthophyll (TXA)	mean	1.514	1.373	1.328	1.377	1.074	1.325	1.388
	p value		0.0014	0.004	0.282	0.0068	0.3302	0.6006
Provitamin A (PVA)	mean	1.245	0.978	0.949	0.865	0.729	0.872	0.734
	p value		<0.0001	<0.0001	0.0019	0.0028	0.0597	0.0133
Total Carotenoids (TCAR)	mean	2.821	2.398	2.305	2.292	1.907	2.162	2.066
	p value		<0.0001	<0.0001	0.0218	0.004	0.063	0.0573
Retinol (RE)	mean	2.234	2.372	2.356	2.310	2.333	2.835	1.739
	p value		0.0062	0.118	0.6316	0.672	0.011	0.0152
α-Tocopherol (AT)		23.57	23.93	23.63	23.22	19.76	25.52	20.03
	p value		0.455	0.3172	0.7613	0.0339	0.3658	0.1003
β-γ-Tocopherol (BT)	mean	3.106	2.808	2.718	2.496	2.338	3.347	2.050
	p value		0.0007	0.0027	0.007	0.0231	0.495	0.0047
Total Tocopherol (TTO)	mean	26.97	27.08	25.93	26.12	22.24	29.08	22.30
	p value		0.841	0.155	0.576	0.0173	0.3696	0.0488
Number		2890	311	140	41	17	16	12

Data are geometric mean values. Differences were estimated using ANOVA after adjusting for age, sex, and smoking status. Serum levels of total carotene and total xanthophyll were sums of α-carotene, β-carotene and lycopene levels, and β-cryptoxanthin, zeaxanthin&lutein and canthaxanthin levels, respectively. Serum levels of provitamin A, total tocopherol, and total carotenoid were sums of α and β-carotenes and β-cryptoxanthin levels, α-tocopherol and β- and γ-tocopherol levels, and total carotene and total xanthophyll levels, respectively.

serum values of TCA, TXA, PVA, and TCAR, except for CX, were significantly lower for the dead due to all causes or to cancer of all sites than for the survivors among the cohort, while those of RE were higher for the deceased due to all causes (Table 2). However, there were no apparent differences in serum values of RE and AT between the survivors and the deceased due to cancer of all sites, although those of BT were significantly lower. Serum values of AC, BC, LY, CR, BT, TCA, PVA and TCAR were significantly lower for those who succumbed to lung or stomach cancer than for those who survived. Those of CX, TXA, AT, and TTO were also significantly lower for the dead due to stomach cancer. Serum values of BC, LY, TCA, PVA, and TCAR, except for TXA including CR, ZL and CX, were marginally or significantly lower for the deceased due to colorectal and liver cancer than for the survivors, while those of RE were significantly higher for the dead due to colorectal cancer. Moreover, the values of RE, BT and TTO also appeared to be lower for the deceased due to liver cancer than for the survivors in spite of tending to show high serum values for colorectal cancer.

Hazard ratios per serum values of carotenoids, retinol, and tocopherols for mortality

The hazard ratios per each logarithmically transformed serum value of carotenoids, including TCA, PVA, TXA, and TCAR, for mortality from all causes or cancer of all sites tended to be lower according to the increase in those serum values (Table 3). Those of carotenoids for mortality from cancer, except for CR and CX, proved to be statistically significant in both models. However, serum values of RE, AT, BT, and TTO were not significantly associated with hazard ratios either for mortality from all causes or from cancer of all sites. Serum values of carotenoids, RE and tocopherols, except for CR, CX and AT, also tended to be inversely associated with hazard ratios for lung cancer mortality. The hazard ratio per each high serum carotenoid value was found to be lower for stomach cancer death, while those of CR, CX, TXA, and TTO were statistically significant in model 1. Those of carotenoids also tended to be inversely related to hazard ratios for colorectal cancer death, and those of BC, TCA, PVA and TCAR were shown to be significantly lower in both models. High serum values of RE appeared to be associated with high hazard ratios for colorectal cancer death, while high serum values of AT, BT, and TTO also tended to be related to high hazard ratios in model 1. However, our study indicated that the hazard ratios per each high serum value of carotenoids, RE, and tocopherols, except for xanthophylls, tended to be lower for

Table 3. Hazard Ratios per Serum Values of Carotenoids, Retinol and Tocopherols for Deaths from All Causes and Cancers

Components		All Causes (n=311)		Cancer of All (n=140)		Lung (n=41)		Stomach (n=17)		Colorectal (n=16)		Liver (n=12)	
(μmol/l)	Model	HR (95% CI)	p*	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
α-Carotene	1	0.76 (0.63-0.91)	0.003	0.70 (0.53-0.91)	0.008	0.65 (0.41-1.06)	0.081	0.94 (0.45-2.01)	0.867	0.61 (0.29-1.36)	0.223	0.36 (0.17-0.84)	0.020
(AC)	2	0.79 (0.65-0.95)	0.013	0.71 (0.54-0.94)	0.017	0.62 (0.39-1.03)	0.065	1.19 (0.55-2.55)	0.670	0.49 (0.22-1.16)	0.104	0.47 (0.21-1.25)	0.126
β-Carotene	1	0.79 (0.68-0.92)	0.003	0.76 (0.60-0.95)	0.016	0.96 (0.63-1.46)	0.849	0.71 (0.38-1.35)	0.293	0.42 (0.22-0.82)	0.012	0.37 (0.18-0.78)	0.009
(BC)	2	0.81 (0.69-0.95)	0.010	0.76 (0.60-0.96)	0.023	0.91 (0.58-1.41)	0.664	0.81 (0.41-1.60)	0.549	0.36 (0.18-0.73)	0.005	0.38 (0.17-0.88)	0.023
Lycopene	1	0.78 (0.68-0.91)	0.001	0.71 (0.58-0.89)	0.002	0.80 (0.54-1.19)	0.278	0.64 (0.34-1.18)	0.156	0.59 (0.31-1.12)	0.109	0.59 (0.28-1.23)	0.162
(LY)	2	0.80 (0.69-0.93)	0.003	0.72 (0.57-0.90)	0.003	0.78 (0.52-1.17)	0.232	0.71 (0.37-1.34)	0.292	0.50 (0.25-0.98)	0.042	0.69 (0.30-1.57)	0.377
Total Carotene	1	0.74 (0.62-0.89)	0.005	0.70 (0.54-0.91)	0.007	0.87 (0.54-1.40)	0.562	0.70 (0.34-1.48)	0.350	0.41 (0.19-0.89)	0.023	0.34 (0.14-0.80)	0.014
(TCA)	2	0.77 (0.64-0.92)	0.004	0.71 (0.54-0.92)	0.012	0.82 (0.50-1.35)	0.441	0.86 (0.40-1.84)	0.688	0.33 (0.15-0.73)	0.007	0.38 (0.14-1.00)	0.051
βCryptoxanthin	1	0.81 (0.69-0.95)	0.009	0.86 (0.67-1.09)	0.203	1.01 (0.66-1.56)	0.956	0.49 (0.25-0.95)	0.036	0.80 (0.38-1.67)	0.548	1.13 (0.50-2.53)	0.772
(CR)	2	0.84 (0.71-0.99)	0.045	0.88 (0.68-1.13)	0.317	0.99 (0.63-1.56)	0.969	0.58 (0.28-1.18)	0.135	0.66 (0.30-1.43)	0.289	1.97 (0.82-4.80)	0.130
Zeaxanthin&Lutein	1	0.76 (0.62-0.92)	0.006	0.66 (0.49-0.89)	0.007	0.90 (0.52-1.55)	0.715	0.54 (0.22-1.28)	0.161	0.62 (0.25-1.48)	0.285	0.83 (0.29-2.27)	0.717
(ZL)	2	0.78 (0.63-0.95)	0.013	0.67 (0.49-0.91)	0.040	0.90 (0.52-1.55)	0.709	0.62 (0.25-1.47)	0.282	0.56 (0.23-1.34)	0.197	1.04 (0.34-3.05)	0.944
Canthaxanthin	1	0.81 (0.69-0.95)	0.009	0.86 (0.67-1.09)	0.203	1.01 (0.66-1.56)	0.956	0.49 (0.25-0.95)	0.036	0.80 (0.38-1.67)	0.548	1.13 (0.50-2.53)	0.772
(CX)	2	0.89 (0.76-1.04)	0.142	0.92 (0.93-1.17)	0.502	1.05 (0.69-1.61)	0.826	0.50 (0.27-0.97)	0.040	0.76 (0.37-1.56)	0.454	0.87 (0.37-2.13)	0.768
Total xanthophyll	1	0.71 (0.57-0.88)	0.002	0.65 (0.47-0.91)	0.016	0.98 (0.54-1.78)	0.956	0.37 (0.14-0.97)	0.043	0.53 (0.19-1.41)	0.207	0.83 (0.27-2.53)	0.746
(TXA)	2	0.74 (0.59-0.92)	0.008	0.67 (0.47-0.94)	0.019	0.97 (0.53-1.77)	0.927	0.45 (0.17-1.21)	0.115	0.44 (0.16-1.19)	0.106	1.27 (0.38-4.12)	0.693
Provitamin A	1	0.74 (0.62-0.89)	0.001	0.72 (0.56-0.94)	0.016	0.90 (0.56-1.47)	0.671	0.60 (0.29-1.25)	0.170	0.43 (0.20-0.95)	0.037	0.42 (0.18-1.03)	0.057
(PVA)	2	0.77 (0.64-0.92)	0.004	0.71 (0.54-0.92)	0.012	0.82 (0.50-1.35)	0.441	0.86 (0.40-1.84)	0.688	0.33 (0.15-0.73)	0.007	0.38 (0.14-1.00)	0.051
Total Carotenoid	1	0.67 (0.53-0.83)	0.0003	0.60 (0.43-0.84)	0.003	0.91 (0.50-1.68)	0.768	0.44 (0.17-1.15)	0.093	0.35 (0.13-0.93)	0.004	0.43 (0.14-1.34)	0.144
(TCAR)	2	0.69 (0.55-0.87)	0.002	0.61 (0.43-0.86)	0.005	0.87 (0.46-1.64)	0.677	0.56 (0.20-1.49)	0.247	0.26 (0.09-0.74)	0.011	0.65 (0.19-2.20)	0.490
Retinol	1	0.91 (0.66-1.25)	0.546	0.89 (0.56-1.43)	0.637	0.54 (0.22-1.30)	0.169	0.65 (0.17-2.54)	0.536	4.19 (1.12-13.8)	0.033	0.10 (0.02-0.48)	0.005
(RE)	2	0.98 (0.69-1.39)	0.928	0.96 (0.57-1.61)	0.876	0.56 (0.20-1.51)	0.255	0.88 (0.19-3.76)	0.870	3.44 (0.78-13.0)	0.101	0.25 (0.04-1.47)	0.126
α-Tocopherol	1	0.99 (0.72-1.37)	0.987	0.72 (0.44-1.17)	0.189	0.94 (0.38-2.27)	0.898	0.25 (0.06-1.04)	0.056	1.59 (0.41-5.27)	0.490	0.25 (0.04-1.33)	0.105
(AT)	2	1.15 (0.79-1.64)	0.465	0.71 (0.40-1.26)	0.243	1.05 (0.35-2.94)	0.924	0.27 (0.05-1.49)	0.136	0.82 (0.16-3.63)	0.810	0.27 (0.03-2.31)	0.241
β-+γ-Tocopherol	1	0.88 (0.70-1.10)	0.266	0.79 (0.56-1.10)	0.156	0.68 (0.37-1.26)	0.216	0.55 (0.22-1.43)	0.218	1.75 (0.64-4.97)	0.281	0.31 (0.11-0.92)	0.035
(BT)	2	0.93 (0.73-1.18)	0.527	0.80 (0.56-1.14)	0.211	0.65 (0.35-1.25)	0.195	0.66 (0.24-1.85)	0.424	1.35 (0.47-4.03)	0.585	0.40 (0.13-1.31)	0.125
Total Tocopherol	1	0.98 (0.71-1.36)	0.912	0.70 (0.42-1.14)	0.151	0.91 (0.36-2.25)	0.835	0.23 (0.05-0.97)	0.046	1.69 (0.42-5.95)	0.448	0.20 (0.03-1.12)	0.062
(TTO)	2	1.14 (0.77-1.65)	0.510	0.67 (0.36-1.22)	0.193	0.99 (0.32-2.91)	0.986	0.24 (0.04-1.40)	0.115	0.85 (0.16-4.05)	0.852	0.22 (0.02-1.95)	0.176

*p for trend. Hazard ratios (HR) and 95 confidence intervals (95% CI) were calculated using Cox proportional hazard model adjusting for age, sex, and smoking status (molde 1) and for age, sex, smoking status, alcohol consumption, and serum values of total cholesterol, triglyceride, and alanine aminotransferase activity (model 2). Serum levels of carotenoids, retinol, tocopherols, total cholesterol, triglyceride and ALT activity were transfered to the logarithmic values.

mortality rates from liver cancer and those of BC showed to be statistically significant in both models. However, serum values of TCAR, which reflected the total serum contents of carotenoids, were not found to be inversely or significantly associated with hazard ratios for mortality rates of lung, stomach, or liver cancer, except for colorectal cancer in both models. In addition, those of AT and BT were not shown to be inversely or significantly related to hazard ratios for mortality rates from all causes, cancer of all sites, lung, stomach, or colorectal cancer.

Hazard ratios of divided serum levels of carotenoids, retinol, and tocopherols for mortality from all causes or cancer of all sites.

High serum levels of carotenoids except for CX were associated with low hazard ratios for mortality from all causes in model 1. High serum levels of carotenoids except for CX were also marginally or significantly associated with low hazard ratios for deaths from cancer of all sites, compared to low serum levels, and the hazard ratio trend was statistically significant for those of AC, BC, LY, TCA, PVA and TCAR. High serum levels of xanthophylls such as CR and ZL also tended to be related to low hazard ratios for cancer deaths including stomach cancer. However, high serum levels of carotenoids, including LY, CR, ZL, and CX, did not appear to show a low hazard ratio for deaths from lung cancer. Those of BC, LY, TCA, PVA and TCAR were marginally or significantly related to low hazard ratios for deaths from colorectal cancer, and the hazard ratio trend for serum BC levels was statistically significant. Low hazard ratios for deaths from liver cancer were shown to be marginally associated with high serum levels of BC, CX, and PVA in spite of a few deaths from liver cancer. The hazard ratios for high serum levels of LY, TCA, AT, BT and TTO could not be calculated because deaths from liver cancer were not detected among the high rank of serum levels. However, high serum RE levels were not demonstrably related to low hazard ratios for mortality from all causes, cancer of all sites and lung, or stomach cancer. High serum RE levels were significantly associated with high hazard ratios for deaths from colorectal cancer, although those were inversely related to hazard ratios for liver cancer deaths. High serum levels of AT, BT and TTO also did not appear to be associated with low hazard ratios for deaths from all causes, cancer of all sites, and lung or stomach cancer. In contrast, high serum levels tended to be related with high hazard ratios for deaths from colorectal cancer.

High serum carotenoid levels except for CX were also marginally or significantly associated with low hazard ratios for mortality from all causes or cancer of all sites in model 2. No results demonstrated that hazard ratios for lung or stomach cancer were lower for high serum levels of carotenoids than for low serum levels. Hazard ratios for liver cancer tended to be lower for high serum levels of BC and PVA, although the hazard ratios for high serum levels of LY, TCA, AT, BT and TTO could not be calculated because deaths from liver cancer were not detected among the high rank of serum levels. In addition, hazard ratios for deaths from colorectal cancer appeared to be lower for high serum levels of BC, LY, PVA and TCAR than for low serum levels. Moreover, high serum levels of BC, LY, PVA, and TCAR were marginally or significantly associated with low hazard ratios for deaths from colorectal cancer in both models. In contrast, those of RE, AT and BT tended to be positively associated with hazard ratios for death from colorectal cancer, though not significantly so.

Comparison of serum levels of carotenoids, retinol and tocopherols at baseline between the survivors and the dead due to cardiovascular disease.

Table 5. Comparison of Serum Carotenoid, Retinol, and Tocopherol Levels between Survivors and Dead due to Cardiovascular Disease in the Followed Subjects

Components (μmol/l)	Alive	Death due to CV	H	IHD	Stroke
α-Carotene	0.122	0.095	0.097	0.104	0.087
(AC) p value		0.001	0.031	0.212	0.005
β-Carotene	0.732	0.545	0.606	0.617	0.462
(BC) p value		0.004	0.212	0.324	0.003
Lycopene	0.290	0.213	0.219	0.217	0.198
(LY) p value		0.001	0.037	0.060	0.011
Total Carotene	1.221	0.825	0.992	1.017	0.792
(TCA) p value		0.001	0.096	0.204	0.001
β-Cryptoxanthin	0.329	0.257	0.278	0.290	0.243
(CR) p value		0.007	0.210	0.432	0.033
Zeaxanthin&Lutein	1.063	1.016	1.193	1.060	0.967
(ZL) p value		0.509	0.818	0.993	0.361
Canthaxanthin	0.043	0.041	0.043	0.045	0.040
(CX) p value		0.500	0.966	0.703	0.509
Total Xanthophyll	1.516	1.392	1.483	1.471	1.327
(TXA) p value		0.178	0.882	0.800	0.166
Provitamin A	1.252	0.953	1.033	1.051	0.847
(PVA) p value		0.002	0.128	0.228	0.003
Total Carotenoid	2.831	2.396	2.570	2.587	2.194
(TCAR) p value		0.012	0.331	0.430	0.012
Retinol	2.220	2.381	2.456	2.428	2.466
(TE) p value		0.126	0.102	0.210	0.130
α-Tocopherol	23.27	24.75	25.82	24.93	23.46
(AT) p value		0.131	0.062	0.288	0.932
β+γ-Tocopherol	3.123	3.274	3.511	3.855	2.951
(BT) p value		0.337	0.118	0.016	0.579
Total Tocopherol	26.69	28.56	29.99	29.32	26.80
(TTO) p value		0.081	0.029	0.125	0.971
Number	2759	75	39	30	31

Data were represented as geometric mean values and ranges of 10% and 90% in parenthesis.Differences in mean values were estimated using ANOVA after adjusting for age, sex, and smoking status.Serum levels of total carotene and total xanthophyll were sums of α-carotene, β-carotene and lycopene levels, and β-cryptoxanthin, zeaxanthin&lutein and canthaxanthin levels, respectively. Serum levels of provitamin A, total tocopherol, and total carotenoid were sums of α-and β-carotenes and β-cryptoxanthin levels, α-tocopherol and β- and γ-tocopherol levels, and total carotene and total xanthophyll levels, respectively.

Serum values of carotenoids except for xanthophylls appeared to be significantly lower for the dead due to cardiovascular disease than for the survivors among cohort subjects, although those of ZL, CX and TXA tended to be only marginally lower (Table 5). Those of carotenoids, except for CR, ZL and CX, were lower for the dead due to heart disease than for the survivors, and those of AC and LY were statistically significant. Serum values of carotenoids except for CX were found to be lower for the dead due to ischemic heart disease than for the survivors, in common with the trend for heart disease. Serum values of those carotenoids were lower for the dead due to stroke than for the survivors and serum values of carotenoids, except for ZL, CX, and TXA, appeared to be statistically significant. In addition, serum values of RE and tocopherols tended to be higher for the dead due to cardiovascular disease, including heart disease, than for the survivors. Those of tocopherols, especially BT, were marginally or significantly higher for the dead due to heart disease, but not to stroke.

Hazard ratios per serum values of carotenoids, retinol and tocopherols for mortality from cardiovascular disease

The hazard ratios per each logarithmically transformed serum value of carotenoids, except for xanthophylls including ZL and CX, appeared to be lower according to an increase in those serum values for mortality from cardiovascular disease in both models (Table 6). Those per serum values of BC, TCA, and PVA were statistically significantly lower in both models. However, serum values of any carotenoids were not found to be significantly associated with the hazard ratios for mortality from heart disease, including ischemic heart disease, although the hazard ratios appeared to be below 1.0 for hazard ratios per each serum value. In contrast, hazard ratios for mortality from cardiovascular disease, including heart disease, tended to elevate higher according to the increase in serum values of RE and tocopherols. In addition, the hazard ratios for deaths from ischemic heart disease were shown to be significantly higher for serum BT values, while serum levels

Table 6. Hazard Ratios per Serum Carotenoid, Retinol and Tocopherol Values for Deaths from Cardiovascular Disease

Components		Cardiovascular Disease (75)		Heart Disease (39)		Ischemic Heart Disease (30)		Stroke (31)	
(μ mol/l)	Model	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
α-Carotene	1	0.69 (0.47-1.00)	0.049	0.63 (0.37-1.06)	0.083	0.72 (0.39-1.32)	0.285	0.64 (0.36-1.12)	0.120
(AC)	2	0.71 (0.48-1.04)	0.082	0.60 (0.34-1.05)	0.073	0.72 (0.38-1.37)	0.315	0.65 (031-1.12)	0.125
β-Carotene	1	0.68 (0.50-0.94)	0.019	0.72 (0.46-1.12)	0.143	0.69 (0.42-1.15)	0.158	0.59 (0.36-0.96)	0.035
(BC)	2	0.71 (0.51-0.99)	0.043	0.72 (0.45-1.15)	0.166	0.71 (0.42-1.22)	0.216	0.60 (0.37-0.98)	0.040
Lycopene	1	0.77 (0.57-1.03)	0.076	0.74 (0.49-1.12)	0.157	0.70 (0.44-1.12)	0.138	0.74 (0.47-1.17)	0.200
(LY)	2	0.79 (0.58-1.08)	0.123	0.70 (0.45-1.08)	0.104	0.67 (0.40-1.09)	0.104	0.81 (0.51-1.30)	0.390
Total Carotene	1	0.65 (0.45-0.92)	0.017	0.67 (0.41-1.12)	0.124	0.66 (0.38-1.18)	0.158	0.55 (0.32-0.95)	0.034
(TCA)	2	0.67 (0.46-0.97)	0.034	0.65 (0.39-1.11)	0.114	0.66 (0.36-1.21)	0.176	0.57 (0.33-0.99)	0.047
β-Cryptoxanthin	1	0.72 (0.51-1.00)	0.050	0.75 (0.47-1.20)	0.227	0.77 (0.45-1.31)	0.332	0.72 (0.43-1.19)	0.201
(CR)	2	0.76 (0.53-1.09)	0.134	0.73 (0.44-1.19)	0.209	0.77 (0.43-1.36)	0.364	0.81 (0.46-1.41)	0.454
Zeaxanthin/Lutein	1	0.76 (0.51-1.15)	0.200	0.88 (0.50-1.55)	0.664	0.81 (0.42-1.53)	0.518	0.68 (0.36-1.28)	0.240
(ZL)	2	0.79 (0.52-1.19)	0.266	0.87 (0.49-1.55)	0.649	0.82 (0.42-1.58)	0.554	0.72 (0.37-1.37)	0.316
Canthaxanthin	1	0.89 (0.66-1.21)	0.463	0.98 (0.64-1.52)	0.942	1.06 (0.65-1.74)	0.822	0.85 (0.53-1.36)	0.490
(CX)	2	0.92 (0.67-1.27)	0.612	0.97 (0.62-1.52)	0.890	1.06 (0.63-1.78)	0.834	0.89 (0.54-1.48)	0.649
Total Xanthophyl l		10.69 (0.44-1.08)	0.105	0.80 (0.42-1.50)	0.491	0.73 (0.35-1.50)	0.400	0.62 (0.31-1.25)	0.179
(TXA)	2	0.72 (0.45-1.16)	0.179	0.79 (0.40-1.51)	0.471	0.74 (0.35-1.57)	0.433	0.67 (0.32-1.40)	0.289
Provitamin A	1	0.63 (0.44-0.91)	0.014	0.64 (039-1.08)	0.096	0.62 (0.34-1.12)	0.110	0.57 (0.32-1.00)	0.049
(PVA)	2	0.66 (0.45-0.98)	0.038	0.63 (0.37-1.10)	0.103	0.63 (0.34-1.18)	0.147	0.59 (0.33-1.05)	0.072
Total Carotenoid	1	0.60 (0.38-0.95)	0.028	0.68 (0.36-1.28)	0.232	0.64 (0.31-1.32)	0.227	0.49 (0.24-1.00)	0.050
(TCAR)	2	0.62 (0.38-1.01)	0.054	0.65 (0.33-1.28)	0.211	0.63 (0.29-1.37)	0.245	0.50 (0.24-1.05)	0.067
Retinol	1	1.10 (0.57-2.10)	0.767	1.67 (0.68-3.96)	0.259	1.53 (0.55-4.10)	0.413	1.16 (0.41-3.18)	0.780
(RE)	2	1.14 (0.56-2.25)	0.721	1.45 (0.55-3.63)	0.449	1.24 (0.40-3.53)	0.700	1.57 (0.53-4.47)	0.413
α-Tocopherol	1	1.37 (0.72-2.53)	0.338	1.78 (074-3.99)	0.194	1.29 (0.46-3.38)	0.616	0.91 (0.32-2.43)	0.848
(AT)	2	1.47 (0.69-2.99)	0.313	1.28 (0.45-3.40)	0.629	0.78 (0.23-2.51)	0.681	1.38 (0.40-4.17)	0.603
β+γ-Tocopherol	1	1.51 (0.95-2.43)	0.082	1.87 (0.98-3.62)	0.060	2.68 (1.26-5.77)	0.010	1.07 (0.53-2.22)	0.854
(BT)	2	1.55 (0.94-2.58)	0.084	1.68 (0.84-3.42)	0.142	2.47 (1.10-5.61)	0.028	1.14 (0.53-2.46)	0.745
Total Tocopherol	1	1.58 (0.81-2.97)	0.175	2.16 (0.88-4.97)	0.091	1.70 (0.60-4.52)	0.313	0.95 (0.33-2.64)	0.928
(TTO)	2	1.79 (0.82-3.72)	0.139	1.66 (0.57-4.50)	0.347	1.12 (0.32-3.71)	0.856	1.47 (0.41-4.64)	0.365

Hazard ratios were calculated using Cox proportional hazard model adjusting for age, sex, smoking status and alcohol consumption (model 1) and for age, sex, smoking staus, alcohol consumption, BMI (body mass index), systolic blood pressure,and serum values of total cholesterol, triglyceride, and alanine aminotransferase activity (model 2). Serum levels of carotenoids, retinol, tocopherols, triglyceride, total cholesterol, and ALT activity were transformed to the logarithmic values.

of tocopherols were not positively associated with hazard ratios for stroke in model 1.

Hazard ratios of divided serum levels of carotenoids, retinol, and tocopherols for mortality from cardiovascular disease

High serum levels of carotenoids, except for xanthophylls such as ZL and CX, appeared to be marginally or significantly associated with low hazard ratios for mortality from cardiovascular disease, compared to those at low levels, while those of carotenoids except for CR were not significantly associated with low hazard ratios for the dead due to heart disease, including ischemic heart disease, in model 1. High serum levels of carotenoids, except for ZL and CX, were also significantly associated with low hazard ratios for stroke, and the trends of BC, PVA, and TCAR were statistically significant. Moreover, high serum levels of RE, AT, BT, and TTO tended to be related to high hazard ratios for cardiovascular disease, including heart disease, compared low serum levels, but those levels did not appear to be related to high hazard ratios for stroke.

High serum levels of carotenoids were apparently associated with low hazard ratios for deaths from cardiovascular diseases in model 2, and LY and CR levels were statistically significant. Those of carotenoids were not significantly related to low hazard ratios for deaths from heart disease, while those of CR tended to have low hazard ratios for heart disease, including ischemic heart disease. High serum levels of carotenoids turned out to be marginally or significantly associated with low hazard ratios for deaths from stroke, and the trends of BC, PVA, and TCAR were also statistically significant, as they were in model 1. However, those of CX were not associated with low hazard ratios for mortality from stroke, which was also true of RE as same as tocopherols.

Discussion

The geographic district studied was located in a rural area of southern Hokkaido, Japan, with a population of 20,067 inhabitants (9,902 males and 10,165 females) at the end of 1982, but that had declined to 18,365 participants (8,934 males and 9,431 females) by 1995. Individuals who moved from the study area during the follow-up period accounted for from 1,072 to 1,326 inhabitants (about 6%) per year. Mortality rates from all causes, cancer, heart disease, and stroke in this district were 787.6, 180.2, 133.8 and 164.7 per 100,000 in 1989, respectively. Crude mortality rates from these diseases in the study area were higher by about 22.3% for all causes, 3.8% for cancer, 4.4% for heart disease, and 67.2% for stroke, compared to Japanese death rates nationwide. The CHEP offered to inhabitants over 39 years old every August from 1982 to the present (Ito et al., 1997, 2002). The inhabitants of this area are mainly engaged in dairy farming, commercial fishing, and commerce.

In this study cohort, serum levels of carotenoids, including xanthophylls, RE, and tocopherols have begun to be determined since August 1989 by HPLC method, as previously reported (Ito et al., 1990). The validity of serum carotenoid determination according to that assay method was less than 15% CV for day to day variation (Ito et al., 1990). Serum levels of carotenoids, RE and tocopherols obtained against the selected same residents appeared to be little different from those estimated over a 3-year period (Ito et al., 2000). Serum levels among the study subjects were slightly different from those seen in other Japanese populations (Ito et al., 1997) and differed from those of Caucasians (Ito et al., 1999) and Europeans, especially serum LY levels (Olmedilla et al., 2001). Moreover, those serum levels of carotenoids such as AC, BC, CR and ZL that were affected by various lifestyle factors such as habits of food intake, favorite behavior and physical exercise, and had been reported to be lower among current smokers and regular alcohol drinkers (Aoki et al., 1987; Stryker et al., 1988; Micozzi et al., 1992; Ito et al., 1991, 1994; Suzuki et al., 2000). Serum levels of carotenoids and tocopherols are also associated with serum TC levels and serum enzyme activities related with liver function, since serum carotenoids are carried by lipoprotein in the blood and metabolized in the liver (Bendich et al., 1987, 1990; Olsen, 1999). Serum levels of AC and BC were also inversely associated with systolic blood pressure (Ito et al., 1994). We estimated the risk of mortality from cancer or cardiovascular disease using Cox's proportional hazard model after controlling for sex, age, and other confounding factors such as smoking status, alcohol consumption, and serum levels of TC, TG and ALT activity in this study.

In the present study, analyses indicated that high serum levels of most carotenoids except for CX were marginally or significantly associated with a low risk of mortality from cancer of all sites and colorectal cancer. High serum BC levels in particular significantly tended to reduce the risk for cancer of the lung, colorectal or liver in model 2. However, no clear relation to lung cancer deaths was observed for serum levels of xanthophylls such as CR and ZL. Moreover, the clear relation between the mortality from stomach or liver cancer and serum levels of xanthophylls, including CR and ZL, was not found by stepwise analyses, because of the few deaths from stomach or liver cancer in each rank group. This suggests that further study using many more death cases is needed to clarify the effects of serum carotenoids in reducing the risk of mortality from stomach or liver cancer.

Some reports have found that serum levels of carotenoids such as BC were elevated by a high intake of vegetables and fruits (Micozzi et al., 1992; Yeum et al., 1996; Al-Delaimy et al., 2005; Ozasa et al., 2005). We too have obtained results showing that the partial coefficients between the intake frequencies of vegetables such as spinach, carrots or tomatoes and serum carotenoid levels in our cohort subjects were 0.21- 0.31 for BC, 0.17-0.29 for TCA, 0.20-0.31 for PVA, 0.18-0.29 for ZL, 0.18-0.31 for TXA, and 0.20-0.33 for TCAR, respectively. In contrast, the intake frequencies of vegetables were negatively and significantly associated with serum RE levels (partial coefficients: -0.08-

0.17, p< 0.05), as has been previously reported (Suzuki et al., 2000). High intakes of vegetables and fruits can increase the serum levels of carotenoids by about a few tenths of a percent, and bio-factors such as other carotenoids and vitamins (including vitamin B and C) appear to play a role in preventing a rise in lung cancer incidence (Comstock et al., 1997). In addition, there have been some reports that high serum levels of carotenoids, including BC, LY, CR and ZL, were significantly associated with reducing the risk of incidence or mortality from cancer, including lung (Eichholzer et al., 1996; Yuan et al., 2001; Holick et al., 2002; Ito et al., 2003), gastric (stomach) (Kumagai et al., 1998; Yuan et al., 2004) or colorectal cancer (Ito et al., 2005; Wakai et al., 2005). Previous studies have demonstrated that, compared to control, serum levels of carotenoids such as BC are lower in stomach or lung cancer (Choi et al., 1999; Eichholzer et al., 2000,). Other studies of Japanese inhabitants showed no clear trend toward reducing the risk of lung (Ito et al., 2005) or colorectal cancer among women with the highest serum BC levels (≥1.21 _mol/l)(Wakai et al., 2005). Although the mechanisms for preventing carcinogenesis by carotenoids such as BC are complex, BC (which has a particularly high levels of provitamin A activity) is considered to be a crucial factor. For instance, the finding that BC has antioxidant activity and enhances immunity related to protecting against carcinogenesis suggests that it also protects against oxidative stress such as damage to cell membranes and DNA caused by activated oxygen species and free radicals (Bendich, 1990, 2004). According to some reports, most carotenoids possess antioxidant activity (Gerster, 1992; McCall et al., 1999; Stahl and Sies, 2003), and carotenoids such as AC and BC can enhance cell-mediated immune responses (Bendich, 1990, 2004; Hughes, 1999). That finding is consistent with the inverse association between mortality from cancer of some sites and the high serum levels of carotenoids such as AC and BC observed among the inhabitants followed in the present study.

In contrast, intervention trials have found that a high-dose administration of synthetic BC is associated with an increased incidence of lung cancer in smokers (The ATBC Study Group, 1994) and industrial workers (Omenn et al., 1996). A long trial conducted by American physicians revealed no inverse association between synthetic BC administration and lung cancer incidence (Hennekens et al., 1996). A high dose of synthetic BC administration was found to elevate serum BC levels more than 10-fold, and to then produce prooxidant activities (Palozza, 1998) or to induce the carotenoid-breakdown product arising oxidative attack in biological systems (Siems et al, 2005) . It has been reported that synthetic BC administration also increases the levels of cell proliferation indicators such as c-jun and c-fos proteins in the lungs of ferrets (Wolf, 2002). Thus, the available data suggest that high-dose administration of synthetic BC alone is associated with a high risk of lung cancer incidence (Wang and Russell, 1999). There was a report that BC consumed together with fat is incorporated effectively into the body (Prince and Frisoli, 1993). We previously found that, after synthetic BC administration by our staffs, serum levels of thiobarbituric acid–reactive substances (TBARS; a class of lipid peroxides) were significantly elevated and more than 4-fold greater than serum BC levels (Ito et al., 1996), although serum TBARS levels were inversely associated with serum levels of carotenoids such as AC and BC in the inhabitants (Ito et al., 1994). In the present study, the hazard ratios of lung cancer mortality for the subjects with high serum BC levels may be due in part to the prooxidant effects of BC, since synthetic BC has been available in Japan since 1990.

In this study, the hazard ratios for mortality from colorectal or stomach cancer were lower for subjects with high serum CR levels, but not to a statistically significant degree, a similar result to that for minor serum levels of CX. There was a report that mandarin juice, which is rich in CR, had chemo-preventive effects against mouse lung tumorigenesis (Kohno et al., 2001). It was also indicated that high serum CR levels had been associated with a reduced risk of colorectal cancer (Eichholzer et al., 1996; Yuan et al., 2001). In light of these reports, our finding that serum CR levels have an inverse association with mortality from colorectal cancers should be followed, since the full potential for application of this protective substance in the prevention of cancer mortality remains unclear. In addition, although there were some reports that serum levels of ZL and CR were not inversely associated with a risk for stomach cancer (Tubono et al., 1999; Yuan et al., 2004) and that high serum levels of ZL did not appear to be correlated with a lower risk for lung cancer in this study, high serum ZL levels did tend to be associated with a lower risk for stomach or colorectal cancer. The ZL rich in green leafy vegetables possesses both antioxidant activities and an immune-promoting function (McCall et al., 1999; Hughes, 1999), and these fiber-rich foods also simulate an active movement of digestive organs such as the colon (Potter, 1996: Park et al., 2005). The bioavailability of lutein, a major carotenoid in green-leafy vegetables, was reported to be 5 times higher than that of BC (van het Hof et al., 1999). Thus, another study with a large population and measurements of serum levels of other carotenoids such as xanthophylls is needed to further clarify the results obtained in the present study.
No apparent associations except for liver cancer were found in this study between the risk of lung cancer and high serum levels of AT and RE. However, high serum RE levels tended to be associated with a high risk for colorectal cancer, while appearing to involve a low risk for liver cancer. There was a review of nine population studies showing that only a few reports of lung cancer death had lower serum levels of RE and AT (Comstock et al., 1992). In a previous follow-up study of Japanese subjects, we found that higher serum RE levels were not significantly associated with an increasing risk for mortality from cancer of all sites (Ito et al., 2002), lung and stomach cancer (Ito et al., 2005), findings similar to those in the present results. High serum AT levels were reportedly associated with a low risk of stomach cancer (Taylor et al., 2003), and high serum RE levels were said to

be related to a low risk of hepatocellar carcinoma, results similar to those on high serum levels of carotenoids such as AC and BC (Yuan et al., 2006). The available evidence suggested that higher serum RE levels also reduced the risk of lung cancer (Yuan et al., 2001; Holick et al., 2002) or colorectal cancer (Wakai et al., 2005) by preventing certain processes of carcinogenesis. Although high serum RE levels tended to be related to a low risk of mortality from stomach or liver cancer, our study ruled out the possibility that high serum levels of tocopherols including AT, an antioxidant substance (Ricciarelli et al., 2001), might be associated with reducing the risk of cancers such as lung or colorectal cancer. A further study with a much larger cohort will be needed to explain this discrepancy in the results about cancer prevention by tocopherols due to the small number of cancer cases.

The results of the present study indicated that high serum levels of carotenoids such as AC and BC were significantly associated with reducing the risk for mortality from cardiovascular disease including stroke. In addition, high serum levels of CR rich in mandarin oranges were also inversely related with a low risk for mortality from stroke. However, high serum levels of xanthophylls such as ZL and CX were apparently not associated with reducing the risk for mortality from cardiovascular disease including heart disease and stroke. Some reports indicate that bio-factors including antioxidants and vitamins such as vitamin C rich in citrus fruits appear to play a role in the prevention of early cardiovascular incidences (Palace et al., 1999; Marchioli et al., 2001; Knekt et al., 2004; Hung et al., 2004). A recent review also showed that serum levels of carotenoids such as AC, BC and LY were associated with cardiovascular disease (Arab and Steck, 2000; Voutilainen et al., 2006). It had been reported that high serum BC levels tended to be related to a low risk for cardiovascular disease (Sesso et al., 2005), coronary heart disease (Morris et al. 1994), and myocardial infarction (Hak et al., 2003). High serum levels of PVA such as AC and BC at baseline were significantly associated with reducing the risk for ischemic stroke (Hak et al., 2004; Chang et al., 2005). Although it has been shown that high serum LY levels in men were not related to a low risk of cardiovascular disease (Sesso et al., 2005) and myocardial infarction (Hak et al., 2003), low serum levels of LY were also shown to be associated with an increased risk of cardiovascular disease in women (Sesso et al., 2004), atherosclerotic vascular events (Rissanen et al., 2001), and atherosclerosis (Rissanen et al., 2003). Moreover, in the intervention studies reporting supplemental BC intakes, the highest BC intake lowered the risk of coronary heart disease events (Yochum et al., 2000; Knekt et al., 2004). Although it has been reported that the risks of cardiovascular disease or coronary heart disease were not reduced in a follow-up study after stopping BC administration among the male subjects who smoked or were exposed to asbestos (Goodman et al., 2004; Tornwall et al., 2004), in a Physician Health Study, high serum BC levels in men were shown to involve a low risk for myocardial infarction (Hak et al., 2003).

It is well known that carotenoids such as BC exert an antioxidant activity and protect against oxidative stress, as mentioned above (Bendich, 1987; McCall et al., 1999; Krinsky, 2001; Stahl and Sies, 2003). In addition, most carotenoids possess bioactivities related to antioxidants and an anti-inflammation which in turn is a reduced risk of cardiovascular disease (van Herpen-Broekmans et al., 2004). Some reports also indicated that low serum levels of carotenoids such as AC and BC were associated with inducing the inflammatory mediators (Hu et al., 2004; Walston et al., 2005). These findings are consistent with the inverse association between mortality from cardiovascular diseases and high serum levels of AC and BC observed among the rural inhabitants in the present study.

However, a high excess of BC intake has been indicated to exhibit a tendency to lose its effectiveness as an antioxidant (Palozza, 1998; Young and Lowe, 2001; Siems et al., 2005). We mentioned above that, after synthetic BC administration, serum TBARS levels were significantly elevated to more than 4-fold higher than serum BC levels (Ito et al., 1996). Since an induced lipid-peroxidation in the body was shown to be associated with a high risk for cardiovascular disease, the high hazard ratios for mortality from cardiovascular disease among the subjects in BC administration studies (Lee et al., 1999; Tornwall et al., 2004; Goodman et al., 2004) might be due in part to excessively high serum BC levels.

It was shown in this study that high serum RE levels were not associated with reducing the risk of mortality from cardiovascular disease, as is also true of high serum levels of tocopherols. AT has been well known as an essential nutrient for reproduction and possesses a potent antioxidant property (Ricciarelli et al., 2001). Moreover, the biological activity of AT is believed to be associated with the prevention of chronic diseases such as cancer or cardiovascular disease by providing protection from oxidative stress (Brigelius-Flohe et al., 1999). However, some reports conclude that high intakes of AT or highest serum AT levels do not prevent cardiovascular events (Morris et al., 2003; the HOPE Study Investigators, 2000, 2005; Lee et al., 2005), stroke (Cherubini et al., 2000), as same results that highest serum RE levels did not show to be associated with reducing the risk of cardiovascular disease (Morris and Carson, 2003; Hak et al., 2004). In addition, AT supplementation (400 IU/day) from natural sources in patients at high risk for cardiovascular events also had no apparent effect on cardiovascular outcomes (the HOPE Study investigators, 2000). These findings are consistent with the inverse association between mortality from cardiovascular diseases and high serum levels of tocopherols observed among inhabitants in the present study.

In conclusion, the present results indicate that serum carotenoids, especially provitamin A such as AC and BC are associated with reducing the risk of death from cancer, especially colorectal cancer. In addition, the risk of liver cancer appears to be lower for subjects with higher serum RE levels. Serum levels of carotenoids such as AC and BC,

in some CR and ZL cases, appear to be particularly promising biomarkers to predict cancer mortality among inhabitants of a rural area of Japan. In addition, the present results demonstrate that the lifestyles enhanced by increasing serum carotenoid levels may also prove to be particularly promising biomarkers to predict mortality from cardiovascular disease such as stroke among people in rural areas of Japan.

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