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 Ohnō, 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.

ELSEVIER

The Impact of Changes in Marital Status on the Mortality of Elderly Japanese

CHISATO NAGATA, MD, NAOYOSHI TAKATSUKA, MD, AND HIROYUKI SHIMIZU, MD

PURPOSE: To assess the impact of changes in marital status on the mortality of elderly Japanese men and women.
METHODS: In a baseline survey conducted in 1992, 2039 male and 1466 female residents in Takayama City, Gifu, who were married and aged 65 years or over responded to a detailed health and lifestyle questionnaire. Information regarding deaths of subjects and their spouses, the causes of death, and whether the subjects and spouses moved away from the city between 1992 and 1999 was obtained from the National Vital Statistics and the residential registers of the city. A proportional hazard model was used including marital status as time- varying independent variable.
RESULTS: During the study period, six men and two women became separated/divorced and 151 men and 448 women became widowed. Widowhood was not significantly associated with mortality in men. Duration of widowhood was significantly inversely associated with mortality in women ($p = 0.04$). A significant decreased hazard ratio (=0.40, $p = 0.04$) was observed for women widowed for 3 years or more.
CONCLUSIONS: We found no evidence indicating that widowed men and women have an increased mortality rate. Instead, the data suggested a decreased mortality rate among long-term widowed women.
Ann Epidemiol 2003;13:218–222.

KEY WORDS: Marital Status, Mortality, Japanese Women, Longitudinal.

INTRODUCTION

The association between marital status and mortality has been an issue of great importance to public health. The impact of changes in marital status on mortality has been examined in several longitudinal studies. Examples are cohort studies of American, Finnish, Swedish, and British men and women (1–5). Little is known about Japanese men and women in this regard. Most of the longitudinal studies suggest an increase in mortality following widowhood (1, 4, 6, 7). However, the effect appears to vary across age, sex, and duration of bereavement. It is likely that the loss of a spouse also affects mortality differently by ethnicity.

This study included married Japanese men and women to examine the effect of the loss of a spouse on subsequent mortality. We took account of a wide range of potential confounders.

From the Department of Public Health, Gifu University School of Medicine, Gifu, Japan (C.S., N.T., H.S.).
Address correspondences to: Chisato Nagata, M.D., Department of Public Health Gifu University School of Medicine, 40 Tsukasa-Machi, Gifu 500-8705, Japan. Tel.: +81-58-267-2247; Fax: +81-58-265-9020. E-mail: chisato@cc.gifu-u.ac.jp
Supported in part by a grant from the Ministry of Education, Science, Sports, and Culture, Japan.
December 17, 2001; revised April 24, 2002; accepted May 8, 2002.

METHODS

The individuals in this analysis were part of the Takayama Study (8), a prospective population study of residents in Takayama City, Gifu, Japan. A detailed description of the study design has been reported elsewhere. In the baseline survey conducted in 1992, about 30,000 men and women aged 35 years and over completed a detailed health and lifestyle questionnaire. The response rate was about 90%.

Although the questionnaire did not specifically identify each subject's spouse, the following 5 conditions were used to identify married couples: (i) The pair consisted of a man and a woman with the same household number; (ii) The difference in their ages was less than 15 years; (iii) Neither of them marked his/her marital status as widowed, separated, divorced, or never married; (iv) Reported number of children did not differ between the men and the women; (v) He or she belonged to the only one pair. The baseline population included 3290 men and 4660 women aged 65 years or over. Among them, we identified 2436 men and 1716 women who were married. In this study, we did not include a younger population because previous studies have suggested that the effect of the loss of a spouse differs between people under 65 years of age and those over 65 (1, 9, 10), and the number of deaths among those under 65 years of age during the study period was small.

360 Park Avenue South, New York, NY 10010
1047-2797/03/$–see front matter
PII S1047-2797(02)00409-X

The questionnaire asked for information about demographic characteristics, smoking and drinking habits, diet, exercise, and medical and reproductive histories. Dietary history was assessed using a validated 169-item semiquantitative food frequency questionnaire (11). Exercise was assessed by asking the average hours per week spent performing various kinds of activities during the past year. The details are described elsewhere (12).

Deaths of subjects and their spouses, as well as the causes of death, during the follow-up period (1992 to 1999) were confirmed using data from the National Vital Statistics. The Statistics and Information Department of the Japanese Ministry of Health and Welfare obtains information on deaths and codes the causes of death using the International Classification of Diseases (ICD-10). Permission to review the data regarding dates and causes of deaths was obtained from the Management and Coordination Agency, Japan. Information concerning subjects who moved away from Takayama City during the course of the study was obtained from the residential registers of the city.

This study was approved by the local institutional review board.

The major end point of this study was all-cause mortality. We also considered disease-specific endpoints, including mortality from cancer (ICD-10 codes C00-D48), cardiovascular disease (ICD-10 codes I00–I99) and all other causes. The hazard ratios and their 95% confidence intervals (CIs) of all-cause and cause-specific mortality according to marital status were computed using Cox proportional hazard models. Length of follow-up was calculated for each subject as the number of years elapsed from the study entry (September 1, 1992) until the date of death from any cause, the date on which the person moved out of Takayama, or the end of study (December 31, 1999). We regarded a subject whose spouse moved away from Takayama city based on the information obtained from the residential registry as being separated/divorced. Marital status was considered a time-varying independent variable from married to widowed at the time of death of the spouse or married to separated/divorced at the time of spouse's move. A non-proportionality in the effect of widowhood on mortality was tested by incorporating the interaction with the logarithm of time into the model. Age was always included into the models as a covariate. The potential confounding effects of education, body size, diet, smoking and drinking habits and disease history were examined by including them into the models.

For analysis, we excluded subjects who reported on the baseline questionnaire that they had a previous history of cancer (55 men and 94 women), stroke (103 men and 31 women) or ischemic heart disease (239 men and 125 women). Hence, the analytic population at baseline consisted of 2039 men and 1466 women aged 65 to 94 years. Among them, 36 (1.8%) men and 32 (2.2%) women moved out of Takayama City during the study period. Analysis was conducted separately for men and women.

RESULTS

During the 7 years of follow-up, 151 men and 448 women became widowed and six men and two women became separated/divorced.

Baseline characteristics by change of marital status are shown in Table 1.

In men, separation/divorce was significantly associated with increase in mortality after controlling for age (Table 2). Among the 4 deaths associated with separation/divorced, 3 occurred within the first 3 years of the follow-up period (hazard ratio = 6.29, 95% CI 2.02–19.6). Widowhood was not significantly associated with mortality. The effect of duration of widowhood on mortality was not statistically significant ($p = 0.56$). The cause of spouse's death (cancer, cardiovascular disease, or all other diseases) was not significantly associated with mortality. Additional adjustment for number of children, years of education, occupation (administrative or professional and others), smoking status, and alcohol intake did not alter the results substantially.

Two women became separated/divorced, but both were alive at the end of the follow-up period. Widowhood was inversely associated with mortality, although this association was not statistically significant. The hazard ratio we estimated for widowhood was a sort of average effect over the range of time observed in data. The test of non-proportionality showed a significant trend for decreasing mortality rate associated with increasing duration of widowhood ($p = 0.03$). According to this model incorporating the interaction with widowhood and time (duration of widowhood), the hazard ratio for widowhood was 1.87 (95% CI 0.68–5.20) at its onset and 1.00 at 2.8 years after the onset. The table also shows the hazard ratios for widowhood in a categorical fashion (widowed for <3 years and 3+ years). Significantly decreased hazard ratio was associated with widowhood for 3 years or more after adjustment for number of children, years of education, and smoking status. Age at widowhood was not significantly associated with mortality. The cause of a spouse's death was not significantly associated with mortality.

The effect of duration of widowhood on mortality was significantly different between men and women ($p = 0.001$ for interaction term involving sex, time and widowhood in the model in a pooled sample of men and women).

Two out of six separated/divorced men died from heart disease. Separation/divorce was significantly associated with mortality from cardiovascular disease mortality in men

TABLE 1. Baseline characteristics of study subjects by change in marital status

	Men			Women	
	Married (n = 1869)	Separated/divorced (n = 6)	Widowed (n = 151)	Married (n = 1016)	Not married[a] (n = 450)
Age (yrs)	71.3 ± 5.4	73.6 ± 7.8	74.3 ± 6.8	69.4 ± 4.1	71.8 ± 5.0
Body mass index (kg/m^2)	21.6 ± 2.8	21.0 ± 1.9	21.3 ± 2.9	21.8 ± 3.4	21.6 ± 3.3
Alcohol intake (mL)	30.3 ± 33.6	32.1 ± 40.1	22.3 ± 30.1	4.9 ± 11.1	4.9 ± 11.7
Exercise (METs[b] · h/w)	18.9 ± 31.3	10.0 ± 10.1	15.0 ± 24.4	12.5 ± 21.2	10.6 ± 18.8
Smoking (%)					
Current	41.8	16.7	39.4	6.5	6.8
Former	42.2	66.7	44.4	2.8	4.6
No. of children (%)					
0	4.0	0	3.4	7.7	5.9
1–2	48.6	50.0	39.6	40.0	33.7
3–4	39.8	33.3	43.0	43.8	42.5
5+	7.6	16.7	14.1	8.0	17.9
Years of education (%)					
<9 yrs	62.1	16.7	66.4	70.5	69.0
10–11 yrs	24.5	50.0	19.9	24.3	24.7
12+ yrs	13.4	33.3	13.7	5.2	6.3
Occupation (%)					
Administrative/professional	26.0	66.7	24.5	20.8	19.4

Data presented as mean ± standard deviation unless otherwise noted.
[a]Including 448 widowed and 2 separated/divorced women.
[b]Metabolic equivalents.
[c]Husband's occupation for women.

(hazard ratio = 10.3, 95% CI 2.55–41.9) after controlling for age. The longer duration of widowhood was associated with the lower rate for each cause-specific mortality in women, but none of the associations was statistically significant.

Exclusion of subjects with a history of cancer, stroke, or ischemic heart disease at baseline may have removed the effect of having a spouse to take care of an ill partner. However, inclusion of these subjects into the analysis did not alter the results substantially; a significantly increased hazard ratio (=6.15) was observed for separation/divorce in men and the trend for decreasing mortality rate with the longer duration of widowhood in women was statistically significant (p = 0.047).

DISCUSSION

We found no evidence indicating that widowed men and women have increased mortality. Our data rather suggested a decreasing trend in mortality with the longer duration of widowhood in women. Most previous studies have shown an increased risk of mortality for widowed persons, although the risk increase was greater in men than in women (1, 4, 6, 7). Some studies (5, 9, 13) have reported no excess of mortality among widowed females compared with married females. One study reported by Smith and Zick (10) showed a significantly lower mortality risk for female widows aged 65 or over than that for comparably aged married women when their husbands died after a long-term illness. An increased mortality rate after separation or divorced was observed in the study reported by Johnson et al (1), but not in the study reported by Ebrahim et al (4).

Adjustments for various potential confounders did not substantially alter the relationship between mortality and change of marital status in this study. This finding agreed with those of other studies (2, 4, 7, 9, 14).

Our study had several strengths. The prospective design, using initially married persons, eliminated potential biases including selection and recall bias, which may occur if the subjects had been recruited after the onset of widowhood or separation/divorce. We were able to take into account of various potential confounders prior to widowhood or separation. The identification of deaths and causes of deaths was accurate.

Our study had some limitations. Some persons who actually were married may have been excluded from the baseline. Among 2359 men and 1709 women who reported being married at the baseline survey, 223 (9.4%) men and 186 (10.9%) women did not satisfy the inclusion. In 136 men and 114 women out of them, there was a disagreement about the number of children with their potential spouses. In 3 women and 13 men, age difference from their potential spouses was between 15 and 20 years (husbands were older than wives). When we reanalyzed data including these 149 men and 117 women, the results were not altered substan-

TABLE 2. Hazard ratios (HRs) and 95% confidence interval (CI) of all-cause mortality according to change in marital status

Change in marital status	No.	No. of deaths	Age-adjusted HR (95% CI)	Adjusted[a] HR (95% CI)
Men				
Married	1869	477	1.00	1.00
Separated/divorced	6	4	5.78 (2.16–15.5)	6.65 (2.46–18.0)
Widowed	151	34	1.01 (0.71–1.45)	1.02 (0.71–1.46)
Women				
Married	1016	111	1.00	1.00
Widowed	448	26	0.74 (0.47–1.17)	0.69 (0.44–1.09)
<3 years		20	0.90 (0.55–1.48)	0.86 (0.52–1.40)
3+ years		6	0.44 (0.19–1.04)	0.40 (0.17–0.96)
p for trend[b]			0.06	0.04

[a]Men: adjusted for age, number of children, years of education, smoking status (current, former, and never-smokers), occupation (administrative/professional and the others), and alcohol intake. Women: adjusted for age, number of children, years of education, and smoking status.
[b]Trend for mortality rate in relation to duration of widowhood.

tially; in men, the hazard ratios for separated/divorced and widowhood were 5.86 (95% CI 2.17–15.8) and 1.05 (95% CI 0.74–1.47), respectively. In women, the hazard ratio for widowhood for 3 years or more was 0.43 (95% CI 0.19–0.95). Therefore, misclassification of marital status at baseline would not have greatly affected the relationship of mortality to change of marital status.

We could not confirm the separated/divorced status of each subject. If one member of couple moved away from the city, the couple was classified as separated/ divorced. If the couple lived in the same city after divorce or separation, they were misclassified as married. Since being separated/divorced was associated with a higher mortality for men in this study, this misclassification may have led to an underestimation of the effects of widowhood as well as separation/divorce on mortality. However, the divorce rate was likely to be very low in this study population. Based on the Vital Statistics of Japan (15), in 1995 the divorce rate was 0.41 and 0.28 per 1000 married men and women aged 65 to 69, respectively. It is therefore unlikely that bias due to misclassification of separated /divorced as married was substantial. It is also possible that the separated/divorced category should have included those whose spouses did not actually move out of the city. We do not know whether the mortality rate for these subjects was identical to the rate for married persons.

We were unable to consider remarriage. However, the rate of remarriage was also very low: 0.74 and 0.35 per 1000 men and women aged 50 or over, respectively, in the general Japanese population (16).

The size of the sample in the present study was inadequate to assess the short-term effects of the loss of a spouse or the individual effect of every cause of death.

The lack of information concerning whether wives who moved out of the city were still living made it difficult to interpret the subsequent excess in mortality in men. The spouses may have moved away because they had a serious illness. In that case, the observed increased mortality may not have been due to separation/divorce. It is also possible that the spouse's move was due to the terminal illness of the husband. We were unable to obtain detailed information on changes in health status, including the onset of terminal illness.

We were also unable to obtain information on income as a potential confounder. However, the longest held occupation was more likely to be administrative or professional jobs in separated/divorced subjects compared with married subjects. It is unlikely that their excess risk of death was due to low economical status. The observed negative effect of widowhood duration on mortality for women also cannot be explained by the confounding effect of economical status because it is unlikely that the direction of association between economical status and mortality should differ between husbands and their wives.

Social networks, social support, the presence of co-residents other than the spouse, and marital stress, which could not be measured in this study, may have been confounders. However, again, it is unlikely that these factors were associated with the deaths of subjects and their spouses in contrary directions. It is possible that women started seeking social support after the spouse's death, which would partially explain a reduced mortality rate of women widowed for 3 years or more compared with the mortality rate of married women. Understanding the gender differences in marital roles and stress, and ways of coping with bereavement in Japanese men and women is needed to interpret the present findings. The historical context of this cohort should also be considered.

REFERENCES

1. Johnson NJ, Backlund E, Sorie P, Loveless CA. Marital status and mortality: the national longitudinal mortality study. Ann Epidemiol. 2000;10:224–238.

AEP Vol. 13, No. 4
April 2003: 218–222

2. Martikainen P, Valkonen T. Mortality after death of spouse in relation to duration of bereavement in Finland. J Epidemiol Community Health. 1996;50:264–268.
3. Mellström D, Nilsson Å, Odén A, Rundgren, Svanborg A. Mortality among the widowed in Sweden. Scand J Soc Med. 1982;10:33–41.
4. Ebrahim S, Wannamethee G, McCallum A, Walker M, Shaper AG. Marital status, change in marital status, and mortality in middle-aged British men. Am J Epidemiol. 1995;142:834–842.
5. Cheung YB. Marital status and mortality in British women: a logitudinal study. Int J Epidemiol. 2000;29:93–99.
6. Mendes De Leon CF, Kasl SV, Jacobs S. Widowhood and mortality risk in a community sample of the elderly: a prospective study. J Clin Epidemiol. 1993;46:519–527.
7. Goldman N, Korenman S, Weinstein R. Marital status and health among the elderly. Soc Sci Med. 1995;40:1717–1730.
8. Shimizu H. The Basic Report on Takayama Study. Gifu, Japan: Department of Public Health, Gifu University School of Medicine; 1996.
9. Helsing K, Szklo M. Mortality after bereavement. Am J Epidemiol. 1981;114:41–52.
10. Smith KR, Zick CD. Risk of mortality following widowhood: age and sex differences by mode of death. Soc Biol. 1996;43:59–71.
11. Shimizu H, Ohwaki A, Kurisu Y, Takatsuka N, Ido M, Kawakami N, et al. Validity and reproducibility of a quantitative food frequency questionnaire for a cohort study in Japan. Jpn J Clin Oncol. 1999;29:38–44.
12. Suzuki I, Kawakami N, Shimizu H. Reliability and validity of a questionnaire and physical activity in epidemiological studies. J Epidemiol. 1998;8:152–159.
13. Davis MA, Neuhaus JM, Moritz DJ, Segal MR. Living arrangements and survival among middle-aged and older adults in the NHANES I epidemiologic follow-up study. Am J Public Health. 1992;82:401–406.
14. Kaprio J, Koskenvuo M, Rita H. Mortality after bereavement: a prospective study of 95,647 widowed persons. Am J Public Health. 1987;77:283–287.
15. Statistics and Information Department. Minister's Secretariat, Ministry of Health and Welfare. Divorces. Special report of vital statistics. Health and Welfare Statistics Association; 1995.
16. Statistics and Information Department. Minister's Secretariat, Ministry of Health and Welfare. Vital Statistics of Japan, Vol.1. Health and Welfare Statistics Association; 1995.

British Journal of Cancer (2003) 88, 1038–1043

www.bjcancer.com

Height, weight, and alcohol consumption in relation to the risk of colorectal cancer in Japan: a prospective study

N Shimizu*[,1], C Nagata[1], H Shimizu[1], M Kametani[2], N Takeyama[3], T Ohnuma[3] and S Matsushita[2]

[1]Department of Epidemiology and Preventive Medicine, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500-8705, Japan; [2]Department of Internal Medicine, Takayama Red Cross Hospital, 3-11 Tenma-cho, Takayama 506-8550, Japan; [3]Department of Internal Medicine, Takayama Kumiai Hospital, 5-68 Ojin-machi, Takayama 506-8502, Japan

Epidemiology

Colorectal cancer incidence in relation to body size, smoking, and alcohol consumption was studied in a cohort of 29 051 city residents of Japan. In 1992, each participant completed a self-administered questionnaire on sociodemographic characteristics, drinking, cigarette smoking, diet, exercise, and reproductive and medical histories. The response rate was 92%. From 1993 to 2000, 161 men and 134 women were diagnosed with colorectal cancer at two major hospitals in the city. Relative risks and 95% confidence intervals were calculated by using Cox proportional hazard models. A positive relation between height and colorectal cancer was seen in both sexes, controlling for age, body mass index (BMI), smoking and drinking habits, and years of education. The findings were statistically significant only for men (relative risk 2.13 for the tallest compared with the shortest height tertile; 95% confidence interval = 1.26–3.58). Body mass index was also associated positively with colon cancer risk for men, whereas the pattern for women was not clear. There was a positive association between pack-years of cigarette smoking and the risk of rectal cancer in men. A positive dose–response relation between alcohol consumption and colon cancer risk was observed for men and women.
British Journal of Cancer (2003) **88**, 1038–1043. doi:10.1038/sj.bjc.6600845 www.bjcancer.com

Keywords: colorectal cancer; height; body mass; Japanese

Colorectal cancer has become the third leading cause of cancer-related mortality in Japan (Ministry of Health, Labor and Welfare, 1999), and its incidence has been increasing more rapidly than in other industrialised countries for several decades (WCRF, AIRC, 1997). This appears to be related to lifestyle changes, such as, for example, diet.

Body size, as well as diet, has been investigated in relation to the occurrence of the cancer. Height is affected not only by genetic factors, but also by nutritional condition during childhood and adolescent. Some studies (Albanes *et al*, 1988; Chute *et al*, 1991; Bostick *et al*, 1994; Giovannucci *et al*, 1995; Robsahm and Tretli, 1999; Smith *et al*, 2000), but not all (Okasha *et al*, 2000), have reported a positive association between height and colon cancer. Results of studies on body mass index (BMI) in relation to colorectal cancer have been also inconsistent. There are relatively few prospective studies of colorectal cancer among Japanese (Nomura *et al*, 1985; Kono *et al*, 1987; Hirayama, 1989; Akiba, 1994), and only Nomura *et al* (1985) investigated BMI, which was positively associated with an increased risk for colon cancer among Japanese male immigrants to Hawaii. We have therefore conducted a prospective study, among Japanese people, of colorectal cancer in relation to height, BMI, alcohol consumption, and smoking habit.

*Correspondence: Dr N Shimizu; E-mail: g2103006@guedu.cc.gifu-u.ac.jp
Received 8 October 2002; revised 30 December 2002; accepted 10 January 2003

MATERIALS AND METHODS

A cohort was established in September 1992 with residents in Takayama, Japan, who were 35 years old or older (Shimizu, 1996). A self-administered questionnaire covering sociodemographic characteristics, drinking, smoking, diet, exercise, and reproductive and medical histories was distributed to 36 990 residents, of whom 34 018 (92.0%) responded. Subjects who left four out of nine two-page spreads of questionnaire or more all blank ($n = 595$, 1.7%), and those who inadequately reported to the questionnaire ($n = 1871$, 5.5%) were excluded from the cohort. The fixed cohort consisting of 31 152 subjects was defined.

The 1992 questionnaire sought details of current height, weight, and weight at age 21 years. The intraclass correlation coefficients between self-reported and measured height and weight in a subsample were 0.93 and 0.97 in both sexes, respectively.

Diet was assessed by a semiquantitative food-frequency questionnaire that contained 169 food items, covering average consumption frequencies and serving sizes of selected food items during the previous year. Individual nutrient intake was estimated based upon the frequency of intake and portion size using the *Standard Tables of Food Composition in Japan*, 5th edition, published by the Science and Technology Agency of Japan. Details including results of validity tests are described elsewhere (Shimizu *et al*, 1999).

The questions on alcohol use included six types, that is, sake, beer, light beer, shochu (distilled from sweet potatoes, rice, or buckwheat), wine, and hard liquor. For each item, the questionnaire included nine frequency categories (never/less than once a month; once a month; twice or three times a month; once a week; twice or three times a week; four to six times a week; once a day;

twice a day; more than four times a day) and the number of cups, glasses, and bottles consumed. The amount of ethanol was calculated in grams using the *Standard Tables* mentioned above. The correlation coefficients that compare alcohol consumption estimated from the questionnaire with 12 one-day diet diaries at about 1-month intervals over 1 year were 0.72 and 0.64, for men and women, respectively (data not published).

Years of smoking and the number of cigarettes smoked each day were reported. Those who had smoked a total of 20 or more packs of cigarettes in their lifetime were defined as smokers.

Physical activity was based on average hours per week spent performing various kinds of activities; details, including the results of the validity tests, are described elsewhere (Suzuki *et al*, 1998; Shimizu, 2001).

Participants who did not report their height (564 men, 709 women) were excluded from the study, as were those who reported cancer other than nonmelanoma skin cancer (173 men, 532 women) or colorectal adenoma (281 men, 208 women) at baseline. We could not obtain information about diagnosis for 13 men and 15 women who were known to have died of colorectal cancer; thus, these men and women were also excluded from the analysis. The subjects totalled 29 051(13 392 men and 15 659 women).

There were 198 (105 men, 93 women) with colon and 97 (56 men, 41 women) with rectal cancer, all diagnosed histologically at two major hospitals in Takayama City during the follow-up from 1 January 1993 to 31 December 2000. Compared to the number of colorectal cancer occurred in the city according to the annual reports issued by the prefecture government (Hida Public Health Center, 2000), the two hospitals covered about 90% of colorectal cancers in the city each year. Details of subjects who moved away from the city during the study period were obtained from the residential registers, namely, 629 (4.7%) men and 508 (3.2%) women.

We analysed height, BMI, and smoking and drinking habits in relation to colorectal cancer risk with a separate analysis for colon and rectal cancers. Individuals were categorised into tertiles according to the distribution of height and BMI. Variables for smoking and drinking were categorised, and tests for a linear trend were performed on ordinal variables or continuous variables with the use of median values of the category. Person-years were accumulated up to death, loss to follow-up or the end of 2000, whichever occurred first. Cox proportional hazard models were used to calculate hazard ratios. All reported *P*-values were two sided. In all proportional hazard models, adjustments were made for age, and, where indicated in the text and tables, for other known risk factors. All statistical analyses were performed using PC-SAS (SAS Institute, SAS/STAT user's guide, Version 8.2, SAS Institute, Cary, NC, USA).

RESULTS

The mean ages (standard deviations) of participants were 54.1 (12.2) for men and 55.1 (13.0) for women. The average heights of each age strata were comparable to those of the general population in Japan. The distribution of various factors possibly related to colorectal cancer by height and sex is shown in Table 1. Age varied inversely with height. The percentages of ever-smokers and persons with higher education increased across the strata of height. Taller men and women consumed more calories and were more physically active. The incidence rates in this cohort by age strata were comparable to those in the general population in Japan (The Research Group for Population-based Cancer Registration in Japan, 2002).

Positive relations between height and colon cancer are seen for men, but were weaker and nonsignificant for women (Table 2). For men, the relative risks (RRs) of colon cancer from the shortest to the middle and tallest height categories were 1.75 (95% confidence interval, CI = 1.07–2.85) and 2.13 (95% CI = 1.26–3.58), respectively, with a *P* for trend of 0.004, after controlling for age, BMI, alcohol consumption, smoking, and education. For women, the point estimates of colon cancer risk for the middle and the tallest categories of height were moderately but not significantly elevated. Further adjustments for physical activity did not substantially alter the results (RRs of colon cancer for the tallest height tertile compared with the shortest tertile adjusted for age, BMI, alcohol consumption, smoking, education, and physical activity = 2.07 (95% CI = 1.21–3.52) and 1.56 (95% CI = 0.85–2.88), for men and women, respectively). We further performed adjustments for nutrient intake, such as total energy and dietary fibre. The results were not modified by the adjustments.

Table 3 shows that BMI was positively associated with the risk of colon cancer for men, whereas the association for women was weak, controlling for age, height, alcohol consumption, smoking, and education. There was no significant association between height and the risk of rectal cancer in both sexes. Further adjustment for physical activity did not substantially alter the results. Our additional analysis for BMI calculated using self-reported weight at age 21 years did not demonstrate any associations or trends (data not shown).

Table 1 Distribution of various factors by sex and height

	Height (cm)					
	Men			Women		
	Short ⩽162	Medium 163–167	Tall ⩾168	Short ⩽150	Medium 151–154	Tall ⩾155
Number	4863	3932	4597	6393	3643	5623
Age (years)[a]	60.2 (11.9)	53.8 (11.0)	48.0 (10.0)	61.9 (13.0)	53.2 (10.9)	48.8 (10.4)
Height (cm)[a]	157.5 (4.41)	164.9 (1.37)	171.7 (3.46)	146.0 (4.51)	152.6 (1.03)	158.3 (3.12)
Weight (kg)[a]	55.4 (7.43)	61.1 (7.46)	67.1 (8.80)	47.1 (6.99)	51.4 (6.58)	54.7 (7.12)
Body mass index ($kg\,m^{-2}$)[a]	22.3 (2.87)	22.5 (2.72)	22.7 (2.79)	22.1 (3.14)	22.1 (2.80)	21.8 (2.76)
Diabetes mellitus (%)	7.1	6.5	5.0	3.6	2.7	1.8
Cholecystectomy (%)	1.2	0.7	0.7	1.2	1.5	0.8
Education < 12 years (%)	74.9	56.7	61.4	83.1	64.2	48.4
Physical activity score ($METs^{b}$–hour $week^{-1}$)[a]	26.5 (40.0)	27.8 (41.5)	29.4 (44.2)	16.8 (28.1)	20.7 (30.4)	22.6 (32.0)
Total energy intake ($kcal\,day^{-1}$)[a]	2468 (839)	2610 (874)	2723 (880)	2006 (761)	2169 (789)	2230 (576)
Alchol intake ($g\,day^{-1}$)[a]	36.9 (38.7)	42.7 (41.9)	44.7 (42.8)	6.18 (14.2)	7.48 (16.3)	9.54 (19.3)
Ever-smokers (%)	81.5	83.2	85.2	14.6	16.5	20.8

[a]Values are means (s.d.). [b]METs=metabolic equivalents.

Table 2 Relative risks for colorectal cancer according to height

	Men, height (cm)			
	Low, ⩽162	**Medium, 163–167**	**High, ⩾168**	***P* for trend**
Colon cancer				
Person-years	36 584	30 108	35 315	
Number	36	36	36	
Age-adjusted RRs (95% CI)	1.00	1.73 (1.08–2.78)	2.09 (1.26–3.46)	0.003
Multivariate RRs (95% CI)	1.00	1.75 (1.07–2.85)	2.13 (1.26–3.58)	0.004
Rectal cancer				
Person-years	36 527	30 064	35 190	
Number	23	24	12	
Age-adjusted RRs (95% CI)	1.00	1.91 (1.06–3.45)	1.23 (0.58–2.60)	0.31
Multivariate RRs (95% CI)	1.00	1.87 (1.02–3.44)	1.21 (0.57–2.61)	0.37
	Women, height (cm)			
	Low, ⩽150	**Medium, 151–154**	**High, ⩾155**	***P* for trend**
Colon cancer				
Person-years	43 749	29 299	39 673	
Number	43	25	25	
Age-adjusted RRs (95% CI)	1.00	1.50 (0.88–2.56)	1.44 (0.84–2.48)	0.15
Multivariate RRs (95% CI)	1.00	1.56 (0.87–2.81)	1.48 (0.81–2.70)	0.18
Rectal cancer				
Person-years	43 658	25 588	39 596	
Number	19	10	12	
Age-adjusted RRs (95% CI)	1.00	1.10 (0.49–2.45)	1.15 (0.51–2.59)	0.74
Multivariate RRs (95% CI)	1.00	1.27 (0.53–3.06)	1.30 (0.52–3.21)	0.56

Multivariate=age, BMI, alcohol consumption, smoking, and years of education. CI=confidence interval.

Table 3 Relative risks for colorectal cancer according to BMI

	Men, BMI ($kg\,m^{-2}$)			
	⩽21.2	**21.3–22.4**	**⩾23.6**	***P* for trend**
Colon cancer				
Person-years	32 685	33 839	33 772	
Number	26	35	43	
Age-adjusted RRs (95% CI)	1.00	1.47 (0.88–2.44)	1.98 (1.21–3.25)	0.007
Multivariate RRs (1) (95% CI)	1.00	1.46 (0.87–2.46)	2.07 (1.26–3.42)	0.004
Multivariate RRs (2) (95% CI)	1.00	1.57 (0.92–2.68)	2.11 (1.26–3.53)	0.005
Rectal cancer				
Person-years	32 669	33 687	33 728	
Number	24	18	16	
Age-adjusted RRs (95% CI)	1.00	0.85 (0.46–1.56)	0.85 (0.45–1.60)	0.60
Multivariate RRs (1) (95% CI)	1.00	0.76 (0.40–1.44)	0.79 (0.41–1.52)	0.47
Multivariate RRs (2) (95% CI)	1.00	0.80 (0.41–1.55)	0.83 (0.42–1.64)	0.59
	Women, BMI ($kg\,m^{-2}$)			
	⩽21.6	**21.7–23.0**	**⩾23.1**	***P* for trend**
Colon cancer				
Person-years	35 100	35 905	36 373	
Number	28	25	36	
Age-adjusted RRs (95% CI)	1.00	1.06 (0.61–1.82)	1.14 (0.68–1.90)	0.62
Multivariate RRs (1) (95% CI)	1.00	1.08 (0.58–2.00)	1.26 (0.72–2.22)	0.39
Multivariate RRs (2) (95% CI)	1.00	1.02 (0.55–1.91)	1.22 (0.69–2.15)	0.48
Rectal cancer				
Person-years	25 046	35 836	36 290	
Number	14	12	15	
Age-adjusted RRs (95% CI)	1.00	0.77 (0.35–1.71)	0.92 (0.44–1.92)	0.86
Multivariate RRs (1) (95% CI)	1.00	0.86 (0.36–2.02)	0.98 (0.44–2.20)	0.99
Multivariate RRs (2) (95% CI)	1.00	0.84 (0.36–1.99)	0.83 (0.35–1.99)	0.68

Multivariate (1)=adjusted for age, height, alcohol consumption, smoking, and years of education. Multivariate (2)=adjusted for age, height, alcohol consumption, smoking, years of education, and physical activity. CI=confidence interval.

The associations between total alcohol intake and colorectal cancer are shown in Table 4. Relative risks of colon cancer for individuals in the highest alcohol consumption compared with abstainers were 2.52 (95% CI = 1.00–6.38) and 1.73 (95% CI = 0.98–3.05) for men and women, respectively, controlling for age, height, BMI, smoking, and education. However, a significant positive relation with rectal cancer risk was restricted to women. In the analysis by type of beverage, sake consumption was significantly associated with colon cancer risk in men (the RR from the abstainers to the highest sake consumption category = 1.91; 95% CI = 1.10–3.32).

Men smoked more than 20 pack-years of cigarettes were associated with elevated rectal cancer risk (Table 5). No pattern of changing risk was observed in women who smoked more than 10 pack-years.

DISCUSSION

These data indicated that being tall or overweight is associated with an increased risk of colon cancer in men, while alcohol consumption was associated with colon cancer risk in both sexes.

Six other prospective studies (Albanes *et al*, 1988; Chute *et al*, 1991; Bostick *et al*, 1994; Giovannucci *et al*, 1995; Robsahm and Tretli, 1999; Smith *et al*, 2000), but not all, support our finding that being tall significantly elevates the risk of colorectal cancer. One possibility is that taller people may have longer intestines (Hirsch *et al*, 1956) and have a greater rate of cell division within the tissue (Albanes and Winick, 1988); thus, more colon cells may be at risk. Greater exposure to mitogenic factors, such as growth hormone, insulin, insulin-like growth factors, and sex steroids, could also result in increased cancer risk. Height has been related to elevated risk of several specific cancers, including breast (Swanson *et al*, 1988) and prostate (Smith *et al*, 2000). Animal experiments have shown that low-energy diets from early age shorten overall animal length and reduce cancer risk (Kritchevsky, 1995). In humans, wartime food deprivation for pubescent women was associated with lower breast cancer rates than in younger and older cohorts (Tretli and Gaard, 1996). Furthermore, an association has been reported between higher caloric intake during childhood and higher rates of cancer (Frankel *et al*, 1998). Height is partly determined by total caloric intake during childhood and adolescence; so its association with colon cancer may indicate that total caloric intake during childhood is relevant for a later colon cancer risk.

Evidence has long suggested that people with excess body weight are at higher risk of colorectal cancer (Lew and Garfinkel, 1979; Nomura *et al*, 1985; Albanes and Taylor, 1990). Our study, as well as others (Garfinkel, 1985; Wu *et al*, 1987; Russo *et al*, 1998; Robsahm and Tretli, 1999; Murphy *et al*, 2000), shows a positive association between the risk of colon cancer of BMI in men but not in women. Other case–control studies (Slattery *et al*, 1997; Caan *et al*, 1998) and a cohort study (Ford, 1999) have shown a significant positive association between the risk of colon cancer and BMI in both sexes, although this was weaker among women in the two case–control studies. Central obesity, which may increase colon cancer risk by acting as a tumour-growth promoter or mitogen (Giovannucci, 1995; Bjorntorp, 1991), is more common among males. Thus, BMI may simply be a more accurate indicator of central obesity for men than for women (Murphy *et al*, 2000). Another possibility is a protective effect of oestrogen.

Our study also shows a significant positive dose–response relation between alcohol consumption and colon cancer risk in both sexes, although not significant in women as found in other studies. In a prospective study in California, the RR of colorectal cancer was 2.42 (95% CI = 1.3–4.5) in men who drank more than 30 ml day^{-1} compared to nondaily alcohol drinkers, while the RR among women who consumed more than 30 ml day^{-1} relative to the same group was weaker and not significant (RR = 1.45, CI = 0.8–2.6) (Wu *et al*, 1987). Similar results were obtained when the cases of rectal cancer were omitted. Klatsky *et al* (1988) prospectively studied and found a positive relation between the

Epidemiology

Table 4 Relative risks for colorectal cancer according to alcohol consumption

	Men, alcohol intake			
	No alcohol	**≤36.7 g day^{-1}**	**>36.7 g day^{-1}**	**P for trend**
Colon cancer				
Person-years	8088	46 494	47 424	
Cases, *n*	5	45	58	
Age-adjusted RRs (CI)	1.00	1.94 (0.77–4.90)	2.96 (1.17–7.46)	0.007
Multivariate RRs (CI)	1.00	1.79 (0.71–4.55)	2.67 (1.06–6.76)	0.01
Rectal cancer				
Person-years	8114	46 379	47 289	
Cases, *n*	8	20	31	
Age-adjusted RRs (CI)	1.00	0.57 (0.25–1.30)	1.12 (0.50–2.51)	0.06
Multivariate RRs (CI)	1.00	0.59 (0.25–1.42)	1.17 (0.50–2.73)	0.06
	Women, alcohol intake			
	No alcohol	**≤3.75 g day^{-1}**	**>3.75 g day^{-1}**	**P for trend**
Colon cancer				
Person-years	36 265	36 394	36 416	
Cases, *n*	34	28	32	
Age-adjusted RRs (CI)	1.00	0.87 (0.51–1.49)	1.52 (0.92–2.53)	0.04
Multivariate RRs (CI)	1.00	1.07 (0.58–1.96)	1.78 (1.00–3.18)	0.03
Rectal cancer				
Person-years	36 131	36 325	36 385	
Cases, *n*	7	15	19	
Age-adjusted RRs (CI)	1.00	1.88 (0.74–4.79)	2.27 (0.91–5.69)	0.17
Multivariate RRs (CI)	1.00	1.20 (0.44–3.26)	1.80 (0.70–4.62)	0.17

Multivariate=adjusted for age, height, BMI, smoking, and years of education.

Table 5 Relative risks for colorectal cancer according to tobacco smoking

	Men, smoking status			
	Never smoked	**Smokers ⩽20 pack-years**	**Smokers >20 pack-years**	***P* for trend**
Colon cancer				
Person-years	16702	42141	37361	
Cases, *n*	16	41	47	
Age-adjusted RRs (CI)	1.00	1.33 (0.78–2.28)	1.43 (0.85–2.41)	0.09
Multivariate RRs (CI)	1.00	1.36 (0.79–2.33)	1.37 (0.81–2.32)	0.19
Rectal cancer				
Person-years	16653	42009	37327	
Cases, *n*	7	16	34	
Age-adjusted RRs (CI)	1.00	1.22 (0.54–2.78)	2.36 (1.13–4.92)	0.03
Multivariate RRs (CI)	1.00	1.33 (0.57–3.12)	2.44 (1.12–5.30)	0.04
	Women, smoking status			
	Never smoked	**Smokers ⩽10 pack-years**	**Smokers >10 pack-years**	***P* for trend**
Colon cancer				
Person-years	82430	8944	7273	
Cases, *n*	68	4	5	
Age-adjusted RRs (CI)	1.00	0.65 (0.24–1.76)	0.97 (0.39–2.40)	0.91
Multivariate RRs (CI)	1.00	0.59 (0.21–1.62)	0.77 (0.30–1.96)	0.54
Rectal cancer				
Person-years	82266	8944	7264	
Cases, *n*	32	4	2	
Age-adjusted RRs (CI)	1.00	1.69 (0.59–4.86)	1.06 (0.25–4.47)	0.47
Multivariate RRs (CI)	1.00	1.76 (0.60–5.14)	0.94 (0.21–4.16)	0.63

Multivariate=adjusted for age, height, BMI, alcohol intake, and years of education.

risk of colon cancer and total alcohol intake in both sexes, although the results in women but not men were statistically significant. On the other hand, a meta-analysis (Longnecker *et al*, 1990) including both prospective and case–control studies showed that the RR of colorectal cancer was 1.10 (95% CI = 1.05–1.14), and the association did not vary according to gender or site within the large bowel. In almost all case–control studies, the use of hospital controls, among which proportions of alcohol-related disease were high, may have biased the results.

There was no association between BMI and rectal cancer risk in both the sexes. Similar finding was obtained in a case–control study reported by Dietz *et al* (1995), that body weight was positively associated with colon cancer but not with rectal cancer. Positive association between BMI and rectal cancer has been reported in some studies (Phillips and Snowdon, 1985; Russo *et al*, 1998), but not in others (Dietz *et al*, 1995; Howe *et al*, 1997). A significant association between alcohol consumption and rectal cancer risk has been reported (Klatsky *et al*, 1988; Hirayama, 1989), but in our study, the association was weak in men. We must mention that the number of cases of rectal cancer might be too small to have meaningful analyses.

Recent prospective studies have suggested a positive association between long-term and heavy smoking and colon cancer (Giovannucci *et al*, 1994; Hsing *et al*, 1998; Terry *et al*, 2001). In our present study, no significant association was observed in relation to the quantity of cigarettes smoked except for rectal cancer risk in men.

A major advantage of our study is that it is population based and prospective, thereby minimising recall bias. Incidence rather than mortality can avoid bias because of a progress of medical treatment as well as effect of disease. Furthermore, the data were analysed adjusting for physical activity, nutrition, and years of education.

However, several limitations must be considered. Firstly, height was self-reported and not measured. Although the intraclass correlation coefficient between self-reported height and measured height in a subsample was relatively high, self-reported information may result in misclassification. However, since the height information was collected before the development of cancer, any misclassification would be nondifferential with respect to the disease. Such random misclassification would tend to attenuate RR estimates, and, thus, would not explain the association between height and colon cancer.

Secondly, height was reported at the baseline. Stature has been reported to be at its peak at the end of the third decade of life (Friedlander *et al*, 1977). Our subjects were 35 years old or over, so their height at the baseline might have been already reduced by ageing or disease and may not have represented their highest. However, age was adjusted for in our analysis, while additional adjustment for pre-existing disease, such as hypertension and diabetes mellitus, did not substantially alter the results.

Another limitation is incomplete detection of colorectal cancers, although details from the two study hospitals appear to cover around 90% of the colorectal cancers in the city reported by the prefectural cancer registry. Some colorectal cancer patients must therefore have been classified as subjects without colorectal cancer. However, it is unlikely that taller or heavier patients were selectively included in the present study. In addition, the study was so large that the effect of misclassification of true cases was minimal.

In summary, this cohort study provides evidence that height and overweight may be associated with an increased risk of colon cancer for Japanese men, and heavy alcohol consumption may increase the risk of colon cancer in both sexes.

ACKNOWLEDGEMENT

We thank the Ministry of Education, Culture, Science, and Technology, Japan, for its partial financial support of this project.

REFERENCES

Albanes D, Jones DY, Schatzkin A, Micozzi MS, Taylor PR (1988) Adult stature and risk of cancer. *Cancer Res* **48**: 1658–1662

Albanes D, Taylor PR (1990) International differences in height and weight and their relationship to cancer incidence. *Nutr Cancer* **14**: 69–77

Albanes D, Winick M (1988) Are cell number and cell proliferation risk factors for cancer? *J Natl Cancer Inst* **80**: 772–774

Akiba S (1994) Analysis of cancer risk related to longitudinal information on smoking habits. *Environ Health Perspect* **102** (Suppl 8): 15–20

Bjorntorp P (1991) Metabolic implications of body fat distribution. *Diabetes Care* **14**(12): 1132–1143

Bostick RM, Potter JD, Kushi LH, Sellers TA, Steinmetz KA, McKenzie DR, Gapstur SM, Folsom AR (1994) Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). *Cancer Causes Control* **5**: 38–52

Caan BJ, Coates AO, Slattery ML, Potter JD, Quesenberry Jr CP, Edwards SM (1998) Body size and the risk of colon cancer in a large case–control study. *Int J Obes* **22**: 178–184

Chute CG, Willet WC, Colditz GA, Stampfer MJ, Baron JA, Rosner B, Speizer FE (1991) A prospective study of body mass, height, and smoking on the risk of colorectal cancer in women. *Cancer Causes Control* **2**: 117–124

Diez AT, Newcomb PA, Marcus PM, Storer BE (1995) The association of body size and large bowel cancer risk in Wisconsin (United States) women. *Cancer Causes Control* **6**: 30–36

Ford ES (1999) Body mass index and colon cancer in a national sample of adult US men and women. *Am J Epidemiol* **150**(4): 390–398

Frankel S, Gunnell DJ, Peters TJ, Maynard M, Smith GD (1998) Childhood energy intake and adult mortality from cancer: the Boyd Orr Cohort Study. *BMJ* **316**: 499–504

Friedlander JS, Costa PT, Bosse R, Ellis E, Rhoads JG, Stoudt HW (1977) Longitudinal physique changes among healthy white veterans at Boston. *Hum Biol* **49**: 541–558

Garfinkel L (1985) Overweight and cancer. *Ann Intern Med* **103**(6): 1034–1036

Giovannucci E (1995) Insulin and colon cancer. *Cancer Causes Control* **6**: 164–179

Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willet WC (1995) Physical activity, obesity, and risk for colon cancer and adenoma in men. *Ann Intern Med* **122**: 327–334

Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Kearney J, Willet WC (1994) A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in US men. *J Natl Cancer Inst* **86**: 183–191

Hida Public Health Center (2000) *Hida no Koushueisei*. pp 66–67 Gifu, Japan: Hida Public Health Center (in Japanese)

Hirayama T (1989) Association between alcohol consumption and cancer of the sigmoid colon: observations from a Japanese cohort study. *Lancet* **2**(8665): 725–727

Hirsch J, Ahrens Jr EH, Blankenhorn DH (1956) Measurement of the human intestinal length *in vivo* and some causes of variation. *Gastroenterology* **31**: 274–284

Howe GR, Aronson KJ, Benito E, Castello R, Cornee J, Duffy S, Gallagher RP, Iscovich JM, Deng-ao J, Kaaks R, Kune GA, Kune S, Lee HP, Lee M, Miller AB, Peters RK, Potter JD, Riboli E, Slattery ML, Trichopoulos D, Tuyns A, Tzonou A, Watson LF, Whittemore AS, Wu-Williams AH, Shu Z (1997) The relationship between dietary fat intake and risk of colorectal cancer: evidence from the combined analysis of 13 case control studies. *Cancer Causes Control* **8**: 215–228

Hsing AW, Mclaughlin JK, Chow W, Schuman LM, Co Chien HT, Gridley G, Bjelke E, Wacholder S, Blot WJ (1998) Risk factors for colorectal cancer in a prospective study among US White men. *Int J Cancer* **77**: 549–553

Klatsky AL, Armstrong MA, Friedman GD, Hiatt RA (1988) The relations of alcoholic beverage use to colon and rectal cancer. *Am J Epidemiol* **128**(5): 1007–1015

Kritchevsky D (1995) The effect of over- and undernutrition on cancer. *Eur J Cancer Prev* **4**: 445–451

Kono S, Ikeda M, Tokudome S, Nishizumi M, Kuratsune M (1987) Cigarette smoking, alcohol and cancer mortality: a cohort study of male Japanese physician. *Jpn J Cancer Res* **78**: 1323–1328

Lew EA, Garfinkel L (1979) Variations in mortality by weight among 750,000 men and women. *J Chronic Dis* **32**(8): 563–576

Longnecker MP, Orza MJ, Adams ME, Vioque J, Chalmers TC (1990) A meta-analysis of alcoholic beverage consumption in relation to risk of colorectal cancer. *Cancer Causes Control* **1**: 59–68

Ministry of Health, Labor and Welfare (1999) *Vital Statistics of Japan 1999 Tokyo, Japan*: Ministry of Health, Labor and Welfare

Murphy TK, Calle EE, Rodriguez C, Kahn HS, Thun MJ (2000) Body mass index and colon cancer mortality in a large prospective study. *Am J Epidemiol* **152**: 847–854

Nomura A, Heilbun LK, Stemmermann GN (1985) Body mass index as a predictor of cancer in men. *J Natl Cancer Inst* **74**: 319–323

Okasha M, McCarron P, McEwen J, Smith GD (2000) Height and cancer mortality: results from Glasgow University Student Cohort. *Public Health* **114**: 451–455

Phillips RL, Snowdon D (1985) Dietary relationships with fatal colorectal cancer among Seventh-Day Adventists. *J Natl Cancer Inst* **74**: 307–317

Robsahm TE, Tretli S (1999) Height, weight and gastrointestinal cancer: a follow-up study in Norway. *Eur J Cancer Prev* **8**: 105–113

Russo A, Franceschi S, La Vecchia C, Dal Maso L, Montella M, Conti E, Giacosa A, Falcini F, Negri E (1998) Body size and colorectal-cancer risk. *Int J Cancer* **78**: 161–165

Shimizu H (1996) A Basic Report on Takayama Study. Gifu, Japan: Department of Public Health, Gifu University School of Medicine

Shimizu H (2001) A supplementary comment on "Reliability and validity of a questionnaire for assessment of physical activity in epidemiological studies" published in Journal of Epidemiology, 1998. *J Epidemiol* **12**: 54

Shimizu H, Ohwaki A, Kurisu Y, Takatsuka N, Ido M, Kawakami N, Nagata C, Inaba S (1999) Validity and reproducibility of a quantitative food frequency questionnaire for a cohort study in Japan. *Jpn J Clin Oncol* **29**(1): 38–44

Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma K, Berry TD (1997) Energy balance and colon cancer beyond physical activity. *Cancer Res* **57**:75–80

Smith GD, Hart C, Upton M, Hole D, Gillis C, Watt G, Hawthrone V (2000) Height and risk of death among men and women: aetiological implications of associations with cardiorespiratory disease and cancer mortality. *J Epidemiol Community Health* **54**: 97–103

Suzuki I, Kawakami N, Shimizu H (1998) Reliability and validity of a questionnaire for assessment of energy expenditure and physical activity in epidemiological studies. *J Epidemiol* **8**: 152–159

Swanson CA, Jones DY, Schatzkin A, Brinton LA, Ziegler RG (1988) Breast cancer risk assessed by anthropometry in the NHANES I epidemiological follow-up study. *J Cancer Res* **48**: 5363–5367

Terry P, Ekbom A, Lichtenstein P, Feychting M, Wolk A (2001) Long-term tobacco smoking and colorectal cancer in a prospective cohort study. *Int J Cancer* **91**: 585–587

The Research Group for Population-based Cancer Registration in Japan (2002) Cancer incidence and incidence rates in Japan in 1997: estimates based on data from 12 population-based cancer registries. *Jpn J Clin Oncol* **32**(8): 318–322

Tretli S, Gaard M (1996) Life style changes during adolescence and risk of breast cancer: an ecologic study of the effect of World War II in Norway. *Cancer Causes Control* **7**: 507–512

Potter JD (Panel Chair), Washington DC, WCRF, AIRC (1997) Colon, rectum. *Food, Nutrition and the Prevention of Cancer: A Global Perspective* pp 216–251

Wu AH, Paganini-Hill A, Ross RK, Henderson BE (1987) Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study. *Br J Cancer* **55**: 687–694

Soy Product Intake Is Inversely Associated with Serum Homocysteine Level in Premenopausal Japanese Women[1]

(Manuscript received 28 September 2002. Initial review completed 25 October 2002. Revision accepted 21 November 2002.)

Chisato Nagata,[2] Hiroyuki Shimizu, Rieko Takami,* Makoto Hayashi,* Noriyuki Takeda† and Keigo Yasuda†

*Department of Public Health, Gifu University School of Medicine, Gifu 500-8705, Japan; *Internal Medicine of Matsunami General Hospital, Gifu, Japan; and †The Third Department of Internal Medicine, Gifu University School of Medicine, Gifu 500-8705, Japan*

ABSTRACT Soybeans, which are an excellent source of folate, vitamin B-6 and minerals, may reduce serum homocysteine level. However, there is a possibility that dietary soy raises the serum homocysteine level because isoflavones, which are weak estrogens contained in soybeans, may exert antiestrogenic effects in a high estrogen environment, such as in premenopausal women. The present study examined a cross-sectional relationship between soy product intake and serum homocysteine level in 201 premenopausal Japanese women. Intakes of soy products, folate, methionine and vitamins B-6 and B-12 were estimated by a semiquantitative food frequency questionnaire. Folate status was also assessed by measuring serum folate. Soy product intake in terms of soy protein as well as soy isoflavone intake was modestly but significantly inversely associated with serum homocysteine level ($r = -0.15$, $P = 0.04$) after controlling for covariates. Soy product intake was also significantly positively correlated with serum folate ($r = 0.15$, $P = 0.04$). Although it is unclear the extent to which each component of soy, such as folate and isoflavones, is associated with the serum homocysteine concentration, this biochemical complex appears to have a favorable effect on homocysteine metabolism in premenopausal women. J. Nutr. 133: 797–800, 2003.

KEY WORDS: • soybeans • isoflavones • homocysteine • premenopausal women

An elevated blood homocysteine concentration has been shown to be an independent risk factor for cardiovascular disease (1). Several studies have identified factors that are associated with homocysteine level in the general population (2–10). Established determinants of blood homocysteine include folate and vitamin B-12 status and serum creatinine concentration (10); this is expected because they serve as cofactors in the enzymatic pathway of homocysteine metabolism (11).

A recent study reported by Bazzano et al. (12) has shown that the consumption of legumes, which are high in bean protein, was significantly inversely associated with the risk of coronary heart disease. Soybeans are an excellent source of folate. It is possible that soy intake reduces the serum homocysteine concentration, which can lead to a decreased risk of coronary heart disease. However, there is a possibility that soy intake raises the serum homocysteine concentration because soybeans are also a unique dietary source of a group of phytochemicals called isoflavones. Although isoflavones are structurally similar to estrogens, it is hypothesized that isoflavones exert antiestrogenic effects in a high estrogen environment, such as in premenopausal women, and estrogenic effects in a low estrogen environment, such as in postmenopausal women (13). Blood homocysteine concentration is related to estrogen status. Premenopausal women or women who are on hormone replacement therapy have lower homocysteine concentrations than postmenopausal women (14,15). Therefore, soy or isoflavone intake in premenopausal women may hinder the beneficial effect of their endogenous estrogen status on homocysteine concentration.

To our knowledge, two intervention studies that examined the effects of soy protein on serum biomarkers have included measurements of serum homocysteine in diabetic subjects (16) and hypercholesterolemic subjects (17). In both studies, there was a decreased plasma homocysteine concentration with soy protein intake compared with casein intake, suggesting a novel, possibly antiatherosclerotic effect of dietary soy. However, the subjects of these studies were men or postmenopausal women. Also, the differences in amino acid composition, including that of methionine, one of the principal dietary precursors of homocysteine, of casein and soy protein may account for the findings.

In the present cross-sectional study, we examined the relationship between soy intake and serum homocysteine concentration in premenopausal Japanese women considering folate, methionine, vitamin B-6 and vitamin B-12 status. A recent update of the Japanese food composition tables enabled us to measure dietary intakes of folate and vitamins B-6 and B-12.

SUBJECTS AND METHODS

Subjects for this study were participants in a health check-up program provided by a general hospital in Gifu, Japan, between September 1996 and August 1997. A total of 291 premenopausal women agreed to participate and completed a self-administered questionnaire that asked about demographic characteristics, smoking and drinking habits, diet, exercise and past medical and reproductive histories (the response rate was 95.7%). To be considered premenopausal, a woman had to have had at least one natural menstrual cycle in the previous 12 mo. Women who had experienced surgical menopause >3 mo before the study were considered to be postmenopausal. To obtain complete data, a nurse epidemiologist interviewed those who returned the questionnaire with incomplete information.

[1] Supported in part by grant from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed.
E-mail: chisato@cc.gifu-u.ac.jp.

Diet was assessed by a semiquantitative food-frequency questionnaire. The women were asked to indicate the average frequency that they consumed 169 food items during the year before the study and the usual serving size of each item. We included nine food items for soy products (miso soup, tofu, deep-fried tofu, fried bean curd, dried bean curd, fermented soy beans, houba-miso, soymilk and boiled soybeans). Soy product intake, as the total amount (g/d) of soy products, was calculated as the sum of these nine food items. Soy protein intake (g/d) was also calculated as the sum of the protein included in these food items. Isoflavone intake (mg/d) from soy products was estimated using isoflavone concentration in these soy foods (18). The intakes of foods and nutrients were estimated from the frequency of ingestion and portion size using the Japanese Standard Tables of Food Composition, fourth and fifth editions, published by the Science and Technology Agency of Japan (19). The Japanese food composition tables are incomplete for amino acid compositions of some foods. When the values were missing for a certain food, they were substituted by those of another nutritionally comparable food. Detailed information on the questionnaire, including its validity and reproducibility, has been described (20,21). For example, the Spearman correlation coefficient comparing estimates of soy product intake from this questionnaire with the estimates from 12 daily diet records kept over a 1-y period was 0.71. The corresponding figures for folate, methionine and vitamins B-6 and B-12 were 0.45, 0.61, 0.59 and 0.24, respectively.

A fasting blood sample was collected from each subject between 9 and 10 AM. The samples were stored at −80°C until assayed. Serum homocysteine was determined by HPLC using the fluorescent conjugate (22). Serum folate was measured with a radioassay (23). Serum creatinine was determined with standard automated clinical chemistry laboratory technique (24). All measurements were conducted at SRL, Inc. (Tokyo, Japan). The intra-assay and interassay CVs were 1.1% and 3.5% for homocysteine, 7.5% and 11.1% for folate and 1.3% and 2.3% for creatinine, respectively.

This study was approved by the local institutional review board.

For statistical analysis, we excluded women who were taking hormone replacement therapy or other hormones (n = 11) and who had a history of cancer (n = 10), ischemic heart disease (n = 1) and endogenous diseases such as diabetes mellitus (n = 9). Of the 260 eligible women, serum creatinine concentrations were measured for 207 women who chose program courses including this measurement; 201 had sufficient sera available for the measurement of serum folate and homocysteine. Their ages ranged from 20 to 54 y.

Spearman correlation coefficients were used to calculate the associations of dietary soy with serum homocysteine and folate. Intakes of soy products and the individual nutrient were log-transformed and adjusted for total energy intake using the method proposed by Willett (25). Previous studies have suggested that age, body size, education, smoking, alcohol use, coffee consumption and serum creatinine concentrations are associated with blood homocysteine concentration. The effects of these potential confounders were examined by including them into the models as covariates. Significant difference was declared at P = 0.05. All of the statistical analyses were performed using SAS programs (26).

RESULTS

The characteristics of the study subjects are given in **Table 1**; 187 (93.0%) women were married, and 12 (6.0%) women were current smokers.

The correlation between soy product intake and serum homocysteine concentration was marginally significant (r = −0.13, P = 0.07), but soy protein and soy isoflavone intakes were significantly inversely correlated with serum homocysteine concentration after controlling for age and total energy intake (r = −0.15, P = 0.04 and r = −0.14, P = 0.04, respectively) (**Table 2**). Additional adjustment for height and weight, years of education, pack-years of cigarette smoking, alcohol and coffee intakes and serum creatinine concentration did not alter the results. Intakes of vitamin B-6 and folate were significantly inversely correlated with serum homocysteine concentration after controlling for covariates (r = −0.17, P = 0.02 and r = −0.17, P = 0.02, respectively), and the correlation for vitamin B-12 was of marginal significance (r = −0.14, P = 0.07). Serum folate was strongly inversely correlated with serum homocysteine concentration (r = −0.46, P = 0.0001).

TABLE 1

Selected characteristics of 201 premenopausal Japanese women[1]

Characteristic	
Age, *y*	42.5 ± 5.3
Height, *cm*	157.1 ± 4.7
Weight, *kg*	53.1 ± 6.3
Body mass index, *kg/m²*	21.5 ± 2.5
Education, *y*	13.1 ± 2.1
Smoking (pack-years), *n*	0.74 ± 3.3
Alcohol intake, *mL/d*	5.6 ± 11.1
Energy intake, *kJ/d*	9,619 ± 3,523
Coffee intake, *mL/d*	210 ± 156
Serum creatinine, *μmol/L*	58.7 ± 6.9

[1] Values are means ± SD.

The correlations between folate status and intakes of soy protein, methionine and vitamins B-6 and B-12 are shown in **Table 3**. Total soy product and soy isoflavone intakes were omitted from the table because these variables were highly correlated with soy protein intake (r = 0.94 and r = 0.995, respectively). The correlations among dietary soy protein, folate and vitamin B-6 were strong. The estimated means ± SD of folate and vitamin B-6 intakes derived from soy products were 64.1 ± 45.0 μg/d and 0.10 ± 0.06 mg/d, respectively.

Further adjustment for folate or vitamin B-6 intake attenuated the correlations between soy intake variables and serum homocysteine concentration (for soy protein intake, r = −0.07, P = 0.34 and r = −0.08, P = 0.27 after additional adjustment for folate and vitamin B-6, respectively). Adjustment for serum folate also attenuated the correlations between soy protein and serum homocysteine (r = −0.10, P = 0.19).

DISCUSSION

We observed that a high intake of soy protein or isoflavones was modestly but significantly associated with a decreased serum homocysteine concentration in premenopausal women. In accordance with other studies (3,5,7–10), serum homocysteine concentration was significantly inversely correlated with dietary and serum folate, and the strong correlation with serum folate concentration suggests that serum folate is an important determinant of serum homocysteine. Vitamin B-6 also plays a role in homocysteine transsulfuration and catabolism (11). We observed a significant inverse correlation between vitamin B-6 intake and serum homocysteine. Similar results have been reported in some studies (10,27,28). However, the correlation with vitamin B-6 was attenuated (r = −0.07) after additional adjustment for serum folate.

Soy intake was significantly inversely correlated with serum folate, and an adjustment for folate status or dietary vitamin B-6 attenuated the correlation between soy intake variables and serum homocysteine. This may suggest that the folate or vitamin B-6 contained in soy products accounts for the apparent association between dietary soy and serum homocysteine concentration. However, it is possible that some other components of soybeans are related to decreased serum homocys-

TABLE 2

Spearman correlation coefficients for serum homocysteine with intakes of soy products, methionine and vitamins B-6 and B-12 and folate status in premenopausal Japanese women[1]

Item			Correlation coefficient[2]	
			*r*1	*r*2
Soy product				
Total, *g/d*	50.7 ± 34.8	(4.9–240)	−0.13	−0.14
Soy protein, *g/d*	9.0 ± 5.4	(1.2–32.7)	−0.15[a]	−0.15[a]
Soy isoflavones, *mg/d*	29.9 ± 23.5	(3.0–179.5)	−0.14[a]	−0.15[a]
Methionine, *g/d*	2.1 ± 0.85	(0.56–6.92)	−0.08	−0.09
Vitamin B-6, *mg/d*	1.70 ± 0.76	(0.53–5.65)	−0.14[a]	−0.17[a]
Vitamin B-12, *μg/d*	10.0 ± 6.2	(1.8–52.1)	−0.14	−0.14
Folate, *μg/d*	520.2 ± 288.6	(125–2,331)	−0.14	−0.17[a]
Serum folate, *nmol/L*	11.8 ± 3.9	(5.2–34.0)	−0.47[b]	−0.46[b]

[1] Values are mean ± SD (range). Mean ± SD and range of serum homocysteine was 7.1 ± 2.9 (3.7–27.8) μmol/L.

[2] *r*1, adjusted for age and energy; *r*2, adjusted for age, height and weight, years of education, pack-years of cigarette smoking, alcohol and coffee intakes and serum creatinine level. [a] $P < 0.05$. [b] $P < 0.01$.

teine concentration. Soy products are also rich in minerals, such as iron, zinc, calcium, phosphorus and potassium. The dietary or plasma concentration of these minerals has been inversely associated with serum homocysteine concentration in some studies (3,28) as well as in the present study (data not shown). Because of strong correlations among intakes of folate, vitamins and minerals when soy products are consumed, it is difficult to associate specific components of soybeans with a decreased serum homocysteine concentration. It is also impossible to entirely rule out confounding with serum folate or intake of folate, vitamin B-6, minerals or other nutrients as an explanation of our finding.

We speculated that the presence of folate or vitamin B-6, if not other components in soybeans, might offset the homocysteine concentration raising effect of soy isoflavones. However, we considered whether soy isoflavones can increase the serum homocysteine concentration in premenopausal women through the antiestrogenic effect. Studies on endogenous estrogen and homocysteine concentrations are scarce. Wouters et al. (29) have reported that plasma homocysteine concentration after methionine loading was negatively correlated with serum estradiol concentration. Our previous cross-sectional and intervention studies have shown that soy intake decreases serum estrogen concentrations in premenopausal women (20,30). However, a decreased serum estrogen concentration may not necessarily lead to a decrease in homocysteine concentration. Considering that the observed correlation between soy isoflavone intake and serum homocysteine concentration was attenuated but remained inverse after adjustment for folate status, we cannot rule out the possibility that dietary soy has an estrogenic effect on homocysteine metabolism in premenopausal women.

Although the present study had limitations in terms of inferring to what extent each component of soy products, such as folate and isoflavones is related to serum homocysteine concentration, we obtained results that do not indicate a homocysteine concentration raising effect of this biochemical complex in premenopausal women. The results instead suggested a favorable effect of soy products as a food group in regard to homocysteine metabolism. There is growing interest in the potential health effects of soy or soy isoflavones. It is worth focusing on this food group to study both the risks and benefits of soy isoflavones. In addition, recent studies on diet and cardiovascular disease risk factors, such as hypertension, have considered the benefits of foods instead of emphasizing single nutrients (31). Although soybeans and soy products are biochemically complex, information about relationships of this food group to diseases or health-related conditions would help consumers to make healthy food choices.

In the present study, the estrogen status of some women may be similar to that of postmenopausal women. Inclusion of these women may have favored the inverse correlation between soy intake and serum homocysteine concentration. However, restricting the study subjects to women who were aged 50 y old or younger and had had a menstrual cycle in the past 3 mo ($n = 187$) did not substantially alter the results ($r = -0.14$, $P = 0.06$ for total soy products, $r = -0.15$, $P = 0.04$ for soy protein and $r = -0.15$, $P = 0.046$ for soy isoflavone in relation to serum homocysteine concentration).

Our dietary questionnaire was designed to measure an individual's relative intakes of foods and nutrients rather than absolute values. The intakes of folate and vitamins B-6 and B-12 may have been overestimated by the questionnaire because in the validity study, these estimates from the questionnaire were 15% (for vitamin B-12) to 26% (for folate) higher than those estimated from the 12 daily diet records over 1 y. On the other hand, the data presented for soy products may have been underestimated because soy product intake estimated from the questionnaire was 20% lower than that estimated from the 12 daily diet records. Although we estimated that 12.3% of total folate intake and 5.9% of total vitamin B-6 intake would be derived from soy products in our study subjects, these values are very likely to be underestimates.

TABLE 3

Spearman correlation coefficients between intakes of soy protein, methionine and vitamins B-6 and B-12 and folate status in premenopausal Japanese women[1]

Item	Soy protein	Methionine	Vitamin B-6	Vitamin B-12	Folate	Serum folate
Soy protein	1.00					
Methionine	0.27[b]	1.00				
Vitamin B-6	0.47[b]	0.51[b]	1.00			
Vitamin B-12	0.26[b]	0.71[b]	0.48[b]	1.00		
Folate	0.56[b]	0.31[b]	0.82[b]	0.32[b]	1.00	
Serum folate	0.15[a]	0.02	0.21[b]	0.005	0.28[b]	1.00

[1] Correlation coefficients are adjusted for age and energy. [a] $P < 0.05$. [b] $P < 0.01$.

We could not measure serum estrogen concentrations because blood samples were not drawn from the subjects on the same day of their menstrual cycle. Tallova et al. (32) reported a change in blood homocysteine concentration during the menstrual cycle. In the present study, adjustment for the day of blood draw in relation to the menstrual cycle did not substantially alter the results ($r = -0.13$, $P = 0.08$ for total soy products, $r = -0.14$, $P = 0.046$ for soy protein and $r = -0.14$, $P = 0.06$ for soy isoflavones in relation to serum homocysteine concentration). To our knowledge, none of the previous studies on lifestyle and homocysteine concentration in premenopausal women have included the measurements of serum estrogens. Interrelationships among dietary soy and endogenous estrogen and homocysteine concentrations should be elucidated in future studies.

LITERATURE CITED

1. Ford, E. S., Smith, S. J., Stroup, D. F., Steinberg, K. K., Mueller, P. W. & Thacker, S. B. (2002) Homocysteine and cardiovascular disease: a systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. Int. J. Epidemiol. 31: 59–70.

2. Nygård, O., Vollset, S. E., Refsum, H., Stensvold, I., Tverdal, A., Nordrehaug, J. E., Ueland, P. M. & Kvale, G. (1995) Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. J. Am. Med. Assoc. 274: 1526–1533.

3. Bates, C. J., Mansoor, M. A., Van der Pols, J., Cole, T. J. & Finch, S. (1997) Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. Eur. J. Clin. Nutr. 51: 691–697.

4. Nygård, O., Refsum, H., Ueland P. M., Stenvold, I., Nordrehang, J. E., Kvåle, G. & Vollset, S. E. (1997) Coffee consumption and plasma total homocysteine: the Hordaland Homocysteine Study. 65: 136–143.

5. Nygård, O. Refsum, H., Ueland, P. M. & Vollset, S. E. (1998) Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am. J. Clin. Nutr. 67: 263–270.

6. Giles, W. H., Kittner, S. J., Croft, J. B., Wozniak, M. A., Wityk, R. J., Stern, B. J., Sloan, M. A., Price, T. R., McCarter, R. J., Macko, R. F., Johnson, C. J., Feeser, B. R., Earley, C. J., Buchholz, D. W. & Stolley, P. D. (1999) Distribution and correlates of elevated total homocysteine: the Stroke Prevention in Young Women Study. Ann. Epidemiol. 9: 307–313.

7. Stolzenberg-Solomon, R. Z., Miller, E. R., III, Maguire, M. G., Selhub, J. & Appel, L. J. (1999) Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population. Am. J. Clin. Nutr. 69: 467–475.

8. Rasmussen, L. B., Ovesen, L., Bülow, I., Knudsen, N., Laurberg, P. & Perrild, H. (2000) Folate intake, lifestyle factors, and homocysteine concentrations in younger and older women. Am. J. Clin. Nutr. 72: 1156–1163.

9. de Bree, A., Verschuren, W. M. M., Blom, H. J. & Kromhout, D. (2001) Lifestyle factors and plasma homocysteine concentrations in a general population sample. Am. J. Epidemiol. 154: 150–154.

10. Jacques, P. F., Bostom, A. G., Wilson, P. W. F., Rich, S., Rosenberg, I. H. & Selhub, J. (2001) Determinants of plasma total homocysteine concentration in the Framingham offspring cohort. Am. J. Clin. Nutr. 73: 613–621.

11. McKinley, M. C. (2000) Nutritional aspects and possible pathological mechanisms of hyperhomocysteinaemia: an independent risk factor for vascular disease. Proc. Nutr. Soc. 59: 221–237.

12. Bazzano, L. A., He, J., Ogden, L. G., Loria, C., Vupputuri, S., Myers, L. & Whelton, P. K. (2001) Legume consumption and risk of coronary heart disease in US men and women. Arch. Intern. Med. 161: 2573–2578.

13. Messina, M. (1999) Legumes and soybeans: overview of their nutritional profiles and health effects. Am. J. Clin. Nutr. 70 (Suppl.): 439S–450S.

14. Lien, E. A., Anker, G., Lønning, P. E., Refsum, H. & Ueland, P. M. (1997) Effects of hormones on the plasma levels of the atherogenic amino acid homocysteine. Biochem. Soc. Trans. 25: 33–35.

15. Morris, M. S., Jacques, P. F., Selhub, J. & Rosenberg, I. H. (2001) Total homocysteine and estrogen status indicators in the Third National Health and Nutrition Examination Survey. Am. J. Epidemiol. 152: 140–148.

16. Hermansen, K., Carstensen, M., Søndergaard, M., Brock, B. & Høie, L. (2001) Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 24: 228–233.

17. Tonstad, S., Smerud, K. & Høie, L. (2002) A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins, and plasma total homocysteine in hypercholesterolemic subjects. Am. J. Clin. Nutr. 76: 78–84.

18. Wakai, K., Egami, I., Kato, K., Kawamura, T., Tamakoshi, A., Lin Y, Nakayama, T., Wada, M. & Ohno, Y. (1999) Dietary intake and sources of isoflavones among Japanese. Nutr. Cancer 33: 139–145.

19. The Science and Technology Agency of Japan. (2001) Standard Tables of Food Composition in Japan, 5th rev ed. Kagawa Nutrition University Press, Tokyo, Japan.

20. Nagata, C., Kabuto, M., Kurisu, Y. & Shimizu, H. (1997) Decreased serum estradiol concentration associated with high dietary intake of soy products in premenopausal Japanese women. Nutr. Cancer 29: 228–233.

21. Shimizu, H., Ohwaki, A., Kurisu, Y., Takatsuka, N., Kawakami, N., Ido, M., Nagata, C. & Inaba, S. (1999) Validity and reproducibility of a quantitative food frequency questionnaire for a cohort study in Japan. Jpn. J. Clin. Oncol. 29: 38–44.

22. Araki, A. & Sako, Y. (1987) Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J. Chromatogr. 422: 43–52.

23. Ishiwata, Y., Endo, N., Ikeda, R. & Yasuda, K. (1995) Fundamental studies on the determination of serum vitamin B-12 and folate using chemiluminanalyzer ACS-180. Jpn. J. Clin. Lab. Automat. 20: 29–37.

24. Asano, K., Iwata, H., Fijimura, K., Ikeda, M., Sugimoto, Y., Matsubara, A., Yano, K., Irisawa, H., Kono, F. & Kanbe, M. (1992) Automated microanalysis of creatinine by coupled enzyme reactions. Hiroshima J. Med. Sci. 41: 1–5.

25. Willett, W. (1990) Implications of total energy intake for epidemiological analyses. In: Nutritional Epidemiology (Willett, W,. ed.), pp. 245–270. Oxford University Press, Oxford.

26. SAS Institute Inc. (1989) SAS/STAT User's Guide, Version 6.04. SAS Institute Inc., Cary, NC.

27. Selhub, J., Jacques, P. F., Wilson, P. W., Rush, D. & Rosenberg, I. H. (1993) Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. J. Am. Med. Assoc. 270: 2693–2698.

28. Shimakawa, T., Nieto, F. J., Malinow, M. R., Chambless, L. E., Schreiner, P. J. & Szklo, M. (1997) Vitamin intake: a possible determinant of plasma homocysteine among middle-aged adults. Ann. Epidemiol. 7: 285–293.

29. Wouters, M. G. A. J., Moorrees, M. T. E. C., Van der Mooren, M. J., Blom, H. J., Boers, G. H. J., Schellekens, L. A., Thomas, C. M. G. & Eskes, T. K. A. B. (1995) Plasma homocysteine and menopausal status. Eur. J. Clin. Invest. 25: 801–805.

30. Nagata, C., Takatsuka, N., Inaba, S., Kawakami, N. & Shimizu, H. (1998) Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J. Natl. Cancer Inst. 90: 1830–1835.

31. Hermansen, K. (2000) Diet, blood pressure and hypertention. Br. J. Nutr. 83: S113 S119.

32. Tallova, J., Tomandl, J., Bicikova, M. & Hill, M. (1999) Changes of plasma total homocysteine levels during the menstrual cycle. Eur. J. Clin. Invest. 29: 1041–1044.