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Selected Abbreviations and Acronyms

BMI = body mass index
HRT = hormone replacement therapy
MET = Metabolic equivalents

previously described in detail (11). In brief, about 92% of all residents aged 35 years old and over in Takayama, Gifu, Japan, responded to a baseline questionnaire including basic demographic information, diet, smoking and drinking habits, exercise, and medical and reproductive histories in September 1992. Diet including alcohol intake during the year prior to the study was assessed using a validated 169-item semi-quantitative food frequency questionnaire. Detailed information on the dietary questionnaire including its validity and reproducibility tests have been described elsewhere (11, 13).

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 (14).

In August 1998 (six years after the initial study), we reinvited to the present study 1500 women who were randomly selected from 6102 women, reported to be premenopausal at baseline, and were known to reside in the city at the end of 1996. We asked their weight and menopausal status using a questionnaire. The onset of menopause was defined as age at the last menstrual period prior to stopping menstruation for 12 months. We sent the same questionnaire with a reminding letter one month after the first one to non-respondents. After all, 1196 women responded to the questionnaire and 23 women were notified to be dead (n = 2) or have moved (n = 21) after 1996. Therefore, the response rate was 81.0%. All study participants completed informed consent forms. Protocol was approved by the local institutional review board.

Subjects for the present study were limited to women aged 40 or over at baseline to study weight change during the perimenopausal period. Out of 881 women aged 40 or over, we excluded those who had menopause by surgery (n = 25) or by radiation/medication (n = 9) and those who did not report their weight (n = 16) or menopausal status (n = 1) from the present analysis. Furthermore, women who reported a history of cancer (n = 2) were excluded. Subsequently, the study comprised of 828 women.

Weight change was calculated from self-reported weights by baseline and follow-up questionnaires. We compared self-reported and measured weights in another sample of women aged 40 to 60 years from the Takayama Study: Intraclass correlation coefficients between self-reported and measured weights were 0.97 in both premenopausal and postmenopausal women.

Weight change over six years was compared between women who remained premenopausal at follow-up and those who had natural menopause during the study period using ANOVA. The other variables examined in relation to weight change were as follows; marital status, years of education, smoking status, nutrient intake, alcohol consumption, age at menarche, number of births, age at first birth, exercise, use of hormone replacement therapy (HRT), and history of chronic diseases. These data were based on baseline questionnaire. Smoking status and HRT use was also asked by follow-up questionnaire. These study variables were categorized and then the covariate-adjusted means of weight change in each category were calculated using ANOVA. Nutrient intakes were adjusted for total energy using the method proposed by Willet (15) before being categorized as tertiary. Tests for linear trend were performed on ordinal variables or continuous variables with use of median values of categories. Age at baseline categorized into 40 to 42,43 to 45,46 to 48, and 49+ years old was included as covariates in all models. Body weight at baseline was also included in all models, as this variable was inversely associated with weight gain in terms of correlation coefficient ($r = -0.18$, $p = 0.0001$). All the statistical analyses were performed using SAS programs (16).

RESULTS

During the 6-year period, 342 women had natural menopause. The means (SD) of age at baseline were 43.0 (2.3) and 47.8 (3.0) for women who remained premenopausal at follow-up and those who had natural menopause during the period, respectively. Weight gain was significantly higher in women who remained premenopausal as compared with those who had natural menopause after controlling for age and weight at baseline (Table 1). Weight change was less in women who were postmenopausal more than 2 years than those in the first or second-year postmenopausal, but the difference was not statistically significant.

Number of births was significantly associated with weight change in women who remained premenopausal after controlling for age and weight at baseline (Table 2). Age at menarche was significantly associated with weight change in women who had natural menopause after controlling for the covariates. The interactions between menopausal status at follow-up and number of births and age menarche were not statistically significant. During the study period, 96 women started HRT. Among them, 40 women had natural menopause during the study period. Weight change was similar between HRT users and non-users during the study period regardless of the menopausal status at follow-up. Smoking status either at baseline or at follow-up was not significantly associated with weight change (data not shown). None of nutrient intakes measured at baseline was significantly associated with weight change (data shown only for selected nutrients).

TABLE 1. Weight and BMI at baseline and weight vhanges from baseline to the follow-up according to menopausal status at the follow-up

	No.	Weight (kg) at baseline[a]	BMI (kg/m2) at baseline[a]	Weight gain[b] (kg)	Distribution of weight change (%)						
					<−10	−10∼−5	−5∼−2	−2∼1	1∼4	4∼7	7kg ≤
Premenopausal	486	53.1 ± 0.39	22.4 ± 0.15	0.41 ± 0.18	1.0	1.4	9.3	37.4	40.3	8.9	1.7
Postmenopausal	342	52.8 ± 0.41	22.2 ± 0.15	−0.18 ± 0.19*	0.9	2.3	15.2	42.4	28.7	7.6	2.9
Years since menopause[c]											
1–2	124	53.4 ± 0.61	22.4 ± 0.23	−0.26 ± 0.29	0	3.2	16.9	45.2	25.8	6.5	2.4
3+	201	52.2 ± 0.54	22.0 ± 0.21	−0.04 ± 0.25	1.5	2.0	12.9	39.8	32.3	8.0	3.5

Values are mean ± standard error based on a regression model.
[a]Adjusted for age at baseline
[b]Adjusted for age and weight at baseline
[c]Age at menopause was missing for 17 postmenopausal women
*$p < 0.05$ for the difference between premenopausal and postmenopausal.

The association of weight change with menopausal status at follow-up was not altered substantially after additional adjustment for age at menarche and number of births, although the difference (0.56 kg) in the means of weight gain between women who remained premenopausal and became postmenopausal was of borderline significance ($p = 0.06$).

Additional exclusion of 20 women who reported a history of diabetes mellitus (n = 11) or cardiovascular diseases such as ischemic heart diseases (n = 10) at baseline strengthened the association of weight change with menopausal status; the difference in means of weight gain between women who remained premenopausal and became postmenopausal was 0.63 kg ($p = 0.04$) after controlling for age, initial weight, age at menarche, and number of births.

DISCUSSION

We observed significantly lower weight gain in women who had natural menopause during the study period as compared with those who remained premenopausal. The distributions of weight change indicate that this difference is not attributable to substantial weight change among a small number of women. These data suggest an association between menopause and weight loss. Although none of the previous studies which longitudinally compared weight change between women who became postmenopausal and remained premenopausal observed significant difference between the two groups (5–7), some showed slight decreased weight gain in women who had menopause (5, 6). Women in the present study gained less compared with those reported in western countries; on average, 0.17 (SD 3.2) kg of weight gain for six years. The effect of menopause on weight may have been diluted by substantial amount of weight gain commonly occurred to premenopausal and postmenopausal women in relatively heavy populations. We also cannot exclude the possibility that the effect of menopause on weight change may vary among the various countries.

Menopause has been associated with change in body composition, a decline in fat-free mass and increase in fat mass, regardless of weight change (17). Lower weight gain observed among women who had menopause in the present study may reflect a great loss of fat-free mass rather than increase in fat-mass. Exercise has been suggested to offset the decline in muscle mass and the gain in body fat (18). In the present study, lower exercise level was associated with greater weight loss in women who had natural menopause during the study period, although this association was not statistically significant. This result may also suggest that relatively small gain in body fat may be characteristics of this study population.

Higher parity was associated with higher weight or weight gain in some studies (19, 20). We found that early age at menarche was associated with weight change in women who had menopause during the study period. However, Björkelund et al. (21) did not observe significant association between menarche age and weight gain in longitudinal study of middle-aged Swedish women.

One of the limitations of the present study is that information on weight was based on self-report at baseline and follow-up. Although our validity study showed that intraclass correlation between self-reported and measured weight was relatively high, the change in weight may be subject to error. However, it is unlikely that such a measurement error is dependent on menopausal status at follow-up as the intraclass-correlation coefficients did not differ by menopausal status. To our knowledge, there are no other available data concerning the accuracy of reporting weight among Japanese women.

We could not obtain information on changes in some lifestyle variables such as diet and exercise and health condition including the onset of diseases during the study period. Change in diet and exercise may have resulted in the lack of their associations with weight change in the present study. If diet and/or exercise were truly associated with weight change, the observed associations may have been due to confounding effects of these factors. To our knowledge, there has been no study addressing change in diet or exercise change around the time of menopause among Japa-

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TABLE 2. Relation of weight changes from baseline to the follow-up to behavioral and reproductive factors according to menopausal status at the follow-up

Variable	Premenopausal No.	Premenopausal Weight gain (kg)	Postmenopausal No.	Postmenopausal Weight gain (kg)
Marital status				
Married	433	0.41 ± 0.26	303	−0.27 ± 0.28
Not married	51	0.31 ± 0.44	38	−0.99 ± 0.61
Years of education				
< 11	176	0.36 ± 0.29	179	−0.55 ± 0.34
12–14	280	0.41 ± 0.28	143	0.00 ± 0.34
15+	30	0.49 ± 0.57	18	−1.22 ± 0.84
Smoking				
Never	384	0.38 ± 0.26	287	−0.18 ± 0.26
Current	70	0.39 ± 0.42	39	−0.94 ± 0.54
Former	17	0.38 ± 0.74	9	−0.20 ± 1.07
Age at menarche (years)				
< 12	127	0.64 ± 0.35	56	0.40 ± 0.49
13–14	259	0.30 ± 0.28	171	−0.30 ± 0.33
15+	95	0.34 ± 0.35	111	−1.03 ± 0.41
	p for trend = 0.45		p for trend = 0.02	
Age at first birth (years)				
< 25	283	0.49 ± 0.29	217	−0.24 ± 0.31
26–30	141	0.37 ± 0.32	92	−0.58 ± 0.42
31+	24	1.34 ± 0.62	20	−0.08 ± 0.83
	p for trend = 0.15		p for trend = 0.85	
Number of births				
0	32	−0.76 ± 0.55	12	−0.84 ± 1.04
1–2	318	0.46 ± 0.28	229	−0.54 ± 0.30
3+	132	0.41 ± 0.32	100	0.18 ± 0.40
	p for trend = 0.04		p for trend = 0.35	
HRT use				
Never	450	0.39 ± 0.26	308	−0.34 ± 0.28
Current	3	1.49 ± 1.65	9	0.56 ± 1.21
Past	18	−0.27 ± 0.70	22	−0.80 ± 0.76
Exercise (METs × h/week)				
< 7.5	226	0.43 ± 0.28	157	−0.83 ± 0.35
7.6–17.5	96	0.36 ± 0.37	68	0.16 ± 0.46
17.6+	164	0.35 ± 0.31	117	−0.11 ± 0.37
	p for trend = 0.78		p for trend = 0.10	
Alcohol (ml/day)				
0	114	0.30 ± 0.34	78	−0.64 ± 0.44
−4.43	184	0.19 ± 0.30	134	−0.44 ± 0.36
4.44+	188	0.66 ± 0.30	130	−0.03 ± 0.37
	p for trend = 0.29		p for trend = 0.24	
Diet				
Total energy (kcal/day)				
< 1,768	162	0.26 ± 0.32	115	−1.00 ± 0.38
1,768–2,174	174	0.43 ± 0.31	101	0.46 ± 0.39
2,175+	150	0.46 ± 0.30	126	−0.47 ± 0.36
	p for trend = 0.80		p for trend = 0.32	
Fat (g/day)				
< 53.5	161	0.65 ± 0.30	115	−0.18 ± 0.38
53.6–63.2	162	0.24 ± 0.31	114	−0.48 ± 0.37
63.3+	163	0.22 ± 0.32	113	−0.36 ± 0.40
	p for trend = 0.16		p for trend = 0.66	

Values are mean ± standard error, based on a regression model controlling for age and weight at baseline and menopausal status at follow-up. Variables listed are measured at baseline.

nese women. In the present study, energy intake and exercise level at baseline did not vary by years left until the onset of menopause among women who had natural menopause during the study period; the age-adjusted means of energy were 2064, 2057, 2159, 2027, and 2158 kcal for women at 1, 2, 3, 4, 5+ years before the onset of menopause, respectively. The corresponding figures for exercise were 32.6, 30.6, 27.0, 25.6, and 28.9 MET × h/week, respectively. Therefore, the changes in these factors during the study period may not be great. Diet and exercise have been implicated as major determinants of weight gain among American women (22, 23). Future study should examine whether the associations between weight change and diet and exercise differ between Japanese and American women.

Age and weight at baseline were similar between respondents and non-respondents; the means of age and weight at baseline were 43.0 years and 52.7 kg in respondents and 42.9 years and 52.5 kg in nonrespondents. However, we cannot exclude the possibility that women who had significant weight gain were more likely to be non-respondents or have reported their weight incorrectly. There has been no available data concerning weight gain and the status of response among Japanese women.

Substantial weight loss may be due to intentional weight loss or underlying disease. Exclusion of nine women who reported loss of 10 kg or over strengthened the association of menopause with weight change; the difference in the means of weight gain between women who remained premenopausal and those who had natural menopause was 0.72 kg ($p = 0.005$).

In this study of Japanese women, on average, modest weight gain during the perimenopausal period was observed. Reproductive factors rather than sociodemographic and behavioral factors appeared to be associated with weight change during this period. Onset of menopause may diminish weight gain during the perimenopausal period. In contrast, early menarche and high parity showed relationship with weight gain.

This study was supported in part by grants 06280108 and 12670349 from the Ministry of Education, Science, Sports, and Culture, Japan.

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British Journal of Cancer (2002) 87, 31–36

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A prospective cohort study of soy product intake and stomach cancer death

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The relationship between intake of soy products and death from stomach cancer was examined in a community-based prospective study of Japanese men and women in Takayama, Japan. Over 7 years of follow-up, 121 deaths from stomach cancer (81 men and 40 women) occurred among 30 304 (13 880 men and 16 424 women) participants who were at least 35 years of age. Diet including the intake of soy products and isoflavones was assessed by a validated semiquantitative food–frequency questionnaire at the beginning of the study. In men, the highest compared to the lowest tertile of total soy product intake was significantly inversely associated with death from stomach cancer after controlling for covariates (hazard ratios=0.50; 95% confidence intervals (CIs) 0.26-0.93, *P* for trend=0.03). Decreased hazard ratios for the highest compared to the lowest tertiles of total soy product intake (hazard ratios=0.49; 95% CI 0.22–1.13) was observed in women, although this association was of marginal significance. These data suggest that soy intake may reduce the risk of death from stomach cancer.
British Journal of Cancer (2002) **87,** 31–36. doi:10.1038/sj.bjc.6600349 www.bjcancer.com
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Keywords: soybeans; isoflavones; stomach cancer; mortality; diet

Epidemiology

The anti-cancer properties of soy isoflavones, i.e., genistein and daidzein, have been demonstrated in experimental studies (Adlercreutz *et al*, 1995). Interest focussed initially on the effects of soy isoflavones on hormone dependent cancers such as breast cancer and prostate cancer, mainly because of their ability to bind to the oestrogen receptor (Martin *et al*, 1978).

An inverse ecological correlation was reported between stomach cancer deaths in 47 Japanese prefectures and intake of soy products and isoflavones estimated from national nutritional survey data, raising the possibility that soy has a potential preventive effect against stomach cancer (Nagata, 2000). Certain analytic epidemiological studies on soy intake and stomach cancer, as reviewed by Messina *et al* (1994) and Wu *et al* (2000). However, soy intake in relation to stomach cancer was not a primary objective of these studies and only a limited number of soy-based items were covered and the association of stomach cancer with intake of isoflavones or soy products as a whole was not assessed.

We have investigated the relationship between soy intake and subsequent death from stomach cancer among Japanese men and women in a cohort study using a validated dietary questionnaire that listed various types of soy foods. Analysis was done separately for fermented and non-fermented soy products, because Wu *et al* (2000) presented the pooled odds ratio/relative risk estimates separately for these two catogories of soy products in a metaanalysis of previous studies on soy intake and stomach cancer.

MATERIALS AND METHODS

The subjects were cohort members from the Takayama Study. The methology of the Takayama Study has been described previously (Shimizu, 1996). Briefly, the cohort included a total of 14 427 men and 17 125 women from Takayama City, Gifu, Japan, who were 35 years of age or older. Each participant completed a self-administered questionnaire in 1992 that was used to collect demographic and general information about smoking, alcohol, diet, exercise and medical and reproductive histories. The response rate was about 90%. Those who had smoked a total of 20 or more packs of cigarettes in their lifetime were defined as smokers.

Dietary history was assessed using a 169-item semiquantitative food-frequency questionnaire. Participants were asked to report the average frequency with which food was consumed in the previous year and the usual serving size of each food item. Nine food items for specific soy products and some others with soy products as ingredients were accounted for in order to estimate the total intake of soy products. Thus, total soy product intake was the sum of the intakes of tofu, miso, soybeans, natto, soymilk, okara, dried-tofu, deep-fried tofu, fried-tofu, fried tofu and minced vegetables/seaweed. The intake of foods and nutrients was estimated from the frequency of ingestion and portion size using the Japanese Standard Tables of Food Composition, 4th revised edition, published by the Science and Technology Agency of Japan. We also estimated the amounts for two main categories of soy products: fermented and non-fermented soy products. The fermented category included miso and natto. The other soy foods measured were non-fermented soy products. Isoflavone (daidzein plus genistein) contents are higher in fermented soy products; according to data summarised by Wakai *et al* (1999), isoflavone concentration per 100 g were 76.6 mg for miso and 67.4 mg for natto, while 24.1 mg for tofu and 36.2 mg for boiled soy beans. Salt is rich in miso, around 10 g per 100 g. The other soy products contain less than 1 g of salt per 100 g. Detailed information on the questionnaire concerning validity and reproducibility tests has been provided elsewhere (Shimizu *et al*, 1999; Nagata *et al*, 2001). For example, the Spearman correlation coefficients comparing estimates from this questionnaire and 12 daily diet records over 1 year were

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Received 5 November 2001; revised 27 March 2002; accepted 10 April 2002

0.75, 0.77 and 0.74 for soy intake in terms of the total amount (g) of soy products, daidzein, and genistein, respectively, in men. Corresponding values for women were 0.68, 0.63, and 0.65, respectively.

We excluded subjects who reported prevalent cancer (n=726; 186 men and 540 women) or a history of total/partial gastrectomy (n=399; 361 men and 161 women) at the baseline. Thus, the resulting baseline population was 30304 (13880 men and 16424 women).

During 7 years of follow-up (1992–1999), deaths and their causes occurring in Takayama City were confirmed with data from the office of the National Vital Statistics. The Statistics and Information Department of the Japanese Ministry of Health and welfare obtain information on deaths and code the causes of death using the International Classification of Diseases (ICD). Permission to review the data regarding dates and causes of deaths was obtained from the Management and Coordination Agency, Japan. Stomach cancer was considered to be the underlying cause of death when the ICD-9 code was 151 or the ICD-10 code was C16. Information concerning subjects who moved away from Takayama City during the course of the study was obtained from the residential registers. During the study period, 648 (4.6%) men and 536 (3.3%) women moved out of Takayama City.

This study was approved by the local institutional review board.

To assess the association between soy intake and death from stomach cancer and to adjust for potential confounding factors, we computed hazard ratios (HRs) and their 95% confidence intervals (CIs) of stomach cancer death that occurred during the 7-year study by using Cox proportional hazard models. The length of follow-up was computed for each subject as the number of years elapsed from the start of the study (September 1, 1992) until the date of death due to stomach cancer, the date of death due to any causes other than stomach cancer, the date on which the person moved out of Takayama, or the end of the study (December 31, 1999). Food and nutrient intake was adjusted for total energy by using the method proposed by Willett (1990) and were converted into categorical variables based on the tertile of their distribution among the entire study population at the baseline. The lowest tertile was the reference category. Initial analysis examined the association between soy intake and death from stomach cancer after controlling for age and total energy. To examine whether the association of soy intake with stomach cancer death could be attributed to non-dietary or dietary variables other than soy product intake, the variables which were significantly associated with stomach cancer death were included in the models as covariates. The variables examined as potential confounders included marital status, body size, education, smoking and alcohol history, history of chronic diseases, use of medication including hormone replacement therapy, reproductive history and dietary components other than soy product intake. Tests for trend were performed on continuous variables using the median value on the categories. All the statistical analyses were performed using SAS programs (SAS Institute, Cary, NC, USA).

RESULTS

During 208951 person-years of follow-up over 7 years, 121 deaths (81 men and 40 women) occurred due to stomach cancer. Characteristics of participants by tertile of soy product intake are shown in Table 1.

In men, there was a statistically significant inverse association between the intake of total soy products and the rate of death from stomach cancer after controlling for age and total energy (Table 2). The intake of non-fermented (but not fermented) soy products was significantly inversely associated with the death rate from stomach cancer. Among non-dietary factors, smoking status and body mass index at about 21 years of age were significantly associated with the rate of stomach cancer death after controlling for age; the HRs (95% CI) for current and former smokers compared to those who never smoked were 2.02 (1.07–3.80) and 1.18 (0.60–2.31), respectively. The HR (95% CI) for the second and highest compared to the lowest tertile of BMI at about 21 years of age were 2.05 (1.14–3.68) and 2.27 (1.22–4.20), respectively. After adjustment for these variables, the inverse associations for total soy product and non-fermented soy product intake remained statistically significant.

For men, of the other dietary components that included total protein, animal protein, vegetable protein, carbohydrate, carotene, crude fibre, salt, vitamin C, vitamin E, vegetables, fruits, rice, processed meats, fish and shellfish, dried fish, pickled vegetables, coffee, and green tea, salt intake was significantly inversely associated with stomach cancer mortality (HR for the highest compared to the lowest tertile=0.53; 95% CI (0.31–0.91)) and rice intake was significantly positively associated with stomach cancer mortality (HR for the highest compared to the lowest tertile=1.81; 95% CI 1.06–3.08) after controlling for age and total energy. Additional adjustment for the intake of salt and rice did not substantially alter the results.

Intake of fermented soy products was not associated with the death from stomach cancer. However, natto (but not miso) was associated with a decreased risk of stomach cancer death (HR=0.70 and 1.19 for the highest tertiles of natto and miso, respectively) after controlling for non-dietary and dietary covariates.

In women, the total amount of soy product intake was significantly inversely associated with death from stomach cancer after controlling for age and total energy (Table 2). Among other dietary and non-dietary variables measured, marital status, age at menarche, BMI at about 21 years of age, and intake of caffeinated coffee were significantly associated with the rate of death from stomach cancer after controlling for age: HR=2.15 for single compared to married; HRs=0.51, 0.41, and 0.18, for age at menarche 13–14, 15–16, and 17+ years compared to <=12 years, respectively; HR=3.36 and 2.16 for the second and the highest compared to the lowest tertile of BMI at about 20 years of age; HR=2.54 for daily compared to rare/never intake of caffeinated coffee. Simultaneous adjustment for these variables did not substantially alter the results. Both fermented and non-fermented soy product intake was inversely associated with the rate of death from stomach cancer, although the associations did not attain statistical significance. Miso intake was nonsignificantly but inversely associated with stomach cancer death in women (HR=0.55, 95% CI 0.23–1.34 for the highest tertile).

We re-analysed data excluding stomach cancer death that occurred during the first 4 years (n=38) in men. The HR estimates did not change substantially: HRs were 0.55, 1.15, and 0.54 for the highest compared to the lowest tertile of total, fermented, and non-fermented soy products, respectively.

DISCUSSION

We observed a significantly reduced rate of death from stomach cancer in the highest compared with the lowest tertile of total soy product intake in men. In particular, a strong inverse association was observed for non-fermented soy products in terms of both the amount (g) and isoflavone intake (mg). A small number of deaths from stomach cancer in women resulted in a lack of statistical power to detect a significant association between soy intake and death from stomach cancer, but the magnitude of HR estimates for the intake of total soy products were similar to that observed for men. These data support the hypothesis that a high intake of soy is associated with decreased risk of stomach cancer.

To our knowledge, this is the first analytic epidemiologic study on stomach cancer to estimate soy and isoflavone intake using a validated questionnaire with a broad range of categories for soy products. Among previous prospective studies on diet and stomach

Table 1 Baseline characteristics of 13 880 men and 16 424 women

Basic characteristics	Men Tertile of total soy product intake			Women Tertile of total soy product intake		
	Low	Middle	High	Low	Middle	High
Median soy product intake (g per day)	49.7	85.6	140.0	46.7	79.5	126.9
Mean age (years)	51.1	54.6	57.6	53.4	55.4	58.2
Mean body mass index (kg per m^2)	22.7	22.5	22.5	22.0	22.0	22.1
Mean BMI at age about 20 years (kg per m^2)	21.3	21.4	21.7	21.0	21.2	21.4
Mean alcohol intake (ml per day)	44.1	41.7	37.8	9.3	7.5	6.2
Mean exercise (METs[a]-h per week)	25.9	27.1	26.7	17.5	19.4	18.6
Married (%)	90.0	91.8	92.5	72.1	76.2	74.5
Years of education ⩾15 years (%)	13.9	10.7	9.6	5.2	4.9	3.7
Current smokers (%)	59.6	53.7	47.7	17.5	11.5	9.7
Former smokers (%)	25.1	30.1	34.1	5.1	4.4	4.1
Age at menarche ⩽12 years (%)				14.8	12.9	9.7
Current users of oral contraceptives (%)				1.0	0.4	0.2
Current users of hormone replacement therapy (%)				1.6	1.7	1.9
Selected dietary variables	**Men (mean±s.d.)**			**Women (mean±s.d.)**		
Soy product intake (g per day)						
Tofu		60.3±45.6			53.5±37.7	
Miso		17.1±11.7			15.0±9.5	
Soybeans		6.3±12.0			6.4±11.2	
Natto		3.7±6.6			3.4±5.8	
Fried tofu		2.2±4.3			2.3±4.3	
Deep fried tofu		5.0±4.0			4.7±3.7	
Dried tofu		0.3±0.7			0.3±0.8	
Fried tofu and minced vegetables/seaweed		3.2±6.2			3.3±6.2	
Okara		0.9±6.9			1.0±6.7	
Soymilk		3.1±24.0			3.3±23.5	
Total		102.1±72.5			93.3±64.0	
Total energy (kcal per day)		2591±868			2103±779	
Total protein (g per day)		94.3±37.5			82.0±33.9	
Carbohydrate (g per day)		352±114			302±108	
Total fat (g per day)		61.0±28.4			55.2±26.5	
Salt (g per day)		14.3±6.2			13.1±5.8	
Coffee (ml per day)		125±148			98±133	

[a]METs, metabolic equivalents.

cancer, six included one or two soy food items (Hirayama, 1984; Nomura *et al*, 1990; Kato *et al*, 1992; Inoue *et al*, 1996; Ahn, 1997; Galanis *et al*, 1998). In a Japanese study, Hirayama (1984) reported a significantly reduced risk of stomach cancer death associated with the daily intake of miso-soup (relative risk (RR)=0.74) compared to those who answered 'never/rare'. No significant association between drinking miso soup and the risk of stomach cancer was reported in other four studies (Kato *et al*, 1992; Inoue *et al*, 1996; Galanis *et al*, 1998). Another soy product, tofu, was included in two studies. In a study of Japanese-American men reported by Nomura *et al* (1990), the RR of stomach cancer was 0.7 (95% CI 0.2–2.3) for those who ate tofu five times per week or more compared to those who ate it one time or less each week. Another study conducted in Korea showed a RR of 0.6 (95% CI 0.40–1.10) for the highest quartile of tofu intake (Ahn, 1997). Most of previous case–control studies included at most only two soy products (miso/soy bean paste or tofu/bean curd) (Wu *et al*, 2000).

Wu *et al* (2000) conducted a meta-analysis of the association of soy product intake with stomach cancer based on previous prospective and case–control studies. They calculated the pooled odds ratio/relative risk estimates separately for fermented and non-fermented soy products. The analysis of 14 studies excluding the study reported by Hirayama yielded an risk estimate of 1.26 (95% CI 1.11–1.43) in association with a high intake of fermented soy products. In contrast, the risk estimate was 0.72 (95% CI 0.63–0.82) for non-fermented soy product intake based on 10 studies. However, they also observed the similar patterns in risk of stomach cancer according to fermented soy product intake and salt intake in these studies. The association of stomach cancer risk with non-fermented soy products was similar to that with vegetables/fruit intake in these studies. Salt and vegetable/fruit intake was directly associated with stomach cancer risk. Therefore, they suggested the possibility that the reported associations for fermented soy products might be affected by the confounding effect of salt intake, and the reported associations for non-fermented soy products might be affected by confounding effects of vegetable/fruit intake. In almost all of the studies in their review, the possible confounding effects of salt, vegetables/fruits, and other dietary factors had not been considered in the soy product analysis. Our study also revealed a difference in the stomach cancer death rate for men based on their consumption of fermented and non-fermented soy products differently associated with the rate of stomach cancer death in men. However, the results on the association between non-fermented soy product intake and death from stomach cancer were not altered after controlling for vegetable or fruit intake in addition to the non-dietary and dietary covariates (for example, after additional adjustment for vegetable intake, HR=0.49 (95% CI 0.26–0.92) in men and HR=0.48 (95% CI 0.20–1.14) in women for the highest compared to the lowest tertile). Additional adjustment for total salt intake also did not alter the results for fermented soy products (HR=0.55 (95% CI 0.23–1.33) for the highest compared to the lowest tertile of intake in women; HR

Table 2 Hazard ratios (HRs) and 95% confidence interval (CI) of stomach cancer death according to soy intake

	Men				Women			
	Tertile of soy product intake			P for trend	Tertile of soy product intake			P for trend
	Low	Middle	High		Low	Middle	High	
Total soy products								
Median intake (g per day)[a]	49.7	85.6	140.0		46.7	79.5	126.9	
No. of death from stomach cancer	29	33	29		16	15	9	
No. of person years	31 656	31 428	31 196		38 201	38 428	38 041	
HR (95% CI)								
Age and energy adjusted	1.00	0.74 (0.44–1.24)	0.46 (0.27–0.81)	0.006	1.00	0.83 (0.41–1.69)	0.42 (0.19–0.95)	0.04
Adjusted[b]	1.00	0.75 (0.45–1.26)	0.48 (0.27–0.83)	0.008	1.00	0.93 (0.45–1.90)	0.49 (0.21–1.12)	0.10
Adjusted[c]	1.00	0.76 (0.44–1.31)	0.50 (0.26–0.93)	0.03	1.00	0.97 (0.47–1.99)	0.53 (0.23–1.22)	0.15
Fermented soy products								
Median intake (g per day)	8.9	18.3	30.9		8.2	16.8	30.9	
No. of death from stomach cancer	24	30	27		16	15	9	
No. of person years	31 595	31 447	31 238		38 052	38 503	38 116	
HR (95% CI)								
Age and energy adjusted	1.00	1.15 (0.67–1.97)	0.89 (0.51–1.54)	0.15	1.00	0.75 (0.36–1.54)	0.49 (0.23–1.07)	0.07
Adjusted[b]	1.00	1.17 (0.69–2.01)	0.91 (0.53–1.58)	0.76	1.00	0.78 (0.38–1.61)	0.52 (0.24–1.13)	0.10
Adjusted[c]	1.00	1.26 (0.72–2.19)	1.05 (0.57–1.93)	0.84	1.00	0.83 (0.40–1.72)	0.56 (0.25–1.24)	0.16
Non-fermented soy products								
Median intake (g per day)	36.7	65.2	112.0		35.3	61.2	102.0	
No. of death from stomach cancer	29	29	23		15	16	9	
No. of person years	31 716	31 385	31 178		38 211	38 373	38 087	
HR (95% CI)								
Age and energy adjusted	1.00	0.73 (0.43–1.22)	0.45 (0.26–0.79)	0.005	1.00	0.92 (0.46–1.86)	0.44 (0.19–1.00)	0.05
Adjusted[b]	1.00	0.73 (0.44–1.23)	0.46 (0.26–0.81)	0.006	1.00	0.97 (0.48–1.96)	0.48 (0.21–1.10)	0.09
Adjusted[c]	1.00	0.74 (0.43–1.28)	0.49 (0.26–0.92)	0.03	1.00	1.01 (0.50–2.04)	0.51 (0.22–1.18)	0.13

[a]Adjusted for total energy. [b]Men: adjusted for age, total energy, smoking status (current, former, and never-smokers) and body mass index at age about 21 years; Women: adjusted for age, total energy, marital status, age at menarche, and body mass index at age about 21 years. [c]Men: adjusted for the variables above and intake of salt and rice; Women: adjusted for the variables above and intake of coffee.

for men are shown in Table 2). These results suggest the negative association found between soy intake and stomach cancer death is not due to confounding with salt or vegetable/fruit intake. However, it is possible that other, non-measured factors, may contribute to some potential confounding. The fact that miso was negatively associated with stomach cancer death in women and that the other fermented soy food, natto, was also negatively associated with stomach cancer death in men, may suggest that the finding for miso in men is due to confounding factors.

Japan remains among the countries showing the highest mortality rates from stomach cancer in the world. The Japanese diet in Japan includes soybean-based foods that are rich in the isoflavones. It could be argued that our findings contradict these observations. The results from the present study imply that the high consumption of soy in Japan should not contribute to its high mortality rate from stomach cancer. We observed significantly positive association between the consumption of rice and stomach cancer mortality in men, and the association was marginally significant in women. High-starch diets have been hypothesised to be associated with stomach cancer (Ji *et al*, 1998). High rice consumption, which is common among the Japanese, may contribute to their high mortality rate from stomach cancer in Japan.

An inverse association of dietary soy with stomach cancer is biologically plausible. Isoflavones have been offered as the primary anticancer soy constituent. Genistein inhibited the growth of human gastric cancer cells (Yanagihara *et al*, 1993). Genistein attenuated gastric carcinogenesis by inducing increased apoptosis and lowering cell proliferation and angiogenesis of antral mucosa and gastric cancers (Tatsuta *et al*, 1999). A diet containing miso inhibited N-methyl-N′-nitro-N-nitrosoguanidine induced stomach tumours (Kim *et al*, 1985; Watanabe *et al*, 1999). It is also possible that other components in soy products might be etiologically important agents. Several laboratory studies have demonstrated that saponins as well as the Bowman–Birk Inhibitor isolated from soybeans have anti-carcinogenic properties (von Hofe *et al*, 1991; Rao and Sung, 1995).

Use of population-based design is one of advantages of the present study. The response rate was high, and the number of people who were censored because they moved out of the city was small. We focused considerable attention on validating the method used for dietary assessment with the use of food records. Our questionnaire enabled us to estimate soy product intake quantitatively and to include careful adjustments for many potentially confounding factors.

There are several limitations in the present study. We examined the relation between soy intake and stomach cancer mortality, not incidence. Thus, our results reflect the potential effect of soy intake on stomach cancer incidence, survival, or both. If soy intake is related differently to incidence than it is to mortality, our results are potentially biased. For example, although the effect of gastric cancer screening on survival is not clear, there is a possibility that a high intake of soy might actually be associated with an increased incidence of stomach cancer combined with a higher participation rate in stomach cancer screening, which could lead to earlier diagnosis and better chances of survival. We obtained the information on history of participation in stomach cancer screening at the baseline but not during the follow-up period. Additional adjustment for history of participation in stomach cancer screening for 3 years prior to entry into the study did not alter the observed association between soy intake and risk of death from stomach cancer. For men, the HRs were 0.59 (95% CI 0.26–0.93), 1.05 (95% CI 0.56–1.96), 0.48 (95% CI 0.25–0.90), and 0.63 (95% CI 0.33–1.21) for the highest compared to the lowest tertiles of total, fermented and non-fermented soy products, and total isoflavones,

respectively. The corresponding HRs for women were 0.52 (95% CI 0.23–1.20), 0.57 (95% CI 0.27–1.22), 0.58 (95% CI 0.26–1.30), and 0.54 (95% CI 0.23–1.27), respectively.

The prospective design of our study and exclusion of those with cancer and gastrectomy at the baseline lessen the possibility that disease status would bias the reporting of exposures. However, disease history obtained at the baseline was based on self-reporting. Those who reported a history of gastric ulcer may have been not informed that they actually had gastric cancer. When we re-analysed data excluding those who had reported a history of gastric ulcer (2041 men), however, the results were not altered substantially. The HRs (95% CI) were 0.50 (0.26–0.97), 1.13 (0.59–2.14), 0.47 (0.24–0.91), and 0.67 (0.34–1.29) for the highest as compared to the lowest tertiles of total, fermented and non-fermented soy products, and total isoflavones, respectively. We also tried to avoid the possibility that underlying stomach cancer should affect the diet by re-analysing data excluding deaths from stomach cancer that occurred during the first 4 years of the study.

The relatively short follow-up was also limitation. Fatal cases that progressed from diagnosis to death in 7 years or less may not have been representative of all stomach cancer cases, or even all stomach cancer deaths.

Adjustment for potential confounders did not modify the HR estimates. However, the high colinearity between various nutrients, foods, and food groups make it difficult to distinguish among the effects.

We could not obtain information on a history of infection with *Helicobacter pylori*, a major risk factor for stomach cancer. Intake of tofu was significantly inversely associated with *H. pylori* infection in a cross-sectional study of Japanese men (Shinchi *et al*, 1997). This study found no association of miso soup intake with *H. pylori* infection, but in another cross-sectional study of Japanese men (Tsugane *et al*, 1994), miso soup intake was significantly positively associated with *H. pylori* infection. High dose of genistein reduced *H. pylori* toxin-induced inflammatory cytokine expression (interleukin-8) (Ding *et al*, 1997). Genistein inhibited growth of *H. pylori* (Bae *et al*, 2001). Infection of *H. pylori* may be a potential confounding factor or a marker of certain confounding factor for the association between soy product intake and stomach cancer. It is also possible that *H. pylori* infection is an intermediate factor between soy product intake and stomach cancer.

Our questionnaire was designed to measure an individual's relative intake of food and nutrients rather than their absolute values. The data presented for food and nutrient intake may have been overestimated by the questionnaire. In the validity study, the estimates for soy product intake were about 20% higher for men and 40% higher for women when they were based on the questionnaire rather than on the estimates, which were based on 12 daily diet records over 1 year. The estimate of total energy was about 11% higher by questionnaire than by the diet records.

In summary, our data support for the notion that soy intake may be associated with risk of death from stomach cancer, either because of its beneficial effect on etiology or survival once stomach cancer occurs. Given the limitations mentioned above and the observational study design, this hypothesis remains tentative. In addition, the reason for a lack of an inverse association for miso, but not natto, is not clear. The potential effect of dietary soy on stomach cancer warrants further study.

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ACKNOWLEDGEMENTS

This study was supported in part by a grant from the Ministry of Education, Science, Sports, and Culture, Japan.

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American Journal of Epidemiology

Vol. 156, No. 9
Printed in U.S.A.
DOI: 10.1093/aje/kwf118

Soy and Fish Oil Intake and Mortality in a Japanese Community

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Received for publication October 18, 2001; accepted for publication June 19, 2002.

The relation between intake of fish and soy products and subsequent all-cause and cause-specific mortality was examined in a cohort of 13,355 male and 15,724 female residents of Takayama, Gifu, Japan. A diet that included soy and fish intake was assessed in 1992 by using a validated semiquantitative food frequency questionnaire. Over 7 years of follow-up, 2,062 participants (1,163 men and 899 women) died. For men, the highest compared with the lowest quintile of total soy product intake was marginally significantly inversely associated with total mortality after adjustment for total energy and nondietary covariates (hazard ratio = 0.83, 95% confidence interval: 0.69, 1.01; p for trend = 0.07). After adjustment for nondietary covariates, a decreased hazard ratio for the highest compared with the lowest quintile of total soy product intake was also observed for women (hazard ratio = 0.83, 95% confidence interval: 0.68, 1.02; p for trend = 0.04). Additional adjustment for dietary factors significantly associated with total mortality did not attenuate these associations. For women but not for men, n-3 fatty acids from fish were significantly inversely associated with total mortality. Results showed that soy intake may have moderate but beneficial effects on total mortality.

cohort studies; diet; fishes; mortality; soybeans

Abbreviations: CI, confidence interval; ICD-10, *International Classification of Diseases*, Tenth Revision.

A high level of consumption of soy and fish is intrinsic to the Japanese diet. Much attention has been paid to the health effects of soy and fish intake. However, evidence has been provided mainly from laboratory studies. Dietary soy or phytoestrogens in soy inhibit various diseases, including cancer, heart disease, and inflammatory disease (1, 2), perhaps mediated by the phytoestrogens' ability to inhibit tyrosine kinase activity (3), angiogenesis (4), and atherosclerosis (5); induce apoptosis (6); scavenge hydrogen peroxide free radicals (7); and bind to estrogen receptors (8). Soy also provides a wide range of potentially healthful nutrients, such as high amounts of protein, fiber, and vitamin E. Fish oil or long-chain n-3 fatty acids also have been considered multipotent compounds. Evidence for the potential of fish oil to counter cancer and cardiovascular disease has been supported by the fact that they have various beneficial functions, including inhibition of eicosanoid biosynthesis derived from arachidonic acid, inhibition of coagulation, promotion of vasodilation, attenuation of inflammation (9, 10), and modification of plasma lipid and lipoprotein concentrations (11). The Japanese guidelines for a healthy diet and lifestyle, "Japan Health 21" (Internet Web site: http://www.kenkounippon21.gr.jp), recommend intake of fish rich in n-3 fatty acids to prevent cancer. Japan has reported the highest life expectancy in the world (12). A high intake of soy or fish oil may contribute to the Japanese people's longevity.

In the current study, referred to as the Takayama Study (13), we examined the associations between intake of soy and fish oil and subsequent mortality in a cohort of Japanese men and women. Although several studies have evaluated the relation of fish intake to total or cardiovascular disease mortality, the results have been inconsistent (14–22). To our knowledge, no previous prospective study has examined the relation between soy intake and total mortality. Although effects of soy intake on cardiovascular disease risk factors have been studied, the direct relation between soy intake and cardiovascular disease has not been assessed in analytic epidemiologic studies. It is worthwhile to study this relation in Japan, where people consume quantities of soy and fish likely to be physiologically meaningful.

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MATERIALS AND METHODS

The Takayama Study, established in 1992, included 14,427 male and 17,125 female residents of Takayama, Gifu, Japan, aged 35 years or older. A detailed description of the study design has been reported elsewhere (13). At baseline, cohort members completed a self-administered questionnaire on demographic characteristics, smoking and drinking habits, diet, exercise, and medical and reproductive histories. The response rate was about 92 percent. Dietary history was assessed by using a 169-item semiquantitative food frequency questionnaire. For each food, participants reported the average frequency of consumption in the previous year and specified the usual serving size. Intake of foods and nutrients was estimated from the frequency of intake and portion size by using the Standard Tables of Food Composition in Japan, published by the Science and Technology Agency of Japan (23).

We included nine specific soy product items and 16 items for fish and fish dishes. These items and some other dishes that include soy or fish as ingredients were taken into account to obtain intake estimates. Thus, total soy product intake was considered the sum of the intakes of tofu, miso, soybeans, natto, soy milk, okara, dried tofu, deep-fried tofu, fried tofu, and fried tofu and minced vegetables/seaweed. Intake of isoflavones from soy products was estimated on the basis of previously published data on isoflavone concentrations in soy foods summarized by Wakai et al. (24). Intake of fish oil (long n-3 fatty acids, eicosapentanoic and docosahexaenoic acids) was estimated by using data published by Sasaki et al. (25).

The ability of our questionnaire to characterize persons according to food and nutrient intake was examined by comparing it with other dietary assessment methods, including a 3-day diet record, four diet recalls over 1 year, and 12 daily diet records over 1 year. Detailed information on the questionnaire, including its validity and reproducibility tests, has been described elsewhere (26). For men, the correlations between the questionnaire and 12 daily diet records over 1 year for intake of soy products, soy isoflavones, eicosapentanoic acid, and docosahexaenoic acid were 0.75, 0.75, 0.52, and 0.58, respectively. The corresponding values for women were 0.68, 0.62, 0.41, and 0.43.

Exercise was assessed by asking study participants to specify the average number of hours per week they spent performing various kinds of activities during the past year. The details are described elsewhere (27).

Deaths (and their causes) occurring in Takayama during the follow-up period (1992–1999) were confirmed with data from the office of 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 by using the *International Classification of Diseases*, Tenth Revision (ICD-10). Permission to review data regarding dates and causes of deaths was obtained from the Management and Coordination Agency, Japan. Information on subjects who moved away from Takayama during the course of the study was obtained from the residential registers. During the study period, 666 (4.6 percent) men and 506 (3.0 percent) women moved out of Takayama.

This study was approved by the local institutional review board.

For this analysis, we excluded subjects who reported on the baseline questionnaire that they had a previous history of cancer (181 men and 540 women), stroke (255 men and 154 women), or ischemic heart disease (636 men and 707 women). Hence, the analytic population at baseline consisted of 29,079 subjects (13,355 men and 15,724 women).

The major endpoint 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. For the category of cardiovascular disease, we also selected ischemic heart disease (ICD-10 codes I20, I21, I24, and I25) and cerebrovascular disease (ICD-10 codes I60–I64, I67, I69, and Q25–Q28) as disease-specific endpoints.

The associations of soy and fish intake with death were examined by using Cox proportional hazards models. Length of follow-up was calculated for each subject as the number of years that had elapsed from 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 the study (December 31, 1999). Food group and nutrient intakes were adjusted for total energy by using the method proposed by Willett (28) and were categorized by quintile (tertile for some disease-specific endpoints) based on their distribution among the entire study population at baseline. The hazard ratios and their 95 percent confidence intervals for all-cause and cause-specific mortality for each category of intake were computed in comparison with the lowest intake category. In the initial analysis, associations of soy and fish intake with mortality, adjusted for age and total energy, were examined. Analyses were also adjusted for other factors significantly associated with all-cause and cause-specific mortality. The variables examined were marital status; body mass index; years of education; smoking; alcohol intake; history of chronic diseases; use of medications, including hormone replacement therapy; reproductive history; and dietary components other than soy and fish intake. Tests for trend were performed on continuous variables by using the median value for the categories. All statistical analyses were conducted by using SAS software programs (29). To eliminate the possibility that the observed association of soy and fish intake with mortality was the result of a preclinical disease condition, we further evaluated the association after excluding deaths (546 men and 426 women) that occurred during the first 4 years of follow-up.

RESULTS

Baseline characteristics of the study population are given in table 1. During 201,160 person-years of follow-up, 2,062 deaths (1,163 men and 899 women) were identified. These deaths were classified by underlying causes into 653 (400 men and 253 women) from cancer (31.7 percent of all deaths), 635 (308 men and 327 women) from cardiovascular disease (30.8 percent of all deaths), and 774 (455 men and 319 women) from all other causes (37.5 percent of all deaths).

TABLE 1. Baseline characteristics of 13,355 men and 15,724 women studied to determine an association of soy and fish oil intake with mortality, Takayama, Japan, 1992

Variable	Men		Women	
	Mean (SD*)	%	Mean (SD)	%
Age (years)	54.0 (12.1)		55.1 (13.0)	
Body mass index (kg/m^2)	22.5 (2.8)		22.0 (2.9)	
Alcohol intake (ml/day)	42.0 (41.5)		7.7 (16.8)	
Exercise (MET*-hours/week)	27.1 (41.5)		18.8 (29.6)	
Married		91.4		75.2
≥15 years of education		11.8		4.7
≥3 children		31.2		34.1
Current smoker		55.0		13.1
Former smoker		28.2		4.4
Hypertension		18.9		17.4
Diabetes mellitus		5.9		2.7
Age at menarche ≤12 years				12.8
Postmenopausal				58.9
Food and nutrient intake/day				
Soy products (g)	102.3 (72.6)		93.3 (63.7)	
Isoflavone (mg)	44.6 (29.9)		41.4 (27.0)	
Fish and shellfish (g)	104.7 (71.7)		82.8 (56.9)	
n-3 fatty acids (mg)	992 (746)		794 (588)	
Total energy (kcal)	2,603 (869)		2,108 (777)	
Total protein (g)	94.5 (37.6)		82.2 (33.9)	
Carbohydrate (g)	353 (114)		302 (107)	
Total fat (g)	61.1 (28.6)		55.4 (26.6)	
Crude fiber (g)	5.1 (2.7)		5.2 (2.8)	
Vitamin C (mg)	115 (85)		122 (86)	
Vitamin D (IU)	293 (209)		232 (160)	
Vitamin E (mg)	8.5 (4.0)		8.2 (3.9)	

* SD, standard deviation; MET, metabolic equivalent.

There was a significant inverse association of soy product intake as well as isoflavone intake with all-cause mortality for men and women after we controlled for age and total energy (tables 2 and 3). Intake of fish oil was inversely significantly associated with all-cause mortality in women but not in men.

The association between soy product intake and all-cause mortality in men was of borderline significance after adjustment for nondietary factors significantly associated with all-cause mortality after adjustment for age and total energy (marital status, body mass index, smoking status, alcohol intake, coffee intake, exercise, and history of hypertension and diabetes mellitus). This association was not altered substantially after additional adjustment for dietary variables significantly associated with all-cause mortality after adjustment for nondietary variables (polyunsaturated fat and vitamin B_1). The hazard ratio for the highest compared with the lowest quintile of soy product intake was 0.86 (95 percent confidence interval (CI): 0.68, 1.09) after controlling for these variables.

The associations of soy product intake and fish oil intake with all-cause mortality in women remained statistically significant after adjustment for nondietary factors (marital status, years of education, body mass index, smoking status, alcohol intake, exercise, age at menarche, menopausal status at baseline, and history of diabetes mellitus). Additional adjustment for dietary factors significantly associated with all-cause mortality (rice/grains/potatoes and vegetables) did not attenuate these associations. For the highest compared with the lowest quintile, the hazard ratio for soy products was 0.84 (95 percent CI: 0.67, 1.04) and for fish oil was 0.73 (95 percent CI: 0.59, 0.90) after we controlled for the covariates. Intake of soy products and fish oil was independently significantly associated with all-cause mortality in women in the multivariate model, which included intake of soy products and fish oil as well as nondietary variables. Hazard ratios for the highest compared with the lowest quintiles of soy products and fish oil were 0.87(p for trend = 0.046) and 0.78 (p for trend = 0.02), respectively.

TABLE 2. Risk of all-cause mortality in men according to quintile of soy product and fish intake, Takayama, Japan, 1992–1999

	Median intake*	No. of deaths	No. of person-years	Risk of all-cause mortality			
				Hazard ratio†	95% CI‡	Hazard ratio§	95% CI
Total soy products							
Quintile of intake (g/day)							
1 (low)	40.6	173	18,367	1.0		1.0	
2	63.9	195	18,340	0.91	0.74, 1.12	0.93	0.76, 1.15
3	85.8	253	18,206	0.97	0.80, 1.17	1.00	0.83, 1.22
4	114.4	263	18,021	0.92	0.76, 1.12	0.96	0.79, 1.16
5 (high)	166.4	279	18,101	0.79	0.65, 0.95	0.83	0.69, 1.01
p for trend				0.01		0.07	
Isoflavones							
Quintile of intake (mg/day)							
1 (low)	18.6	170	18,407	1.0		1.0	
2	29.0	194	18,291	1.00	0.81, 1.23	1.02	0.83, 1.26
3	38.1	234	18,228	0.96	0.79, 1.17	0.96	0.79, 1.18
4	49.7	264	18,046	0.92	0.76, 1.12	0.95	0.78, 1.15
5 (high)	71.5	301	18,064	0.82	0.68, 0.99	0.88	0.72, 1.06
p for trend				0.02		0.10	
Fish							
Quintile of intake (g/day)							
1 (low)	46.2	214	18,292	1.0		1.0	
2	68.1	210	18,232	0.90	0.74, 1.08	0.92	0.76, 1.11
3	86.8	212	18,317	0.88	0.73, 1.07	0.91	0.75, 1.10
4	111.9	243	18,150	0.87	0.73, 1.05	0.90	0.75, 1.09
5 (high)	157.8	284	18,045	0.92	0.77, 1.10	0.94	0.78, 1.12
p for trend				0.40		0.50	
Fish oil							
Quintile of intake (mg/day)							
1 (low)	410	205	18,281	1.0		1.0	
2	602	198	18,315	0.83	0.68, 1.01	0.82	0.67, 0.99
3	788	225	18,186	0.85	0.71, 1.03	0.87	0.72, 1.05
4	1,051	258	18,138	0.88	0.74, 1.06	0.88	0.73, 1.06
5 (high)	1,582	277	18,116	0.88	0.73, 1.05	0.87	0.73, 1.05
p for trend				0.40		0.38	

* Adjusted for total energy.
† Adjusted for age and total energy.
‡ CI, confidence interval.
§ Adjusted for age, total energy, marital status, body mass index, smoking status (never, former, current), alcohol intake, coffee intake, exercise, and history of hypertension and diabetes mellitus.

The inverse association between soy product intake and any cause-specific mortality was not statistically significant for men (table 4), but the hazard ratios did not vary greatly according to cause of death. For women, no cause-specific mortality was statistically significantly associated with intake of soy products or fish oil (table 5).

The respective numbers of deaths from ischemic heart disease and cerebrovascular disease were 63 and 137 for men and 52 and 132 for women. For men, the hazard ratios of death from ischemic heart disease were 0.71 (95 percent CI: 0.39, 1.28; p for trend = 0.29) for the highest tertile of soy product intake and 1.05 (95 percent CI: 0.56, 1.97; p for trend = 0.91) for the highest tertile of fish oil intake after we controlled for total energy and nondietary covariates. The corresponding figures for women were 0.86 (95 percent CI: 0.42, 1.78; p for trend = 0.15) and 0.73 (95 percent CI: 0.37, 1.45; p for trend = 0.37). For men, the hazard ratios of death from cerebrovascular disease were 0.84 (95 percent CI: 0.55, 1.29; p for trend = 0.43) for the highest tertile of soy product intake and 1.19 (95 percent CI: 0.78, 1.81; p for trend = 0.37) for the highest tertile of fish oil intake. The corresponding figures for women were 0.85 (95 percent CI: 0.56, 1.30; p for

TABLE 3. Risk of all-cause mortality in women according to quintile of soy product and fish intake, Takayama, Japan, 1992–1999

	Median intake*	No. of deaths	No. of person-years	Risk of all-cause mortality			
				Hazard ratio†	95% CI‡	Hazard ratio§	95% CI
Total soy products							
Quintile of intake (g/day)							
1 (low)	38.5	176	21,982	1.0		1.0	
2	60.2	180	22,145	1.01	0.82, 1.24	1.05	0.85, 1.29
3	80.0	161	22,026	0.84	0.68, 1.04	0.91	0.73, 1.13
4	103.1	185	21,979	0.86	0.76, 1.05	0.93	0.76, 1.15
5 (high)	148.6	197	21,990	0.76	0.62, 0.94	0.83	0.68, 1.02
p for trend				0.003		0.04	
Isoflavones							
Quintile of intake (mg/day)							
1 (low)	17.9	161	22,023	1.0		1.0	
2	27.6	168	22,145	1.04	0.84, 1.29	1.07	0.86, 1.33
3	35.8	170	22,051	0.89	0.71, 1.10	0.96	0.78, 1.20
4	45.9	172	21,992	0.79	0.64, 0.98	0.85	0.68, 1.05
5 (high)	64.8	228	21,911	0.85	0.70, 1.04	0.94	0.76, 1.15
p for trend				0.02		0.18	
Fish							
Quintile of intake (g/day)							
1 (low)	36.6	207	21,906	1.0		1.0	
2	53.9	177	22,018	0.94	0.77, 1.15	0.93	0.76, 1.14
3	68.8	171	21,979	0.95	0.78, 1.17	0.96	0.79, 1.18
4	88.1	173	22,091	0.92	0.75, 1.13	0.93	0.76, 1.14
5 (high)	122.4	171	22,128	0.82	0.67, 1.00	0.86	0.70, 1.05
p for trend				0.07		0.17	
Fish oil							
Quintile of intake (mg/day)							
1 (low)	332	216	21,838	1.0		1.0	
2	486	179	22,111	0.91	0.74, 1.10	0.92	0.76, 1.13
3	635	163	22,032	0.82	0.67, 1.00	0.84	0.69, 1.04
4	832	178	22,025	0.85	0.70, 1.04	0.90	0.73, 1.09
5 (high)	1,253	163	22,118	0.73	0.59, 0.89	0.77	0.62, 0.94
p for trend				0.002		0.01	

* Adjusted for total energy.
† Adjusted for age and total energy.
‡ CI, confidence interval.
§ Adjusted for age, total energy, marital status, years of education, body mass index, smoking status (never, former, current), alcohol intake, age at menarche, menopausal status, exercise, and history of diabetes mellitus.

trend = 0.45) and 0.87 (95 percent CI: 0.58, 1.30; *p* for trend = 0.49).

Exclusion of deaths that occurred during the first 4 years of follow-up somewhat strengthened the associations between soy product intake and all-cause and cause-specific mortality, except for cardiovascular disease in men; hazard ratios for the highest compared with the lowest quintile of soy product intake were 0.75 (*p* for trend = 0.001), 0.79 (*p* for trend = 0.25), and 0.73 (*p* for trend = 0.04) for mortality from all causes, cancer, and all causes other than cancer and cardiovascular disease, respectively, after we adjusted for total energy and nondietary variables. For women, the hazard ratios for the highest compared with the lowest quintile of soy product intake were 0.88 (*p* for trend = 0.06) and 0.73 (*p* for trend = 0.03) for mortality from all causes and from all causes other than cancer and cardiovascular disease, respectively. The hazard ratios for the highest compared with the lowest quintile of fish oil intake were 0.75 (*p* for trend = 0.05) and 0.51 (*p* for trend = 0.07) for mortality from all causes and cancer, respectively.

TABLE 4. Risk of cause-specific mortality in men according to quintile of soy product and fish oil intake, Takayama, Japan, 1992–1999

	Cancer			Cardiovascular disease			All other causes		
	No. of deaths	Adjusted hazard ratio*	95% CI†	No. of deaths	Adjusted hazard ratio‡	95% CI	No. of deaths	Adjusted hazard ratio§	95% CI
				Soy products					
Quintile of intake									
1 (low)	61	1.0		52	1.0		60	1.0	
2	57	0.78	0.55, 1.12	52	0.86	0.59, 1.27	86	1.15	0.83, 1.61
3	96	1.10	0.80, 1.52	54	0.73	0.50, 1.08	103	1.15	0.84, 1.58
4	84	0.86	0.62, 1.20	72	0.90	0.62, 1.29	107	1.14	0.83, 1.57
5 (high)	102	0.89	0.64, 1.22	78	0.78	0.55, 1.12	99	0.85	0.61, 1.17
p for trend		0.60			0.29			0.20	
				Fish oil					
Quintile of intake									
1 (low)	73	1.0		60	1.0		72	1.0	
2	75	0.87	0.63, 1.21	53	0.74	0.51, 1.08	70	0.82	0.59, 1.15
3	69	0.74	0.53, 1.02	53	0.71	0.49, 1.03	103	1.12	0.83, 1.52
4	84	0.82	0.60, 1.12	71	0.82	0.58, 1.15	103	1.01	0.75, 1.37
5 (high)	99	0.89	0.66, 1.20	71	0.76	0.54, 1.07	107	0.98	0.72, 1.32
p for trend		0.52			0.27			0.77	

* Adjusted for age, total energy, marital status, body mass index, alcohol intake, and smoking status (never, former, current and 1–29 years of smoking, and current and ≥30 years of smoking).

† CI, confidence interval.

‡ Adjusted for age, total energy, marital status, body mass index, alcohol intake, coffee intake, exercise, smoking status (never, former, current), and history of hypertension and diabetes mellitus.

§ Adjusted for age, total energy, marital status, body mass index, alcohol intake, exercise, and smoking status (never, former, current and 1–29 years of smoking, and current and ≥30 years of smoking).

DISCUSSION

Soy product intake showed a moderate but statistically significant inverse association with all-cause mortality in women. A similar reduction in all-cause mortality was observed for men. This dietary component appears to have moderate but beneficial effects on survival. Although we found no statistically significant association of soy product intake with specific causes of death studied, the decreased hazard ratios for all causes except cardiovascular disease in women suggest that soy intake may influence the risk of these diseases, resulting in an overall decrease in all-cause mortality. Significant associations between soy intake and incidence of or mortality from various cancers have been reported in some epidemiologic studies (1).

Intake of fish, a food group, was not significantly associated with all-cause mortality in either men or women. However, we found a significantly inverse association between fish oil intake and all-cause mortality in women. Previous epidemiologic studies have not provided strong evidence for the association between fish intake and cancer (30). In general, a null association between fish intake and cardiovascular disease has been reported in populations with higher levels of fish intake (21). A decreased hazard ratio of mortality from all causes other than cancer and cardiovascular disease was observed for the highest compared with the lowest quintile of fish oil after we excluded deaths that occurred during the first 4 years of follow-up. Future studies should examine the possibility that fish oil may have a beneficial effect on the inhibition of certain diseases, including those other than cancer and cardiovascular disease.

The present study has a number of strengths and limitations. Strengths include the prospective design, use of a general population, a relatively low proportion of subjects lost to follow-up, and consideration of potential confounding from dietary and nondietary factors. Another advantage is that our questionnaire included a wide range of soy products and types of fish used to estimate total intake of soy products and fish oil, respectively. Most previous studies investigating the health effects of soy consumption have included a limited number of items for these dietary components and have not adequately described the validity of the dietary assessment methods used (1). In addition, a recently developed fatty acid composition table (18) enabled us to study the relations of mortality to types of fat consumed.

Although our major interest was all-cause mortality, we had to further infer which causes of death were responsible for the associations we observed. The present study lacked statistical power to conduct meaningful analyses of soy and fish intake and each specific cause of mortality. However, the finding based on all-cause mortality, which reflects the death rate for the common causes of death in the population,

TABLE 5. Risk of cause-specific mortality in women according to quintile of soy product and fish oil intake, Takayama, Japan, 1992–1999

	Cancer			Cardiovascular disease			All other causes		
	No. of deaths	Adjusted hazard ratio*	95% CI†	No. of deaths	Adjusted hazard ratio‡	95% CI	No. of deaths	Adjusted hazard ratio§	95% CI
Soy products									
Quintile of intake									
1 (low)	45	1.0		61	1.0		70	1.0	
2	58	1.22	0.83, 1.80	57	1.04	0.72, 1.50	65	0.94	0.67, 1.32
3	46	0.91	0.60, 1.37	70	1.26	0.89, 1.79	45	0.64	0.44, 0.93
4	54	0.98	0.66, 1.46	70	1.06	0.75, 1.50	61	0.77	0.54, 1.09
5 (high)	50	0.79	0.53, 1.18	69	0.90	0.63, 1.28	78	0.79	0.57, 1.11
p for trend		0.12			0.57			0.11	
Fish oil									
Quintile of intake									
1 (low)	54	1.0		85	1.0		77	1.0	
2	53	1.01	0.69, 1.48	60	0.82	0.59, 1.15	66	0.94	0.68, 1.31
3	46	0.87	0.59, 1.29	57	0.79	0.58, 1.11	60	0.87	0.62, 1.22
4	59	1.08	0.75, 1.57	64	0.86	0.62, 1.20	55	0.77	0.54, 1.09
5 (high)	41	0.70	0.47, 1.05	61	0.77	0.55, 1.00	61	0.80	0.57, 1.13
p for trend		0.15			0.16			0.11	

* Adjusted for age, total energy, number of children, and history of diabetes mellitus.

† CI, confidence interval.

‡ Adjusted for age, total energy, marital status, smoking status (never, former, current), body mass index, exercise, age at menarche, and history of hysterectomy and hypertension.

§ Adjusted for age, total energy, marital status, years of education, smoking status, body mass index, exercise, alcohol intake, and history of diabetes mellitus.

should have considerable public health implications. Another disadvantage of the study is that, because we used mortality rather than incidence as the outcome, we could not determine whether intake of soy or fish oil is related to incidence, to survival, or to both.

We were able to take into account the confounding effects of various nondietary and dietary factors. However, it is possible that other factors that we did not measure may have contributed to confounding. In addition, as in other dietary studies, a high colinearity between various nutrients, foods, and food groups made it difficult to distinguish their effects.

Although our questionnaire showed a moderate to good ability to rank individual relative intake of foods and nutrients, the absolute estimates may have been overestimated. In the validity study, the estimates for total soy product intake were about 25 percent higher for men and 40 percent higher for women when they were based on the questionnaire versus the diet records.

According to the 1991 National Nutritional Survey report (31) covering 5,353 households in Japan, mean daily intake of soy products estimated from a 3-day diet record was 66.4 g (4.6 percent of calories). In the present study, the mean percentages of calories obtained from soy products were 5.4 percent (standard deviation, 2.9 percent) for men and 6.3 percent (standard deviation, 3.2 percent) for women. Considering that the National Nutritional Survey included subjects younger than age 35 years (age distribution was not described in the report) and that soy product intake increased with age (31), soy product intake in our study population would not differ greatly from that for subjects in this survey.

We cannot exclude the possibility that the effects of ill health on diet could not be controlled for by excluding prevalent cancer, stroke, and heart disease or by repeating the analysis and excluding deaths that occurred during the first 4 years of follow-up. Longer follow-up will be needed to evaluate the long-term relation between soy and fish oil intake.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Ministry of Education, Science, Sports, and Culture, Japan.

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

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

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

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

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

Materials and Methods

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

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

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

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

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

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

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

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

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